

# Student Checklist (1A)

This form is required for ALL projects.

1. a. Student/Team Leader: Keaton Danseglio Grade: 12  
Email: keatondan22@gmail.com Phone: 5165191662  
b. Team Member: \_\_\_\_\_ c. Team Member: \_\_\_\_\_
2. Title of Project:  
Transgenerational Effects of Paternal Stress in Drosophila melanogaster
3. School: North Shore HS School Phone: 5162777000  
School Address: 450 Glen Cove Ave, Glen Head, NY, 11545
4. Adult Sponsor: Dr. Molly Mordechai Phone/Email: mordechaim@northshoreschools.org
5. Does this project need SRC/IRB/IACUC or other pre-approval? ☐ Yes ☒ No Tentative start date: July 1, 2019
6. Is this a continuation/progression from a previous year? ☒ Yes ☐ No  
If Yes:  
a. Attach the previous year's ☒ Abstract and ☒ Research Plan/Project Summary  
b. Explain how this project is new and different from previous years on  
☐ Continuation/Research Progression Form (7)
7. This year's laboratory experiment/data collection:  
Cannot be BEFORE 1/1/1907/01/2019 Cannot EXCEED 12 months from start date 11/01/19  
Actual Start Date: (mm/dd/yy) End Date: (mm/dd/yy)
8. Where will you conduct your experimentation? (check all that apply)  
☒ Research Institution ☐ School ☐ Field ☐ Home ☐ Other: \_\_\_\_\_
9. List name and address of all non-home and non-school work site(s):  
Name: LIU CW Post  
Address: 720 Northern Blvd, Greenvale, NY  
Phone/ email: 5162992900
10. Complete a Research Plan/Project Summary following the Research Plan/Project Summary instructions and attach to this form.
11. An abstract is required for all projects after experimentation.

### 3a. Rationale

Lamarckian inheritance of acquired traits as the mechanism for evolution was discredited following the Darwinian notion of natural selection (Darwin, 2004). Darwinian evolutionary models have been largely validated by numerous studies of changes in genes over time (Mitchell & Taylor, 1999). However, more recent studies have shown transgenerational responses resulting from changes in parental environment (Wang *et al.*, 2017). Although genetic inheritance from parent to progeny has been widely studied, less is known about the other components of transgenerational transferal, especially those associated with environment induced epigenetic inheritance (Bale, 2014). Evidence of altered fitness and transgenerational epigenetic modifications in *Drosophila* from changes in parental environment has been recently documented (Sollars *et al.*, 2002; Xing *et al.*, 2007; Seong *et al.*, 2013; Wang *et al.*, 2017). Although challenged by the ‘cleaning slate process,’ where the epigenome is thought to be erased prior to fertilization, epigenetic inheritance has been legitimized by studies indicating that gametes and embryos can circumvent this epigenetic reprogramming process (Xing *et al.*, 2007; Wang *et al.*, 2017).

The present study seeks to develop a system to explore solely the transgenerational transmission of epigenetic information through the infliction of abiotic stresses, starvation and heat shock, in *Drosophila melanogaster*. The effect of paternal sublethal stresses, rather than maternal, was observed as males contribute substantially less to the zygote than females, who transfer their mitochondrial DNA and cytoplasmic components (Cosmides, 1981; Celotto, 2006). *Drosophila* will be used as a model organism to fully isolate the transmission of the epigenome; biases pertaining to parenting style or other variable offspring environmental factors will be minimized. This study will also consider the effect of multiple exposures of the same paternal stress on progressive fitness of progeny.

### 3b. Research Question and Hypothesis

#### 1. Research Question

Are stress-induced epigenetic modifications paternally inherited in *Drosophila melanogaster*?

#### 2. Hypothesis

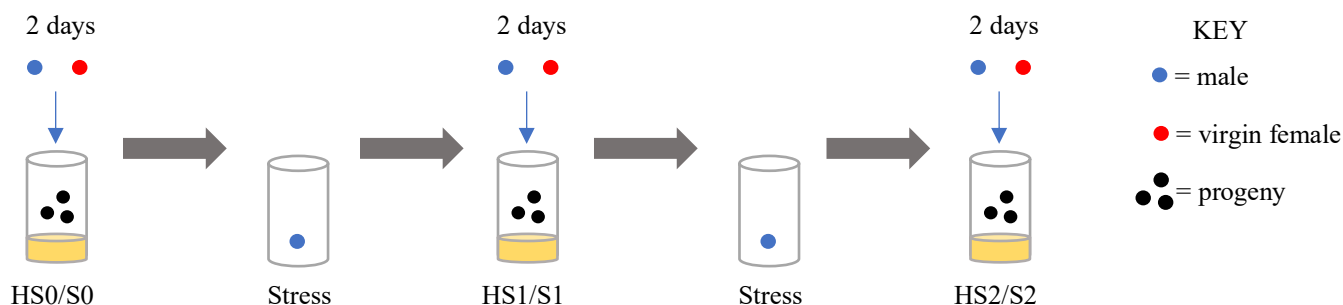
If epigenetic modifications can escape epigenetic reprogramming, then progeny of *Drosophila melanogaster* with paternal stress will have altered fitness relative to those with no paternal stress.

### 3c. Methods

#### 1. Procedure

Two strains of *Drosophila melanogaster* will be used in this study: wildtype ( $w^{1118}$ ) and *Drosophila* insulin-like peptide (DILP) deficient (53197). All stock will be maintained in the laboratory at room temperature ( $23 \pm 1^\circ\text{C}$ ) and will be raised on standard Caltech fly food: 87 g cornmeal, 75 g sucrose, 15 g live yeast (refrigerated), 4.5 g agar, 10 ml fly acid (4.15% phosphoric and 41.8% propionic), and 750 ml water. Flies will be kept on standard food through experimentation. To prevent desiccation during the administration of stress, flies will be placed on a solidified solution of 0.01 g/ml agar. Flies will be euthanized after experimentation in a beaker of 50% ethanol solution.

Flies will be continuously collected as virgins using  $\text{CO}_2$  as an anesthetic throughout experimentation. Virgins will be collected in 2-hour intervals and stored in same sex groups of 5 individuals. A male at the age of 1-3 days post eclosion will be put with a virgin female and allowed time to mate. 48 hours later, both flies will be extracted, and the male will be kept in a storage vial under standard conditions. The following day, the male will be subject to one of the two stresses described below. Immediately after this stress, the male will be transferred into a vial with a new virgin female. Two days later, this process will be repeated; the same stress will be administered to the male and he will be placed with another virgin female in a third vial. This experimental setup is shown in **Figure 1**. Progeny from each vial will be collected and separated based on sex 14 days after the removal of the P generation. The following day, the progeny will be exposed to the same stress that their fathers endured, and survival assays will be conducted. In each trial, 10 males will be used, totaling 30 vials of progeny. 3 trials will be conducted for each strain and stress subgroup (wt heat shock, wt starvation, DILP heat shock, and DILP starvation).



**Figure 1)** Experimental setup for the three stress conditions. Progeny will be extracted 14 days after the removal of the parental generation, and stress assays will be conducted. Key: HS=heat shock; S=starvation; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males with two exposures.

To determine the threshold for sublethal stress infliction, 100 males of each strain at the age of 1-3 days post eclosion will be immersed in a hot water versa-bath (Fisher) set at 37 °C. Flies will be monitored every 30 minutes after initial stress exposure to identify the latest time before observable mortality. These times will be used on the P generation flies as the sublethal heat shock stress. For the survival assays of the progeny in heat shock, the number of knocked flies will be recorded every 30 minutes from hour 2 to hour 4.5. For practicality reasons, 24 hours will be used as the sublethal stress threshold for starvation. For the survival assays of the progeny under starvation, the number of dead flies will be observed in 4-hour intervals between hour 24 and hour 72. All starvations will commence at the same time of day.

## **2. Risk and Safety**

Making fly acid will be done under a hood with goggles and appropriate skin protection. Gloves will be worn when handling hot beakers during food preparation.

## **3. Data Analysis**

For a given trial, the total number of flies that died during each time interval will be determined; these values will be used for comparison between the 3 paternal stress conditions (vials without corresponding offspring across conditions will be excluded from trial totals as no data could be collected for the next generation). Totals from all 3 trials will be analyzed using log rank (Mantel-Cox) tests to compare the survival distributions between different conditions. The three conditions (progeny with no paternal stress, progeny after 1<sup>st</sup> paternal stress, and progeny after 2<sup>nd</sup> paternal stress) will be compared to one another separately, maintaining male and female subgroup totals. Males and females will not be compared to one another.

## **3d. Bibliography**

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Keaton Danseglio

## **Post Research Summary**

No changes made