

Expression of mitochondrial metabolic regulation gene *Sirtuin 2* significantly downregulated in female compared to male *D. melanogaster*

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Summary

- Male and female *D. melanogaster* were exposed to chemical GenX through their diet
- Change in gene expression of *Sirt2* was quantified through qRT-PCR
- Females express significantly less *Sirt2* at baseline compared to males

Abstract

GenX is a synthetic chemical used in food packaging, nonstick coatings, and firefighting foam, but there is limited information on the effects this chemical has on living organisms. To gain a better understanding of the potential impacts of this chemical on the transcriptional level, male and female *D. melanogaster* were exposed to GenX through their diet. The expression of *Sirtuin 2* (*Sirt2*), a gene involved in the regulation of mitochondrial energy metabolism in *D. melanogaster* brains, was analyzed at control conditions between sexes and after exposure to GenX using qRT-PCR. Females upregulated *Sirt2* 1.04-fold (p=0.903) and males upregulated *Sirt2* 1.49-fold (p=0.718) after exposure to GenX, and females express 331-fold less *Sirt2* than males at control conditions (p=0.019). We conclude that females express significantly less *Sirt2* at control conditions compared to males.

Introduction

Hypothesis: GenX exposure will change the expression in *Sirt2* in *D. melanogaster*.

Females will express higher baseline levels of *Sirt2* than males.

***D. Melanogaster*:** 75% of human disease genes share a homolog in *D. melanogaster*. Chosen for their short life span, size, simple maintenance, the impact of GenX can be easily tested using this model organism.

GenX: Unregulated PFAS chemical often used in manufacturing nonstick coatings, plastic hardware, and technology. GenX has no conclusive evidence as to its toxicity or safety levels.

***Sirtuin 2*:** Important in the regulation of mitochondrial energy metabolism, protein deacetylation, determination of adult lifespan, and neurodegeneration. Thought to be preferentially implicated in stress response in females (1).

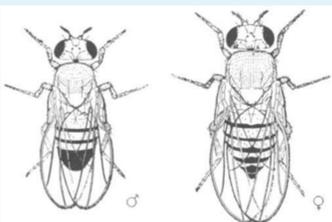


Figure 1. Adult male and female flies. Male flies (left) have a solid black lower abdomen, and female flies (right) have a striped lower abdomen.

Methods

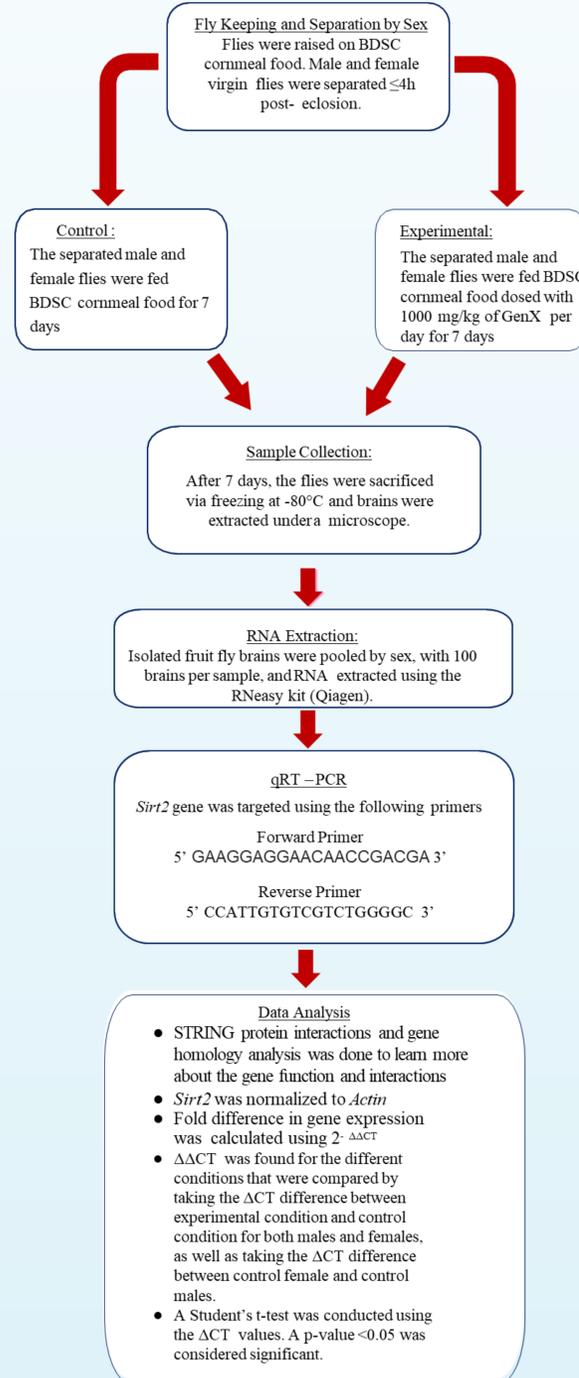


Figure 2 Flowchart of experimental design.

Results



Figure 3. Heatmap showing gene expression fold change in control and experimental conditions for exposure to GenX, including sexual dimorphism differences. CF represents Control Female, EF represents Experimental Female, CM represents Control Male and EM represents Experimental Male. Green indicates downregulation of a gene and red indicates upregulation of a gene. *Sirt2* has a 1.03-fold upregulation in experimental females compared to control females (p-value=0.903), 1.49-fold upregulation in experimental males compared to control males (p-value=0.717) and 331-fold downregulation in control females compared to control males (p-value=0.0194).

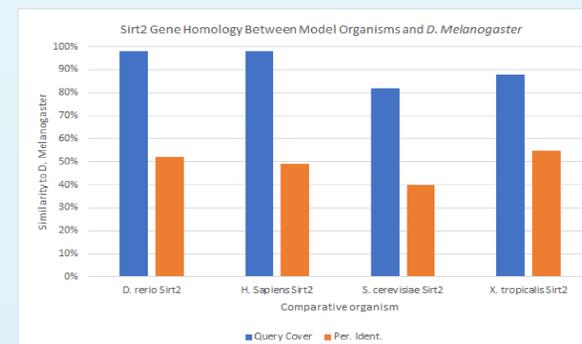


Figure 4: *D. Melanogaster* Sirt2 query cover and percent identity graph. The figure was generated using NCBI protein BLAST and compares Query cover and percent identity to *D. melanogaster* using different species.

Discussion

- *Sirt2* is involved in the determination of lifespan and neurodegeneration.
- There is a 331-fold difference in gene expression between male and female control flies, with a p-value of 0.0194.
- We also report a 1.03 fold difference upregulation in experimental females compared to control females (p-value=0.903), 1.49 fold upregulation in experimental males compared to control males (p-value=0.717). These data are not statistically significant.
- This suggests that exposure to GenX does not affect gene expression of *Sirt2*.
- The sexual dimorphism of the gene in adult flies agrees with previously published data (2).
- *Sirt2* is conserved in other species, which provides the potential for repeating the experiment in other model organisms and to potentially translate these results into human responses.

Study Limitations and Future Directions

- *D. melanogaster* were only exposed to a single dosage of GenX.
 - Future studies can use varying concentrations of GenX.
- *D. melanogaster* were exposed to GenX in their adult life stage.
 - Future experiments can assess GenX exposure during developmental stages
- Only acute exposures were expressed.
 - Future studies can include different endpoints and longer exposures.

References

1. Alexey Moskalev, Ekaterina Plyusnina, Mikhail Shaposhnikov, Lyubov Shilova, Alexey Kazachenok & Alexander Zhavoronkov (2012) The role of D-GADD45 in oxidative, thermal and genotoxic stress resistance, Cell Cycle, 11:22, 4222-4241, DOI: [10.4161/cc.22545](https://doi.org/10.4161/cc.22545)
2. <https://flybase.org/reports/FBgn0038788.html>