The Effects of an Endocrine Disruptor on Courtship Behaviors in *Drosophila Melanogaster*

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Abstract

Drosophila are exposed to an array of environmental factors which can influence their behavior. Common examples are pesticide exposures or low resource availability leading to metabolic stress. This study focuses on factors that disrupt endocrine signaling, namely, Endocrine disruptors (ED's) . ED's can have desirable effects, e.g. when used in food production as insecticides, or undesirable effects, e.g when insecticides affect non-target organisms. If endocrine disruptors fail to affect target insects, they may negatively impact crops. If they affect non-target organisms, ecosystems may be disrupted. Endocrine disruptors can also affect humans via water contamination and their widespread presence in plastics, as well as in cosmetic products. Therefore, understanding environmental and genetic variation in the affects of ED's on endocrine function has widespread importance in the natural environment, in agriculture, and for human health. In our experiment the PI 3-kinase inhibitor 'Wortmannin' is used to understand the effects of pharmacological perturbation of insulin signaling on fruit fly behavior. Our focal question pertains to whether or not an exposure to this endocrine disruptor has any effect on courtship latency and duration in Drosophila. i.e. the amount of time it takes to court, and the length of time courting transpires, respectively. We found that there is no significant effect of Wortmannin on courtship behavior.

Introduction

- Endocrine disruptors are a class of chemicals that interfere with hormone signaling, which include BPA and Wortmannin[1]
- Endocrine disruptors are found both naturally and synthetically, and are used in industry, making understanding their implications on target/non-target organisms important
- The ED Wortmannin interferes in the insulin signaling pathway, phenocopying starvation stress in Drosophila[2]
- Orientation of the male toward the female ("circling") and chasing are activities associated with Drosophila courtship
- The intensity and duration of these activities, included in courtship index and courtship latency, can provide insights into how ED's impact the Drosophila reproductive behaviors

Objectives

- Understand the potential implications of endocrine disruptor exposure on adult fruit fly behavior.
- Troubleshoot behavioral assay and machine learning software for use in future experiments.
- Test whether or not endocrine disruption will cause flies treated with Wortmannin to be less/ more likely to engage in courtship activity.

Media

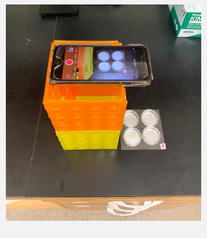
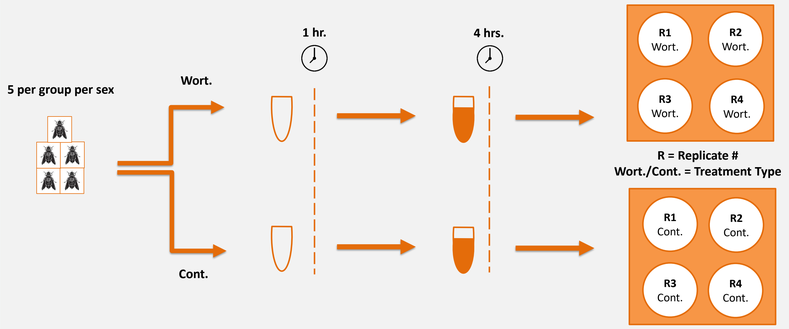


Fig 1: Phone camera, stand and arenas used in Behavioral Assay Setup.

QR Codes: Gifs displaying target courtship behavior

Fruit Fly Mating: https://www.youtube.com/watch?v=58pQsVeADj8. Drosophila Courtship Song – Genetic –University of Leicester: https://www.youtube.com/watch?v=KzWIuhXMUko

Experimental Method



Feeding Assay
- BARCODE Feeding Assay[3] was used to determine how much treatment was consumed, later used to compare amount eaten in each treatment.

BORIS
- BORIS software was used to manually count for chosen behaviors within specified region in arenas.

Statistical Analysis
- Two-sample t tests compared Chase, Circling, Total Behaviors and CI between Control and Wortmannin treatment groups.

Results

	Control	Wortmannin
Chamber 1	14	83
Chamber 2	31	32
Chamber 3	4	39
Chamber 4	53	36

Fig 2: Total Behavior Counts in each chamber per treatment.

	Control	Wortmannin
Chamber 1	0.575	0.867
Chamber 2	0.512	0.320
Chamber 3	0.290	0.337
Chamber 4	0.565	0.453

Fig 3: Courtship Indexes of each chamber per treatment.

	FA1 F	FA1 M	FA2 F	FA2 M
Test Statistic	0.442363	1.381696	0.041620	0.990329
p-value	0.673727	0.216310	0.968152	0.360255

Fig 4: The two-sample t test results comparing the consumption levels of two treatments in males and females.

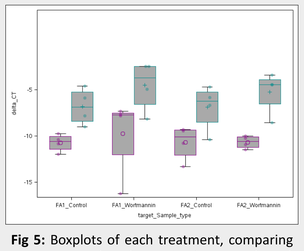


Fig 5: Boxplots of each treatment, comparing the male (cyan) and female (plum) consumption levels after standardization.

Results

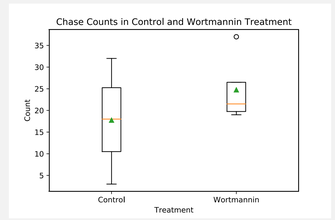


Fig 6: Distribution of the counts of chase behavior(Left: Control, Right: Wortmannin). The mean(green triangle) and median(orange line) are shown. The whiskers show the minimum and maximum values.

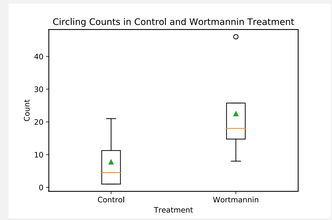


Fig 7: Distribution of the counts of circling behavior (Left: Control, Right: Wortmannin). The mean(green triangle) and median(orange line) are shown. The whiskers show the minimum and maximum values.

	Chase	Circling	Total Behavior	Courtship Index
Test Statistic	5.500	3.500	3.000	7.000
p-value	0.281	0.121	0.097	0.443

Fig 8. The parametric two sample t test results.

	Chase	Circling	Total Behaviors	Courtship Index
Test Statistic	-0.930238	-1.560759	-1.371878	-0.060745
p-value	0.388144	0.169602	0.219185	0.953535

Fig 9: The nonparametric two sample t test results.

Conclusions

- Comparing circling, chase, total behavior, and courtship index, **No significant difference was seen** between the control and wortmannin treatments.
- There was no significant difference in the amount of treatment consumed by the subjects. Males and females both consumed yeast paste with wortmannin in the same amount as the control yeast paste.
- Going forward, the experiment will be re-run, factoring in room for improvement in; *lighting*, *sample size, and arena setup/ analysis.*

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