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±1°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 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).

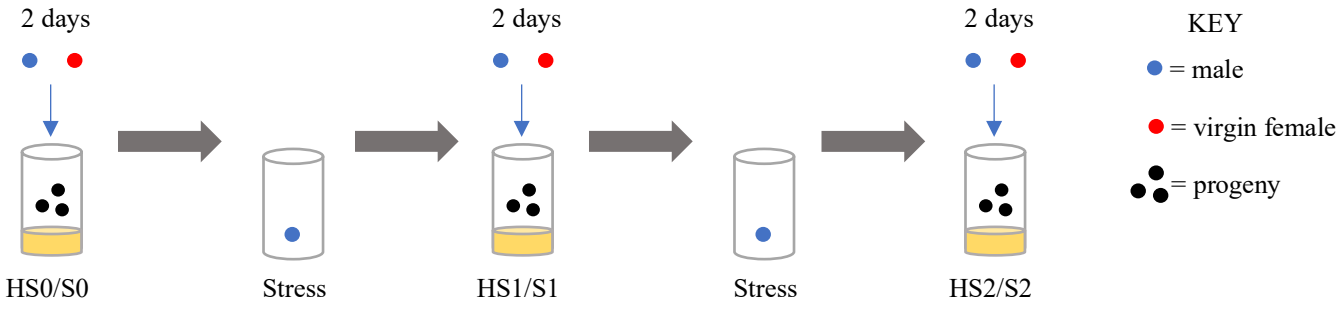

Figure 1) Experimental setup for the three stress conditions. Progeny will be extracted 14 days after the removal of the parental generation, and stress assays will be conducted. Key: HS=heat shock; S=starvation; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males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

Bale, T. L. (2014). Lifetime stress experience: transgenerational epigenetics and germ cell programming. *Dialogues in clinical neuroscience*, *16*(3), 297.

Celotto, A. M. (2006). Mitochondrial Encephalomyopathy in Drosophila. *Journal of Neuroscience*, *26*(3), 810–820.

Cosmides, L. M., & Tooby, J. (1981). Cytoplasmic inheritance and intragenomic conflict. *Journal of Theoretical Biology*, *89*(1), 83–129.

Darwin, C. (2004). *On the origin of species*, 1859. Routledge.

Messerschmidt, D. M., Knowles, B. B., & Solter, D. (2014). DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos. *Genes & Development*, *28*(8), 812–828.

Mitchell, M., & Taylor, C. E. (1999). Evolutionary computation: an overview. *Annual Review of Ecology and Systematics*, *30*(1), 593-616.

Rutherford, S. L., & Henikoff, S. (2003). Quantitative epigenetics. *Nature Genetics*, *33*(1), 6–8.

Seong, K.-H., Maekawa, T., & Ishii, S. (2013). Inheritance of Stress-Induced Epigenetic Changes Mediated by the ATF-2 Family of Transcription Factors. *Stress-Induced Mutagenesis*, 103–118.

Sollars, V., Lu, X., Xiao, L., Wang, X., Garfinkel, M. D., & Ruden, D. M. (2002). Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nature Genetics*, *33*(1), 70–74.

Wang, Y., Liu, H., & Sun, Z. (2017). Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans. *Biological Reviews*, *92*(4), 2084–2111.

Xing, Y., Shi, S., Le, L., Lee, C. A., Silver-Morse, L., & Li, W. X. (2007). Evidence for transgenerational transmission of epigenetic tumor susceptibility in Drosophila. *PLoS genetics*, *3*(9), e151.

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Post Research Summary

No changes made