Continuation/Research Progression Projects Form (7)

Required for projects that are a continuation/progression in the same field of study as a previous project.
This form must be accompanied by the previous year's abstract and Research Plan/Project Summary.

Student's Name(s) Keaton Danseglio

To be completed by Student Researcher: List all components of the current project that make it new and different from previous research. The information must be on the form; use an additional form for previous year and earlier projects.

Components	Current Research Project	Previous Research Project: Year: 2019
1. Title	Transgenerational Effects of Paternal Stress in Drosophila melanogaster	Are the Effects of Caffeine Different in Males and Females?
2. Change in goal/ purpose/objective	Examined the heritability of epigenetic	Examined the physical and physiological effects of caffeine
3. Changes in methodology	Used stress assays (heat shock and starvation) to obtain data	Massed flies, conducted geotaxis assays, and counted the number of flies emerged for data collection
4. Variable studied	Paternal stress on stress resistance of offspring	Caffeine consumption on physical performance, mass, and relative survival rate
5. Additional changes	Used logrank test to analyze data	Used Chi-Squared to analyze data

Attached are:

☑ Abstract and Research Plan/Project Summary, Year 2019

I hereby certify that the above information is correct and that the current year Abstract & Certification and project display board properly reflect work done only in the current year.

Keaton Danseglio — Student's Printed Name(s)

Keaton Danseglio — Signature

01/23/20 — Date of Signature (mm/dd/yy)

Are the Effects of Caffeine in *Drosophila melanogaster* Different in Males and Females?

By: Keaton Danseglio and Mary Sotiryadis

ABSTRACT

Studies of caffeine and its effects have failed to create male and female subgroups and thus lack evidence that suggest the drug affects each sex differently. To fully understand how the drug's effects may vary in *Drosophila melanogaster* based on sex, we quantified flies' relative survival rate, weight, and speed after exposure to the drug during development.Embryos were introduced to various concentrations of caffeinated food (0.00 mg/mL, 0.0625 mg/mL, 0.125 mg/mL, and 0.250 mg/mL)and kept there through larval stage. When the flies emerged from pupae, they were separated and counted on the basis of sex and transferred to non-caffeinated food (0.00 mg/mL). To assess relative survival rate, the number male and female flies that were extracted was used. After 23 days, geotaxis assays were conducted, and *Drosophila* were massed. The study showed no effect of caffeine on the weight of male or female *Drosophila.* However, our results demonstrated a negative effect of the drug on male performance in the highest caffeine concentration (0.250 mg/mL) that was not present in any of the female experimental groups. Interestingly, we noted that significantly less males were extracted from the 0.125 mg/mL group and significantly more females were extracted from the 0.0625 mg/mL group. Overall, the effects of caffeine in *Drosophila melanogaster* were more prevalent in males than they were in females.

Rationale (3a)

Caffeine is an addictive drug that is widely consumed throughout the entire world. Although this stimulant is considered safe, recent studies have uncovered some potentially negative effects of the drug that should be taken into consideration by those who consume it. In high doses, the drug has been linked to an increase in anxiety, sleep deprivation, and in some cases can impair fine motor skills (Smith, 2002). Furthermore, the dependence an individual develops for caffeine will have unfavorable consequences. During caffeine withdrawal, one will experience irritability, headaches, and a lack of concentration (Caffeine Withdrawal Symptoms). In addition to further exploring the negative repercussions of caffeine use, this study will also determine the effect sex has on caffeine susceptibility, particularly in *Drosophila melanogaster*.

Previous studies have shown that caffeine has caused *Drosophila* to be more hyperactive and less able to associate visual cues with a related stimulus (Mustard, 2013). Building on these discoveries can help support that as fruit flies consume more caffeine, they will be more active and less muscularly and neurologically developed.

Research Questions (3b)

How does the sex of a *Drosophila melanogaster* affect its susceptibility to caffeine?

How do varying concentrations of caffeine affect *Drosophila melanogaster* development?

Hypotheses (3b)

If male and female *Drosophila melanogaster* have anatomical and chemical differences that can alter the way their brains react with caffeine, and we test probable effects of caffeine, then each sex's susceptibility to the drug will vary.

If caffeine is a drug that interrupts certain neurological processes, and we raise *Drosophila melanogaster* on varying levels of caffeine and assess their development, then the flies that consume a greater concentration of caffeine will display abnormal growth and behavior.

Procedure (3c)

Food & Caffeine Preparation

In our study, the Caltech fly food recipe will be used to create one liter of food. This recipe consists of 87 grams of cornmeal, 75 grams of sucrose, 15 grams of live yeast, 4.5 grams of agar, and 750 mL of water (Lewis, 1960). These ingredients will be placed in a two liter beaker and then microwaved for a minute. After the minute is up, the food will be stirred to help it thicken and then placed back into the microwave. This process will be repeated for 10-15 minutes, until the food is thick enough. Last, 10 mL of fly acid (a water solution of 0.5 M of phosphoric acid and 8 M of propionic acid) will be added to the food.

The food will be divided into four 500mL beakers, amounting to 250mL of food in each. To make food with a caffeine concentration of 0.0625 mg/mL, 1.25 mL of caffeine stock and 8.75 mL of water will be added to one of the beakers. A concentration of 0.125 mg/mL will be made by adding 2.50 mL of caffeine and 7.50 mL. The caffeine concentration of 0.250 mg/mL will require 5.0 mL of caffeine and 5.0 mL of water. Next, 10 mL of water will be added to the

last beaker, acting as a control group as it receives no caffeine. Different FD&C food coloring dyes will be added to each food mixture to simplify differentiation between the varying concentrations; the 0.0625 mg/mL food will be colored blue, the 0.125 mg/mL food will be made purple, the 0.250 mg/mL food will be colored pink, and the control group (0.000 mg/mL) will be yellow, as no food coloring was added to it. Finally, the food will be poured into 80 vials, each containing about 5 mL, where the fruit flies will later inhabit; 20 vials will be made for each of the four concentrations of caffeine.

Growing Embryos and Sexing Mature Flies

To begin our study, adult w^{1118} *Drosophila melanogaster* will be put in an egg laying chamber that contains yeast paste on a blue agar media (consisting of 5% yeast extract, 5% sucrose and 1% agar); this will serve as a location for female fruit flies to feed and lay their eggs. After two days, the embryos will be collected from the agar media and washed using distilled water. Next, regardless of sex, the embryos will be put into the previously made vials. An equal volume (60 μL) of egg suspension will be put into each vial to maintain consistency within a trial.

The vials will be placed in an incubator set at 29 degrees Celsius to decrease the flies' maturation time. After 10 days, when the flies have consumed caffeine in their larvae stage, the flies will be fully developed. Before being placed into separate bottles, the flies will be extracted from the vials and sedated using a carbon dioxide pad. Then, they will be sexed under a microscope and separated into bottles based on their sex and the concentration of caffeine they were raised on; the food in the bottles they will be transferred to will contain no caffeine. The

separation of males and females will be conducted in order to prevent reproduction, as well as for the purposes of the experiment. The number of male and female flies extracted from each vial will be recorded and each vial will be labeled to allow for their identification.

Weighing

After 23 days, the average masses of the grown flies in each bottle will be recorded three times and compared with those of varying caffeine concentrations. A Cole-Parmer Symmetry PA 120 Series Analytical Balance will be used to weigh the flies. The masses of males will not be compared to those of females because of their anatomical differences; it would be uninformative to compare the two as females are naturally larger than males (The Differences Between the Sexes, 2016).

Conducting Geotaxis Assays

Next, we will conduct geotaxis assays to assess the muscular and neurological development of the flies. In this assay, four empty vials will be marked with three lines that are evenly spaced out; there will be one vial for each concentration. The flies will be placed into the empty vials respective to the concentration they were raised on. They will be given 10 minutes to adjust to this new environment. After this period of adjustment is over, the four vials will be slammed down onto a flat surface. Using an iPhone 6S camera, the flies crawling up the sides of the vials will be videotaped. Because they experience microgravity in their confined environment, fruit flies are inclined to use their legs when moving around in their vials. The

amount of time it takes for half of the flies in each vial to crawl above the specific line will quantified using a timer measuring to the hundredths place.

Risk and Safety (3c)

Potential risks of this experiment include burn damage when making the fly food, unhealthy exposure to hazardous chemicals when dealing with the caffeine, and exposure to high levels of CO_2 gas when sexing the fruit flies. In order to minimize these risks, we will constantly monitor the CO_2 level on the gas pad and wear gloves, goggles, and heat protective accessories when necessary.

Data Analysis (3c)

Chi-Square tests will be conducted to compare the total numbers of *Drosophila*, as well as the number of males and females, extracted from a given caffeine concentration. A linear regression will be determined for the number of flies that will emerge from each vial based on the concentration of caffeine food in that vial. Analyses of variation (ANOVA) will be used to find significance in the data collected from the geotaxis assays and the fruit fly weights. If the ANOVAs indicate significance, unpaired t-tests will be conducted to pinpoint the location of this significance. Also, the median from each concentration's geotaxis assay will be determined and compared to that of other concentrations.

The geotaxis assay videos of each concentration will be examined and the time it takes for half of the flies in the vials to pass the mark will be recorded. The quantified data from the geotaxis assays will be used to conduct 3 unpaired t-tests; each

A Chi Squared test will be conducted for the control group and the three experimental groups to compare the survival rates of the flies

Three t-tests will be conducted to compare the weights of the control group to each experimental group.

Human Participant Research

Not applicable

Vertebrate Animal Research

Not applicable

Potentially Hazardous Biological Agents Research

Not applicable

Hazardous Chemicals, Activities & Devices

Throughout the duration of our study, we will be handling a high concentration of caffeine stock and fly acid. To minimize health risks, we will wear gloves and goggles when handling these substances. The caffeine stock that will be used will be stored in a test tube in the freezer to ensure it does not pose a threat to anyone in the lab. Further, all equipment that comes

in contact with the fly acid or caffeine will be disposed of in a hazardous waste container. By taking these precautions and working under the supervision a mentor, the safety of everybody in the lab will be ensured.

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NO CHANGES MADE