mRNA expression is higher in male relative to female *Drosophila melanogaster* by 14.690-fold for age-regulating gene *Sir2* and by 6.038-fold *for autism-related gene CG1607*

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Summary

- Do females and males regulate aging differently? Are female and male brains functionally different?
- qRT-PCR measured relative expression levels of *Sir2 and CG1607* in female and male flies.
- Male flies showed 14.690-fold higher levels of *Sir2*, and 6.038-fold higher levels of *CG1607* relative to female fruit flies.

Abstract

Sir2 and *CG1607* code for proteins in a family that regulate cellular health and are correlated with aging. The mRNA expression of *Sir2* and *CG1607* in female and male fruit fly brains was quantified through qRT-PCR to examine if there was sexually dimorphic gene expression in fruit flies. Male flies showed upregulation for both genes (14.69-fold for *Sir2* mRNA and 6.038-fold for *CG1607* mRNA) relative to females, highlighting sex-specific differences in male and female brain gene expression.

Introduction

Hypotheses:

- Since female fruit flies tend to live longer than males, *Sir2* gene expression will be higher in female relative to male fruit flies.
- As autism is more highly diagnosed in men, *CG1607* gene expression will be higher in male relative to female fruit flies.

***Drosophila melanogaster* (fruit flies):**

- Model organism for genetics, physiology, and evolution.
- Reproduce quickly, are inexpensive to maintain, are easy to distinguish between sexes [1].
- Share 75% of disease-related genes with humans.

Target genes:

- ***Sir2***
  - Codes for a sirtuin protein [2].
  - Sirtuins are in part responsible for aging, specifically the unravelling of bundled DNA in the epigenome [3].
  - Upregulation of this gene was shown to extend lifespan and affect age-related diseases [3].
- ***CG1607***
  - Associated with autism and affects motor coordination [4]
  - Autism is more highly diagnosed in men [5].

Methodology

Fly Keeping
Flies were raised on a molasses-based diet from Archon Scientific

Separation by Sex
Male and female virgins were separated ≤4 hours post-eclosion and fed with 5% sucrose for 72 hours

Sample Collection
Male and female fruit fly brains were dissected under microscope

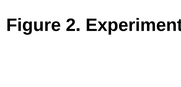


Figure 1. Fluorescent microscope image of fruit fly brain. The fruit fly brain accounts for about 14% of the fly body weight.

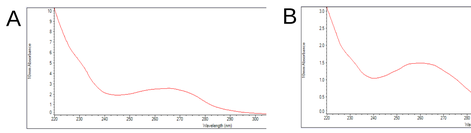
RNA Extraction
- Homogenized brains with glass beads and subjected to TriZol/chloroform RNA extraction
- Separated organic materials with isopropanol
- Treated samples with DNase
- Stored RNA sample at -80℃

qRT-PCR
- qRT-PCR performed in iTaq™ Universal SYBR® Green One Step Kit with 20µl volume
- *Sir2* primers
  - FWD: 5' - CCACATCCTACGGCCATATTC - 3'
  - REV: 5' - TGTTGCAGCTCCTCACTATTC - 3'
- *CG1607* primers
  - FWD: 5' - CGGCAACACGCAATACTTTAC - 3'
  - REV: 5' - ACAATACAGCCACACCGATAC - 3'

Data Analysis
- Calculated ΔCt – the difference between the threshold cycle of the target gene and the threshold cycle of the reference gene
- Fold change: $2^{-\Delta\Delta Ct}$

Figure 2. Experimental design flowchart.

Results



C

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

Figure 3: Total RNA yield and concentration for male and female fruit fly brain samples. (A) Total female RNA yield, (B) total male RNA yield, (C) and total RNA concentration of brain samples.

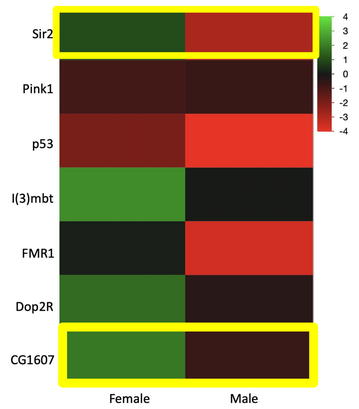


Figure 4. Males have 14.690-fold increased *Sir2* mRNA expression and 6.038-fold increase for *CG1607* relative to males. Red indicates higher gene expression; green indicates lower gene expression. All genes were normalized to *gapdh1*.

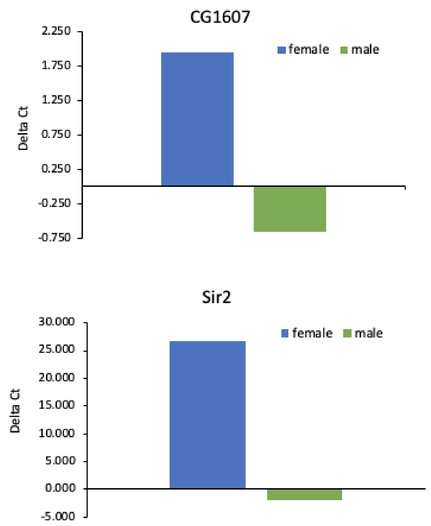


Figure 5. Males have lower ΔCT (threshold cycle) values for both *Sir2* and *CG1607* when normalized to *gapdh1*. ΔCT value of *Sir2* is -2.828 for males and is -2.828 for females; ΔCT values of *CG1607* is -0.648 for males and 1.946 for females. Lower ΔCt value indicates larger amounts of starting RNA.

Discussion & Conclusion

Sir2 and *CG1607* are differentially expressed between female and male fruit flies.

Sir2

- *Sir2* mRNA is expressed 14.690-fold higher in male relative to female fruit flies.
- This contradicts the hypothesis that *Sir2* will be more highly expressed in female fruit flies.
- Mating is shown to have effects on life-span in *Drosophila* [3]. These results reflect the pre-mating mRNA expression of fruit flies.

CG1607

- *CG1607* is 6.038-fold higher in males.
- This supports the hypothesis and agrees with previous studies indicating higher rates of autism in men [5].

These strong differences between males and females under control conditions support the notion that males and females should be studied separately. This study also provides baseline expression levels of *Sir2* and *CG1607* for future studies.

Study Limitations & Future Directions

- Flies were pooled for RNA extraction; individual differences were not accounted for. If possible, RNA from individual flies could be analyzed for this gene.
- Analyzed RNA without accounting for protein expression in different sections of the brain. Protein analysis like western blotting should be performed to verify the protein level differences.
- There was no statistical analysis in this study. More samples should be analyzed to establish significance.
- Only one life stage was studied. Future studies could monitor mature flies or fly larvae to analyze sexual dimorphism of gene expression in other life stages.
- Expose flies to stressors (heat, starvation, chemical toxicants) and quantify gene expression from males and females.

References