

# Effect of Paraphenylenediamine on *NAT2* Gene Expression, Behavior, and L3 Larvae Counts in *Drosophila Melanogaster*

## Introduction

- Drosophila melanogaster* (*D. melanogaster*) is a eukaryotic organism with genetic similarities to humans. This organism also has a short and simple reproduction cycle and is easy to care for. These factors makes it an excellent model organism to study.
- Paraphenylenediamine (PPD) is an arylamine compound commonly found in permanent hair dye, gasoline, and cosmetics (Hein, 2006).
- The *NAT2* gene in humans catalyzes the conversion of PPD to its monoacetyl-PPD and diacetyl-PPD metabolites and is found primarily in the gastrointestinal tract and liver (Chong, 2016).
- Prior research has shown that NAT2 protein has the most association with bladder cancer since it is mostly found in the gastrointestinal tract.

**Hypothesis:** The introduction of 3.4% PPD to *D. melanogaster* will increase the expression of the *NAT2* gene, will create less of a response to a mechanosensation, and will have decreased numbers of third instar (L3) larvae compared to *D. melanogaster* that are exposed to water.

## Methods

**Coding Sequence:** The *NAT2* gene

sequence code was found using Flybase

**Primer Synthesis:** Forward and Reverse Primers for *NAT2* were designed using the Integrated DNA Technologies website.

**Culturing:** *D. melanogaster* cultures were maintained in potato flakes, deionized (DI) water, and yeast pebbles. 1.5 mL of a 3.4% PPD solution was dropped on the experimental group and sat for 24 hours. The control group had 1.5 mL of DI water dropped on that sat for 24 hours.

**Behavioral Assay:** Larvae were subjected to petri dishes with PBS solution. Larvae were then sorted and removed with forceps to be tested for any aversion to a poke in the thorax region.

**Larvae Count:** Larvae were extracted from culture tubes and placed in petri dishes with PBS solution to sort through and count.

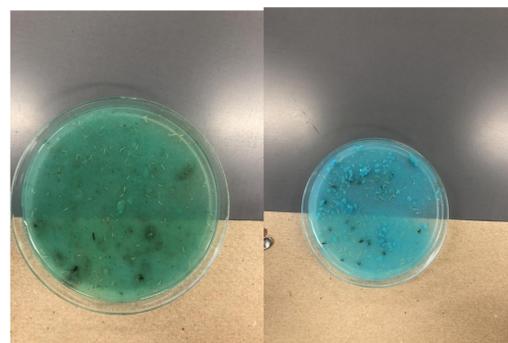
**RNA Extraction:** RNA was isolated and purified with Qiagen's RNeasy Mini Kit.

**Reverse Transcription:** cDNA was synthesized using RevertAid Reverse Transcription Kit.

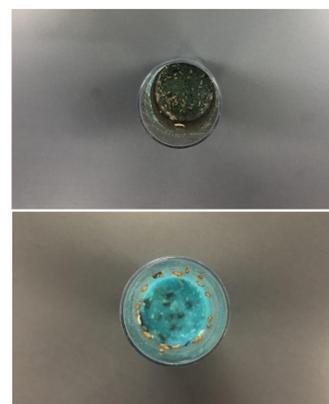
**qPCR:** Powerup SYBR was used for qPCR. *Gapdh* gene expression was used to normalize *NAT2* gene expression.

Annie Galt, Sydney Jones, and Makayla Welch

Department of Biology, Carroll College



**Figure 1:** Fly larvae were extracted from culture tubes after the end of treatment and placed in petri dishes with PBS to be counted and assayed. PPD is on the left, the control is on the right.

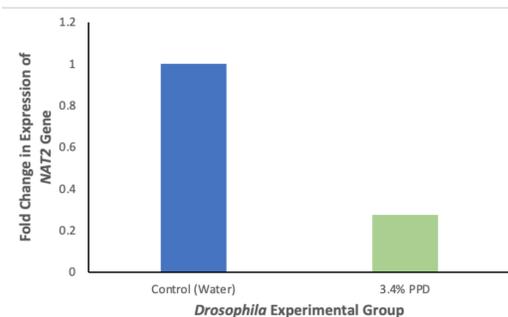


**Figure 2:** Top view of the fly cultures with potato media. PPD is on the top, the control is on the bottom.

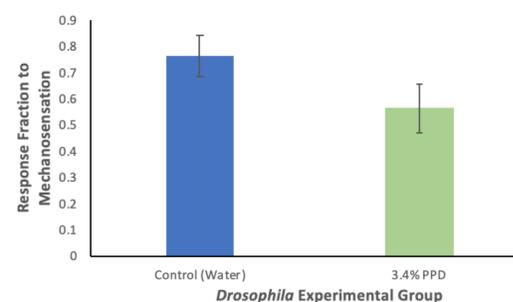


**Figure 3:** Side by side view of fly cultures and their corresponding petri dishes. PPD is on the right, and the control is on the left.

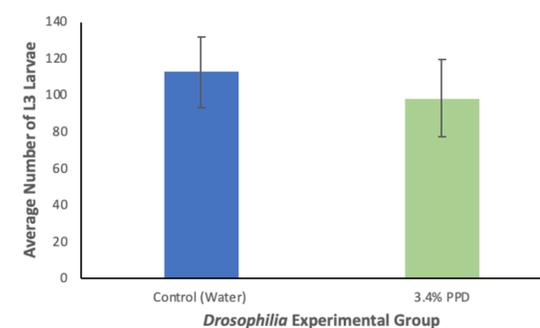
## Results



**Figure 4:** Fold change in expression of *NAT2* between the *D. melanogaster* control group and the experimental group exposed to a 3.4% solution of PPD for 24 hours.



**Figure 5:** Response fraction to Mechanosensation between the *D. melanogaster* control group and the experimental group exposed to PPD ( $p=0.1038$ ,  $df=57$ ).



**Figure 6:** Average number of L3 larvae identified during two rounds of collecting data from the experimental group of larvae. ( $p=0.6631$ ,  $df=2$ )

## Conclusion

- The results of the qPCR showed a decrease in the expression of the *NAT2* gene when *D. melanogaster* were exposed to a 3.4% solution of PPD. These findings do not align with the original hypothesis; however their significance is unknown due to only being able to complete one round of qPCR (Fig.4).
- In both the behavior assay and the larvae count of third instar larva there were no significant differences between the control group and the group exposed to 3.4% PPD (Fig.5 and Fig. 6).
- To further this study, multiple rounds of qPCR would be needed to create more significant and reliable data along with a more stable environment to collect mechanosensation data. Due to unexpected world events, there were some supplies that were not always available, thus improvising was needed.
- Future studies should turn their focus to PPD's effect on a similar gene, the *NAT 1* gene, which detoxifies PPD in other parts of the body such as the skin. The study of both genes could give more insight into PPD's effect in the human body.

## References

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