# *brat* is significantly expressed 107.5-fold more in male than in female *Drosophila melanogaster* at baseline

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## Summary

- Is the gene expression of *brat* affected in both sexes of GenX-exposed *D. melanogaster* (fruit flies)?
- Measured gene expression level of male and female fruit flies via qRT-PCR
- *brat* was significantly upregulated in control males relative to control females
- *brat* expression was not significantly changed due to GenX exposure

## Abstract

Chemicals used in manufacturing can contaminate the environment, posing potential risks to organisms. Furthermore, female and male organisms may have a sexually dimorphic response to stressors. The purpose of this study is to determine whether the toxicant GenX has effect on expression of *brat*, and if there is sexually dimorphic expression of *brat* at baseline. Expression of *brat* was measured using qRT-PCR. After exposure to GenX, *brat* was upregulated in males by 2.86-fold (p-value = 0.405) and downregulated in females by 1.2-fold (p-value = 0.896). At baseline, females downregulated *brat* 107.5-fold compared to males (p-value = 0.0348). Female fruit flies express significantly less *brat* than males at control conditions.

## Introduction

**Fruit Flies:** Share conserved genes with humans, and sexes are easy to differentiate from one another.

**GenX:** chemical GenX is a per- and polyfluoroalkyl substance that is found on non-stick coatings or food packaging.

**Target gene**: *brat*, responsible for coding tumor suppressor proteins that inhibit protein translation and prevent self renewal of cancerous cells.

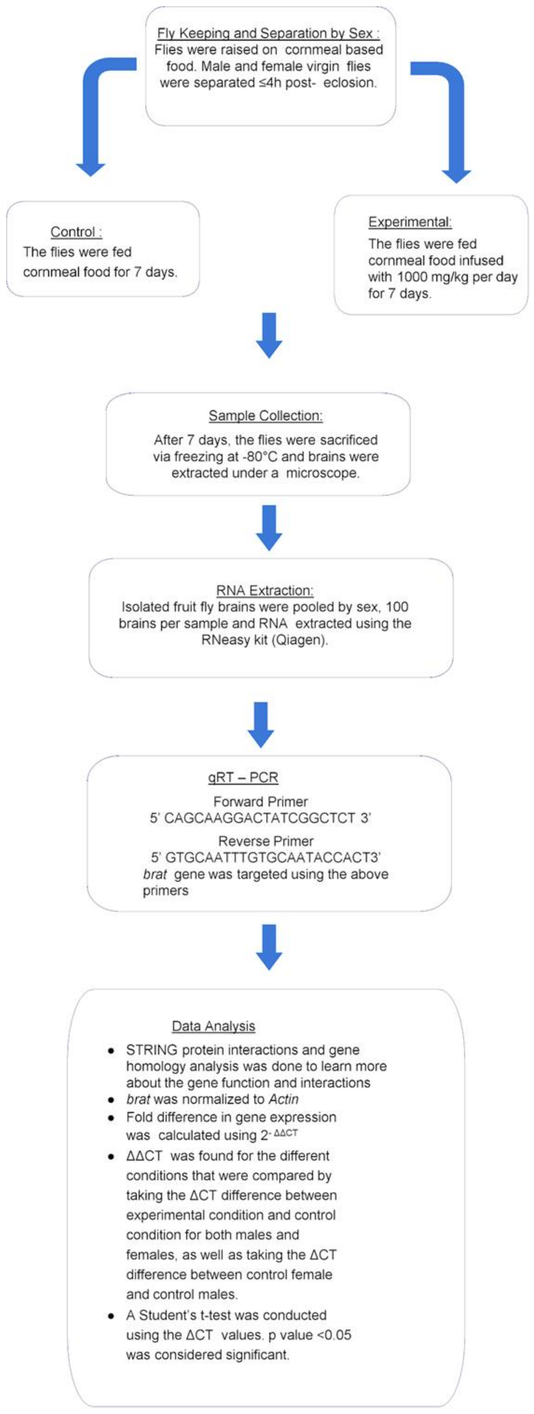
**Hypothesis**: GenX increases the gene expression of *brat* in both sexes of fruit flies. Males express more *brat* than females.

**Figure 1A. Photo of adult female *D.melanogaster.*** Light micrograph with magnification X7.5.

(1B)

**Figure 1B. Photo of adult male *D.melanogaster.*** Magnification X7.5.

## Methodology




## Results

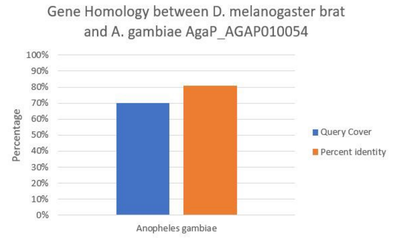
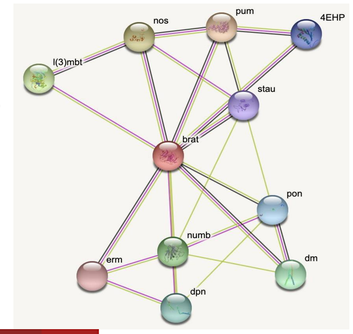



**Figure 3.Bar graph showing gene homology between *D. melanogaster* and *A. gambiae* in terms of query coverage and percent identity for the *brat* gene.** Generated using NCBI Protein BLAST.

**Figure 4 STRING diagram showing proteins that interact with *brat*.** Proteins connected to *brat* are involved in cell cycle regulation. This figure was generated in STRING (https://string-db.org/).



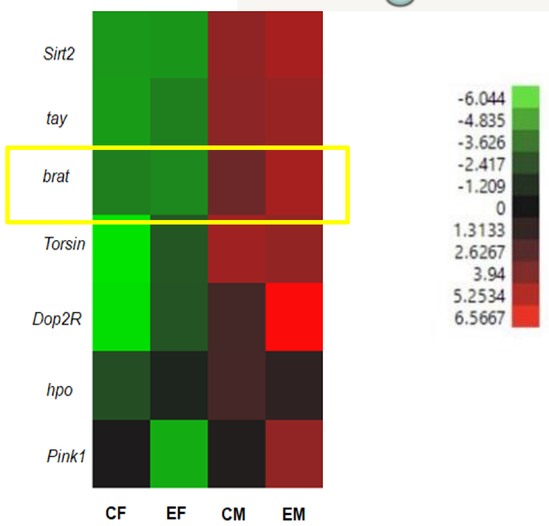




**Figure 5. Heatmap showing changes in gene expression for flies under different conditions.** CF indicates control female, EF indicates experimental female, CM indicated control male, and EM indicates experimental male. Gene upregulation is indicated by red and downregulation is indicated by green. *brat* was downregulated 1.2-fold when comparing experimental female to control female (p-value= 0.896), upregulated 2.855-fold when comparing experimental male to control female (p-value=0.405), and downregulated 107.5-fold when comparing control female to control male (p-value=0.035). This heatmap was generated in JMP Pro 14 using ΔCt values normalized to a housekeeping gene, *actin*.

## Discussion

**We report that there is a 107.5-fold difference in the expression of *brat* between male and female control flies (p-value=0.0347).**

- *brat* showcases sexual dimorphism in its expression in flies. It acts as a tumor suppressor gene, suggesting that there is potentially more cell growth in control male flies compared to control female flies.

- This will have to be verified through increasing the number of replicates and looking at the variation in gene expression in each of the replicates

**Exposure to GenX did not cause a significant change in expression of *brat* in both male and female experimental flies (p-value > 0.05).**

- This suggests that GenX does not cause growth of brain tumors in fruit flies, over the course of the 7 day exposure.

***brat* interacts with several proteins that are involved in cell cycle regulation and transcriptional repressors (figure 4).**

- the potential differences in gene expression of *brat* between males and females could suggest that there is greater transcriptional repression in male fly brains.

## Study Limitations and Future Directions

- The sample was taken from lab-grown *D. melanogaster*. Future experiments can use natural populations of flies instead of strains bred in labs.
- Only one GenX concentration was tested. In the future, we could add different concentrations of GenX to fly food to study the relationship between *brat* and GenX concentrations, to potentially establish a dose response curve.
- Fruit flies were exposed to GenX only in their adult life stages. Future experiments could expose flies to GenX at different times in their life to see if GenX affects growth and development of flies through their life stages.
- Changes in gene expression due to GenX was measured only in the brain. Measure change in gene expression from GenX exposure in other tissues, not just the brain, to see if, and how, GenX exposure affects gene expression in other tissues.

## References