Increasing doses of Benzo[a]pyrene (B[a]P) decreased bacterial diversity in male *Drosophila melanogaster*

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Introduction

Drosophila melanogaster
- Established model organism [9]
- Sexual dimorphism, low cost, short lifespan, and shared evolutionary history with humans [4]
- 60% of *D. melanogaster* genome is homologous with *H. sapiens* [1]

Males vs. Females
- Male and female *D. melanogaster* display differences in things such as sexual selection, reproductive behaviors, and hormones [3]
- Different stressors can result in various biological responses that differ between male and female fruit flies [2,3]

B[a]P (Benzo[a]pyrene)
- Highly toxic polycyclic aromatic hydrocarbon (PAH) that is formed during the incomplete combustion of organic materials, such as coal, oil, and tobacco smoke [5]
- Classified as a potent carcinogen by the International Agency for Research on Cancer (IARC). It is known to induce DNA damage and mutations [6]

Microbiome
- The microbes in your gut can affect how the brain develops and immune system function [7]
- Plays a crucial role in various aspects of fly and human biology, including nutrient digestion and absorption, immune system development and function, and behavior modulation [8]
- 16S Sequencing: A method to identify and classify different bacteria by sequencing the 16S gene that's present in all bacteria but varies between species

Hypothesis
- Bacterial diversity in male *Drosophila melanogaster* will decrease with increased levels of benzo[a]pyrene (B[a]P) exposure.

Materials and Methods

1. Fly Caring
-Fruit flies get proper nutrition with standard fly food mix
-10 males and 10 females housed together and allowed to mate
-Flies were kept on food specific for each condition for 7 days
-50 flies mixed ages combined together for each sample

2. Tissue Collection
-Fruit flies separated by sex into males and females
-Homogenization
(whole fly bodies and bead mill)

3. DNA Extraction
-Qiagen DNeasy Blood and Tissue Extraction Protocol
-Lyse cells with proteinases and buffers to get DNA out of the cells
-Begin separating DNA from other components, using gravitation separation and DNA binding to spin column
-"Wash" the more isolated DNA with Buffer AW1 and AW2
-Once the DNA sample is cleaned, the isolation of the fragment can be appropriately targeted

4. DNA Sequencing
-Library preparation of 16S gene
-Metagenomic 16S sequencing with Oxford Nanopore MINion
-Barcoding for each sample

5. Data Analysis
-Epi2Me program used to categorize species presence in each sample from Nanopore sequencing data automatically
-Figures were used as visualization tools to identify trends for preliminary data
-Data used as a prediction or initial screen for later studies
-No statistical analyses were performed

6. Figure Generation
-Figures made in Google Sheets
-Total numbers of species per sample classified
-Top 12 species used for pie charts

Results

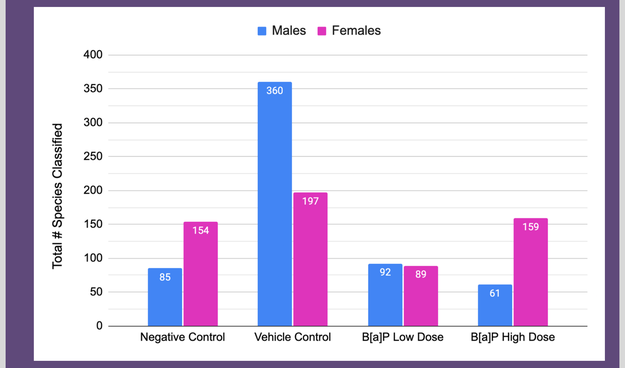

Figure 1. Total number of species classified bar graph. Conditions are shown for both females and males comparing total number of species detected per sample. Figure was generated with Google Sheets.

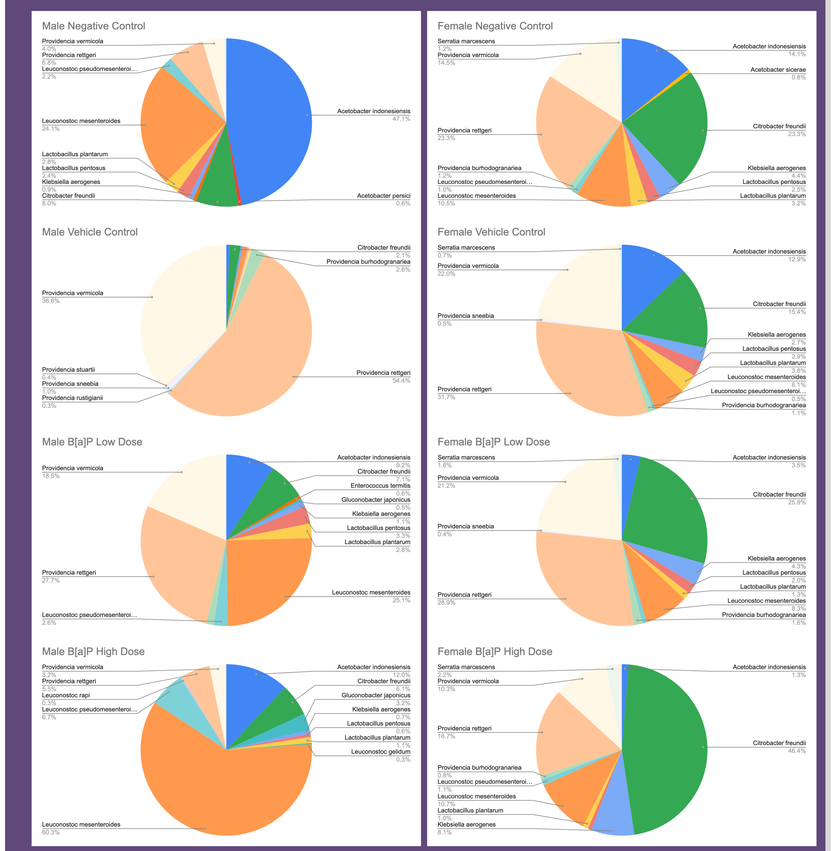

Figure 2. Top 12 abundant species for male and female negative control, vehicle control, B[a]P low dose, and high dose. Conditions are shown for both males and females in a pie chart. Percentages represent abundance through number of reads per species detected and total number of reads per sample. Figure was generated with Google Sheets.

Discussion/Conclusion

Hypothesis was correct: Male B[a]P high dose has less total species classified than male B[a]P low dose.

- Increasing the dose of B[a]P exposure decreases microbiome diversity in males relative to vehicle control, while decreasing (low dose) then increasing (high dose) microbiome diversity in females (Figure 1, 2).
- The sexes have similar levels of microbial diversity at lower B[a]P exposures, with more diversity in females compared to males for the higher dose. However, the females' species composition is more consistent, where the abundance of each species present in males changes more across conditions (Figure 1, 2).
- Increasing B[a]P exposure positively affected *Acetobacter indonesiensis* abundance in males, with the opposite trend in females. While increasing B[a]P exposure negatively effects *Providencia* genus abundance for both sexes, with decreases more dramatic in males. Similarly, while the *Leuconostoc* genus abundance increases with B[a]P dose for both sexes, this effect is more pronounced in males (Figure 2).
- Acetone exposure (vehicle control) increases microbiome diversity for both sexes, with the diversity increasing more dramatically in males compared to females (Figure 1).
- *Acetobacter indonesiensis* abundance decreases in vehicle compared to negative control in males, while the *Providencia* genus abundance increases in vehicle compared to negative control in both sexes with the effect more dramatic in males (Figure 2).
- It's possible that the diversity and species abundance of the female microbiome is more resilient when exposed to acetone and B[a]P.

Limitations/Future Directions

- Only one replicate of extracted DNA per condition was sequenced, meaning that we were unable to determine statistical significance in the data. Also, the age of the flies was not controlled for, being that they were mixed ages. Therefore, we cannot make definitive conclusions from the data, so future experiments can include multiple replicates with male and female flies that are the same ages.
- Samples have different cumulative reads, potentially leading to bias and underrepresentation.
- Only two doses of B[a]P were used, so it was hard to make a conclusion about a dose effect.
- Future studies can use more doses, and a larger range of such.
- Other Further Directions:
  - Look at multiple generations for each condition to see how the microbial diversity is impacted.
  - Look further into how and if the female microbiome is truly more resilient to change, under different conditions.

References