Exposure to Benzo[a]pyrene leads to sex differences in top 3 abundant bacteria species in *Drosophila melanogaster*

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Summary

- Male and female fruit flies exposed to a PAH (benzo[a]pyrene) and acetone (vehicle control)
- Bacterial species in each condition identified with metagenomic 16S sequencing using Oxford Nanopore MINion sequencer.
- Data visualized to compare top 3 and top 12 abundant species

Introduction

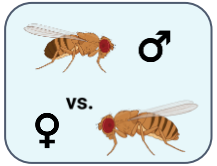
Hypothesis:
The top 3 bacterial abundant species in male and female fruit flies will change with increasing doses of B[a]P [5, 7].

Microbiome:
- Not all bacteria that lives within microbiomes are harmful which could cause fungi and viruses [3]
- They break things down so the organism gets nutrients
- Our microbiomes is essential to our health by assisting our human development like with immune system function and neurodevelopment
- Since our gut microbiota can ferment protein, it helps us with muscle recovery so you don't have to be in pain

Polycyclic Aromatic Hydrocarbons (PAHs):
- PAHs are known carcinogens [2]
- PAHs are contaminants in the environment, found in all places such as soil, water, and air [1]
- Caused by burning many different materials, and are naturally found after volcano eruptions and wildfires [1]
- Benzo[a]pyrene (B[a]P) is a commonly studied carcinogenic PAH

***Drosophila melanogaster* (fruit flies):**
- 60% of their genes are shared with humans [8]
- 75% of genes that causes disease in humans is shared with fruit flies [9]
- Fruit Flies are good organisms for experiments with short life spans and reproduce rapidly [9]
- Fruit flies are established model organisms [9]

Female vs. male significance:
- Female and male fruit flies exhibit sexual dimorphism
- The sexes have many physiological differences such as genetics, immunity, and stress response
- Exposing fruit flies to PAHs to compare effects may be applicable how humans react when exposed to PAHs



Methodology

Fly Husbandry
-10 females and 10 males per vial and allowed to mate
-Proper nutrition (vials with standard fly food mix)

Setting Up Groups (Control and Experimental)
-Mixed ages
-Flies fed food for each condition for 7 days

Sample Collection
-50 flies per sample, separated by sex
-Whole fly body samples

DNA Extraction
-Used Qiagen DNeasy kit
Cell Lysis:
-Destruction of a cell membrane for collection of its contents
Binding of DNA:
-Binding under low salt conditions by removing water from the hydrated molecules in order to remove contaminants from the DNA
Washing:
-Removal of impurities in a way as to not disturb purity of DNA using buffers AW1 and AW2
Purification/Elution:
-Isolated DNA from the mixture with buffer and centrifuge

Nanopore sequencing (16S)
-Library preparation of 16S gene
-16S sequencing with Oxford Nanopore MINion
-Barcoding for each sample

Data Analysis
-Epi2Me program used to analyze species per sample from sequencing data
-No statistical analyses performed

Figure Generation
-Figures made in Google Sheets
-Visualization/prediction tool to find preliminary trends
-Top 3 and top 12 species used

Results

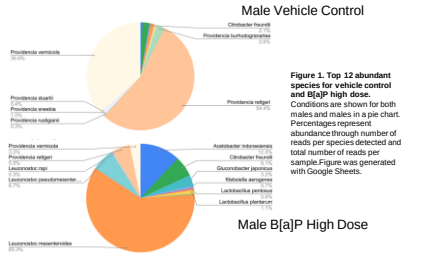


Figure 1. Top 12 abundant species for vehicle control and B[a]P high dose. Conditions are shown for both males and males in a pie chart. Percentages represent abundance through number of reads per species detected and total number of reads per sample.Figure was generated with Google Sheets.

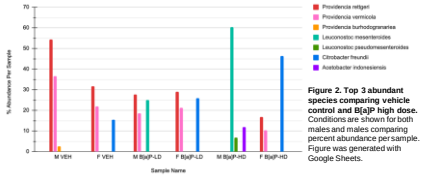


Figure 2. Top 3 abundant species comparing vehicle control and B[a]P high dose. Conditions are shown for both males and males comparing percent abundance per sample. Figure was generated with Google Sheets.

Discussion/Conclusion

All top species of bacteria are: *Acetobacter indosienesis, Citrobacter freundii, Providencia rettgeri, Providencia vermicola, Providencia burhodogranareia, Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides.*

Our hypothesis was partially correct: Increasing dose of B[a]P exposure resulted in a change for which bacterial species were the top 3 most abundant for males, but not for females.

★ *Acetobacter indonesiensis, Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides,* and *Providencia burhodogranaeria* are only seen in the top 3 for male flies (Figure 2).

Vehicle Control:

★ Both sexes share *P. rettgeri* and *P. vermicola* in the top 3 most abundant species. However, the 3rd most abundant for males is *P. burhodogranaeria*, and *C. freundii* for females (Figure 2).
★ Males show a clear dominance of one species, while females have less skewed abundance (Figure 2).

Benzo[a]pyrene Exposure:

★ B[a]P low dose treatment → similar distribution of top 3 species abundance for both sexes (Figure 2).
★ B[a]P high dose → even more pronounced dominance of one species in both sexes, compared to vehicle control (Figure 2).

★ B[a]P high dose treatment → females and males do not share any genuses with each other in the top 3. The dominant species in the top 3 was *Citrobacter* freundii (46.4%) in females and *Leuconostoc mesenteroides* (60.3%) in males (Figure 1).

★ Sex and B[a]P dose influenced the *Providencia* genus: as dose increased → bacterial abundance was reduced in both sexes compared to vehicle control, with a more dramatic effect in males (Figure 1).
  - *Providencia* species are known to cause urinary tract infections and diarrhea in humans [ref]. They're commonly detected in wild fruit flies, and used in host-pathogen studies [ref], although no phenotypic signs of infection in the flies were seen throughout the duration of the experiment.

★ Sex and B[a]P dose influenced the *Leuconostoc* genus: as dose increased → more bacterial abundance compared to vehicle control in both sexes, with a more dramatic effect in males. Also, *L. mesenteroides* and *L. pseudomesenteroides* were only present in the top 3 most abundant for males (Figure 1, 2).
  - *L. mesenteroides* is a bacteria associated with fermentation and commonly found on the skin of fruits and vegetables [ref]. It could be possible that there's a connection between the presence of B[a]P and increased fermentation.

We conclude that exposure to B[a]P will effect the top 3 species of bacteria present in the microbiome of male flies, but only change the abundance of the same top 3 species in female flies; these effects increased with the exposure level of B[a]P.

Limitations / Future Directions

Although no noticeable signs of infection were seen, it is possible that slight internal infections were present in the flies due to the abundant presence of *Providencia* bacteria → future experiments will need to be done to confirm whether or not the flies had an active infection.

Only one replicate of extracted DNA per condition was sequenced → we're unable to determine statistical significance in the data.

The age of the flies was not controlled for, as they were mixed ages → we cannot make definitive conclusions from the data → future experiments can include multiple replicates with male and female flies that are the same ages.

References