Transgenerational Effects of Paternal Stress in *Drosophila melanogaster*

By: Keaton Danseglio

Date: November 2019

School: North Shore High School, Glen Head, New York

Mentor: Dr. Theodore Brummel, Long Island University

ACKNOWLEDGEMENTS

I wish to sincerely thank Dr. Brummel, Professor in Biology, for providing me with the resources and guidance that made this study possible. He gave me the opportunity to express my own ideas through research.

I would like to show my gratitude to Dr. Mordechai, my science research teacher, who has encouraged me throughout my research experience and who has taught me necessary research skills.

I also thank Mr. Klein for reviewing my paper and providing me with useful feedback.

ABSTRACT

It is widely known that gene expression is influenced by the environment. One mechanism for altering gene expression involves epigenetic changes, alterations in the structure of DNA that are inherited by the progeny of these cells. During the formation of sperm and egg cells, it is widely believed that most epigenetic signatures are erased and thus, parental contribution to offspring is largely genetic, dependent on the sequence of DNA rather than its structure. However, recent studies have indicated environment induced modification resulting in epigenetic signatures that are passed on to future generations. Female gametes (eggs) contain a large amount of proteins and other non-genetic components that can influence the development of their offspring, making detection of epigenetic inheritance more difficult. Male gametes (sperm) contribute primarily DNA and thus are better candidates for detecting epigenetic inheritance. Heat shock and starvation are major stressors which produce large changes in gene expression that promote enhanced survival under stressful conditions. Using *D. melanogaster* as a model system, it was found that progeny of males exposed to a sublethal-stress (heat shock or starvation) experienced a reduction in stress resistance that further decreased as the frequency of paternal stress increased. Analyses of mutants in the insulin signaling pathway suggest that insulin signaling is not required for the transgenerational effects observed. Although no molecular analysis was conducted, it was hypothesized that chromatin remodeling explains the observed effects. Additionally, the potential evolutionary advantages of the phenomenon seen in the results is discussed. This study is the only one of its nature that tests transgenerational effects of multiple instances of stress and quantifies these effects on progeny through stress assays of progeny derived from a stressed male germline.

INTRODUCTION

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, 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).

The Target Rapamycin (mTOR) signaling pathway is modulated centrally by the mTOR protein, a serine/threonine kinase responsible for the regulation of growth and maintenance of homeostasis on a cellular level (Laplante & Sabatini, 2012; Yoon, 2017). In the insulin-regulated pathway, a transduction cascade modulated through mTOR is produced that results in binary decision making (Sancak *et al.*, 2007). Under unstressed conditions with ample resources available, mTOR is activated and cell growth is promoted. When the cell is stressed or deprived of resources, mTOR is repressed and a survival state is induced; under such conditions, cellular components are recycled, autophagy is established, protein synthesis is reduced, and repair mechanisms are induced (Ricoult & Manning, 2012). In cases of mTOR repression under mild environmental stress, a large-scale physiological change can occur that increases fitness. This type of advantageous response, known as hormesis, has been shown in *Drosophila* that have experienced longevity in response to therapeutic doses of dietary restriction and frequent sublethal heat shock (Hercus *et al.*, 2003; Calabrase, 2008; Fontana *et al.,* 2010; Kouda & Iki, 2010).

Altered fitness of *Drosophila* has not only been shown in hormesis, but also through the transgenerational epigenetic modification from parental environmental stress (Sollars *et al*, 2002; Xing *et al.*, 2007; Seong *et al.*, 2013; Wang *et al.,* 2017). One study found that 2 days of acute paternal sugar consumption resulted in fly progeny with obesity (Öst *et al.*, 2014). A similar study shows that flies raised on dietary restricted food have progeny with significantly shorter development times than those raised on regular food (Valtonen *et al.*, 2012).

On the contrary, the ‘cleaning slate’ process of the epigenome challenges the legitimacy of the epigenetic transferal hypothesis as the epigenome is thought to be erased prior to and after fertilization. There are three main stages of chromatin states in *Drosophila* development from primordial germ cells to the zygote. First, primordial germ cells undergo a reprogramming process where RNA Polymerase II elongation is inhibited, erasing epigenetic signatures (Strome & Lehmann, 2007). Next, the appropriate

germ cell epigenetic signature for oogenesis or spermatogenesis is expressed, resulting in the production of the respective gametes. After fertilization, epigenetics signatures in the embryo are erased in order to create pluripotency for differentiation during gestation (Messerschmidt *et al*., 2014). Although the resetting of the epigenome undermines the notion of epigenetic inheritance, recent studies have indicated that epigenetic information can circumvent the reprogramming process in the gametes and embryo (Wang *et al*., 2017). One study that correlates overactivation of the JAK kinase with reduction of epigenetic reprogramming provides one viable mechanism for uncomplete epigenetic erasure (Xing *et al.*, 2007).

This present study develops a system to explore solely the transgenerational transmission of epigenetic information through the infliction of abiotic stresses, starvation and heat shock, in *Drosophila melanogaster.* For this reason, 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). This notion has not been widely accounted for in some studies that observed environment induced epigenetic inheritance through the maternal germline (Sollars *et al*, 2002; Villamor & Cnattingius, 2006; Waterland *et al.*, 2008). The use of *Drosophila* as a model organism to fully isolate the transmission of the epigenome minimizes any biases pertaining to parenting style or other variable offspring environmental factors as observed in other studies (Bygren *et al.*, 2014; Kral *et al.*, 2007; Villamor & Cnattingius, 2006). This present study also considers the effect of multiple exposures of the same paternal stress on progressive fitness of progeny. It was hypothesized that if epigenetic modifications resulting from an mTOR, or insulin pathway inhibiting stress could be heritable in *Drosophila melanogaster*, then progeny with paternal stress will have altered fitness relative to those seen in wild type controls.

METHODS

Fly stock and food

Two strains of *Drosophila melanogaster* were used in this study: wildtype (w^{1118}) and Drosophila insulin-like peptide (DILP) deficient (53197). All stock was maintained in the laboratory at room temperature (23±1°C) and was 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 were kept on standard food through experimentation. To prevent desiccation during the administration of stress, flies were placed on a solidified solution of 0.01 g/ml agar. Flies were euthanized after experimentation in a beaker of 50% ethanol solution.

Mating isolation and stress administration

Stock was amplified through the continuous transferal between bottles to ensure an ample supply of emerging flies, which were collected as virgins using CO_2 as an anesthetic. Virgins were 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 was put with a virgin female and allowed time to mate. 48 hours later, both flies were extracted, and the male was kept in a storage vial under standard conditions. The following day, the male was subject to one of the two stresses described below. Immediately after this stress, the male was transferred into a vial with a new virgin female. Two days later, this process was repeated; the same stress was administered to the male and he was placed with another virgin female in a third vial. This experimental setup is shown in **Figure 1**. Progeny from each vial were collected and separated based on sex 14 days after the removal of the P generation. The following day, the progeny were exposed to the same stress that their fathers endured and survival assays were conducted. In each trial, 10 males were used, totaling 30 vials of progeny. 3 trials were conducted for each strain and stress subgroup (w^{1118} heat shock, w^{1118} starvation, 53197 heat shock, and 53197 starvation).

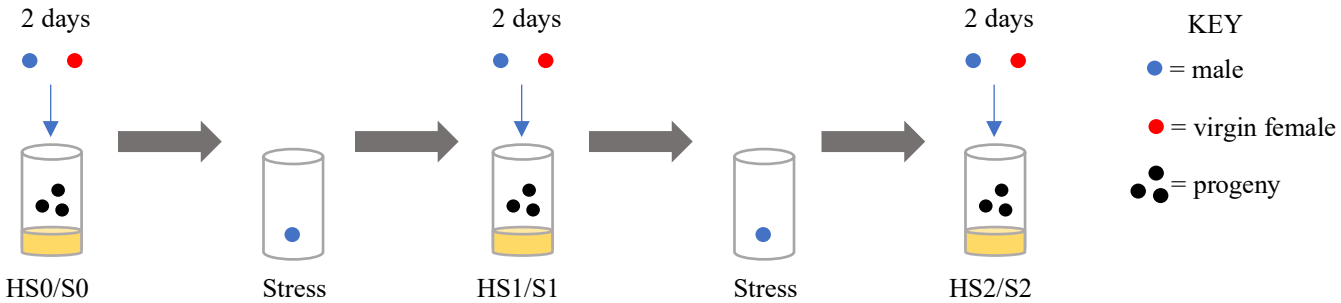


Figure 1) Experimental setup for the three stress conditions. Progeny were extracted 14 days after the removal of the parental generation, and stress assays were conducted. Key: HS=heat shock; S=starvation; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males with two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

Heat shock

100 males of each strain at the age of 1-3 days post eclosion were immersed in a hot water versa-bath (Fisher) set at 37 °C. Flies were monitored every 30 minutes after initial exposure to identify the latest time before significant mortality was observed; 120 minutes for w^{1118} males and 90 minutes for 51397 males. These times were used on the P generation flies as a sublethal stress. For the survival assays of the progeny in heat shock, the number of knocked flies was recorded every 30 minutes from hour 2 to hour 4.5.

Starvation

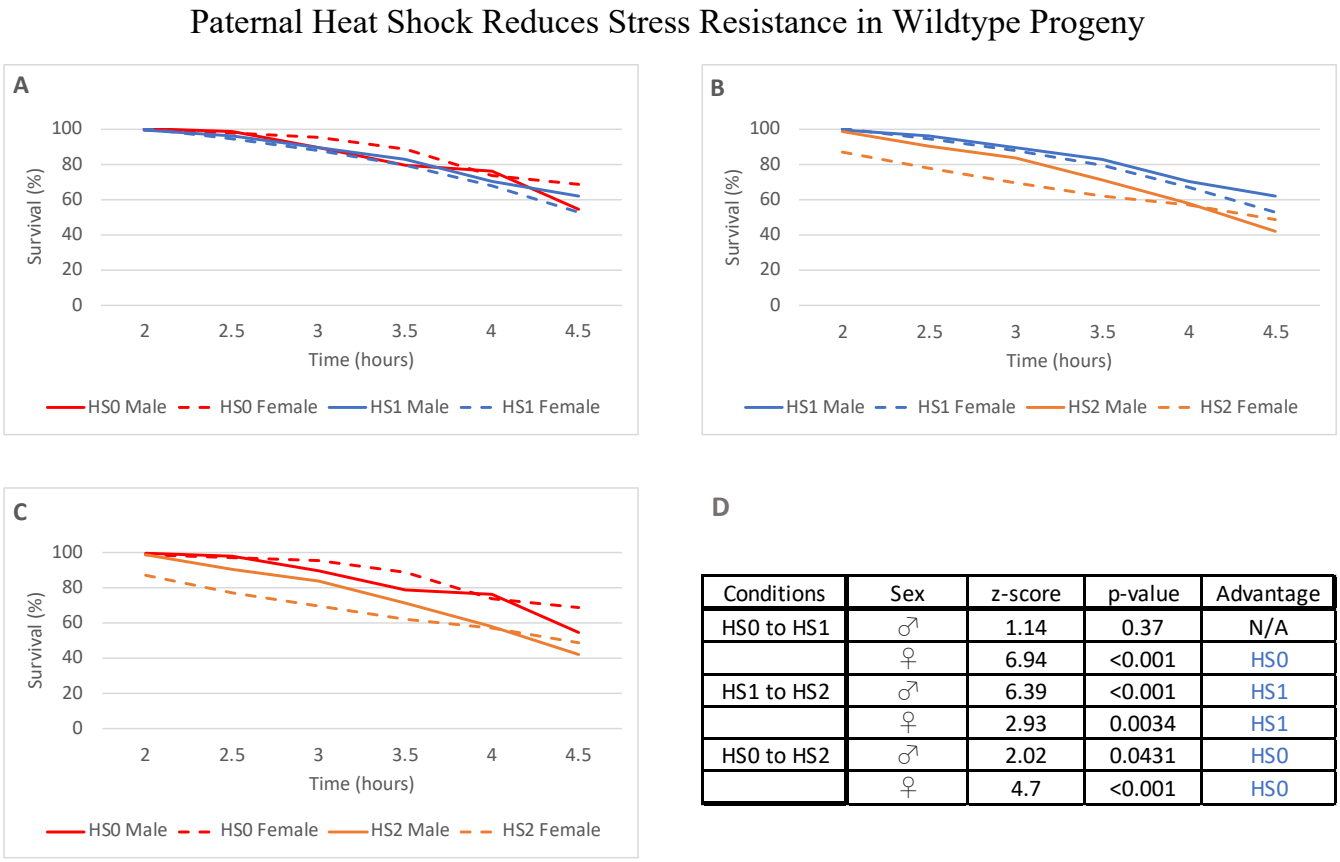
To find the sublethal threshold for starvation when mortality rate was still low, 100 males of each strain at the age of 1-3 days post eclosion were starved and monitored for 48 hours; the majority of flies from both strains survived to hour 24, deeming that time as an appropriate sublethal stress for males in this study. For the survival assays of the progeny under starvation, the number of dead flies was observed in 4-hour intervals from hour 24 to hour 72. All starvations commenced at the same time of day.

Data analysis

For a given trial, the total number of flies that died during each time interval was determined; these values were used for comparison between the 3 paternal stress conditions (vials without corresponding offspring across conditions were excluded from trial totals as no data could be collected for the next generation). Totals from all 3 trials were 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^{st} paternal stress, and progeny after 2^{nd} paternal stress) were compared to one another separately, maintaining male and female subgroup totals. Males and females were not compared to one another.

RESULTS

The general trend shows that progeny whose fathers endured a sublethal stress withstood that stress for a shorter amount of time. The groups with the lower stress resistance were those with two instances of paternal stress (HS2, S2), and those with highest stress resistance were progeny sired by unstressed fathers (HS0, S0). Paternal heat shock of wildtype flies had a significant effect on stress resistance of female progeny after one instance of stress and male progeny after the second. In the HS0 to HS1 comparison, males showed no significant difference whereas females with unstressed fathers were more fit (**Figure 2A**). The HS1 to HS2 and HS0 and HS2 comparisons reveal that progeny of both sexes from HS1 and HS0 groups outperformed their counterparts, respectively (**Figure 2B & 2C**). This significance was found in all heat shock comparisons conducted for the DILP deficient progeny (**Figure 3A, 3B, & 3B**). In all cases, excluding the wildtype male HS0 to HS1 analysis, the log-rank tests showed that progeny with more paternal stress survived shorter in heat shock (**Figure 2D & 3D**).

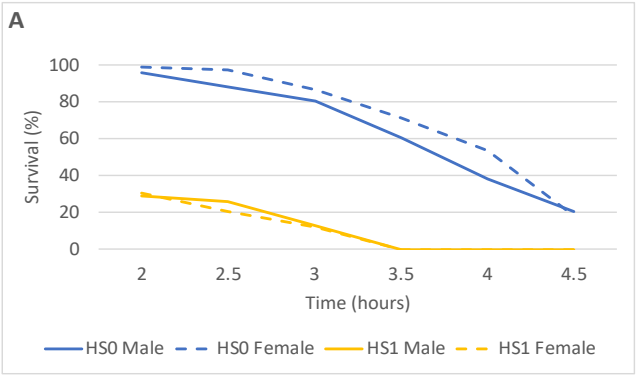


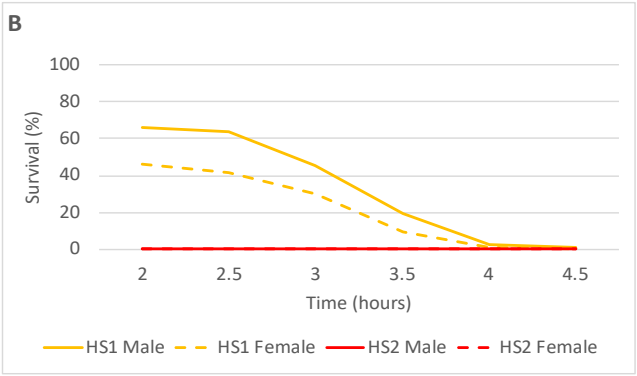
D

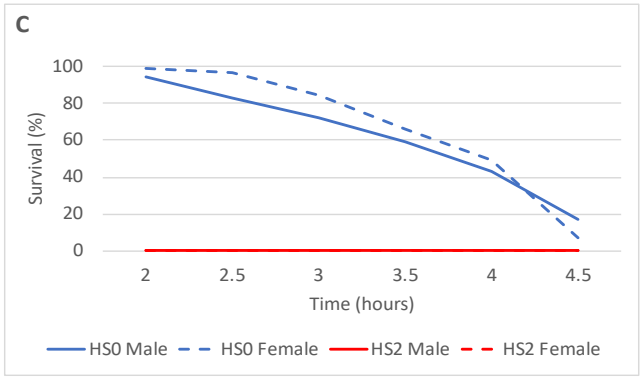
Conditions	Sex	z-score	p-value	Advantage
HS0 to HS1	♂	1.14	0.37	N/A
	♀	6.94	<0.001	HS0
HS1 to HS2	♂	6.39	<0.001	HS1
	♀	2.93	0.0034	HS1
HS0 to HS2	♂	2.02	0.0431	HS0
	♀	4.7	<0.001	HS0

Figure 2) Heat shock assay of wildtype (w^{1118}) *D. melanogaster.* Survival percentages were determined from totals of three trials. Key: HS=heat shock; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males with two exposures**. (A)** HS0 vs. HS1. **(B)** HS1 vs. HS2. **(C)** HS0 vs. HS2. **(D)** Mantel-Cox analyses were used for comparison of survival distributions. 'Advantage' indicates the progeny group that best withstood the stress (blue: higher resistance before paternal stress; red: higher resistance after paternal stress).

Paternal Heat Shock Reduces Stress Resistance in DILP Deficient Progeny







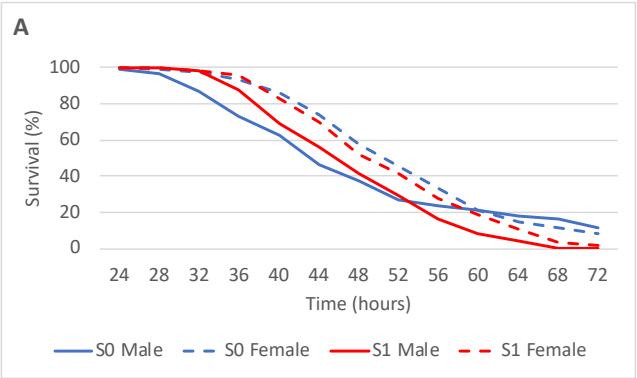
D

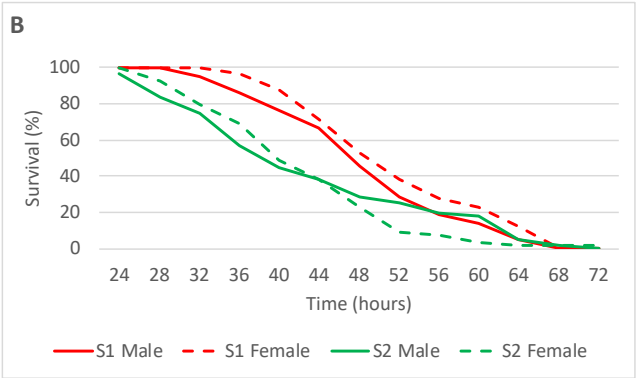
Conditions	Sex	z-score	p-value	Advantage
HS0 to HS1	♂	11.93	<0.001	HS0
	♀	15.48	<0.001	HS0
HS1 to HS2	♂	9.47	<0.001	HS1
	♀	9.14	<0.001	HS1
HS0 to HS2	♂	13.62	<0.001	HS0
	♀	15.03	<0.001	HS0

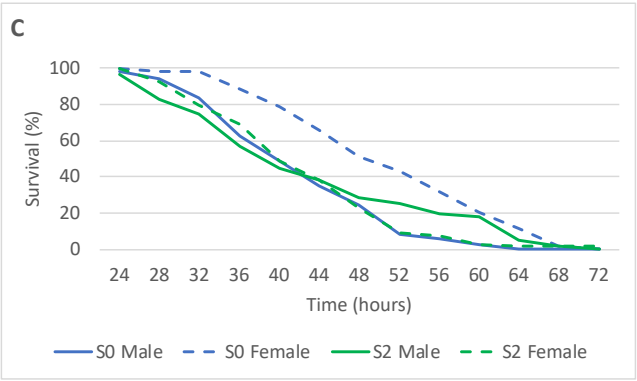
Figure 3) Heat shock assay of DILP Deficient (53197) *D. melanogaster.* Survival percentages were determined from totals of three trials. Key: HS=heat shock; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males with two exposures. **(A)** HS0 vs. HS1. **(B)** HS1 vs. HS2. **(C)** HS0 vs. HS2. **(D)** Mantel-Cox analyses were used for comparison of survival distributions. 'Advantage' indicates the progeny group that best withstood the stress (blue: higher resistance before paternal stress; red: higher resistance after paternal stress).

The trend of altered resistance due to paternal stress is also seen with starvation as a sublethal stress. One instance of paternal starvation of wildtype flies significantly reduced stress resistance of male and female progeny as seen in the S0 to S1 comparison (**Figure 4A**). Further, significant differences were found only in the females when conducting the log rank tests for the HS1 to HS2 and the HS0 to HS2 comparison (**Figure 4B & 4C**). While the log-rank tests revealed reduced fitness for wildtype females sired by stressed fathers, the relationship was less prevalent in males (**Figure 4D**).

Paternal Starvation Reduces Stress Resistance in Wildtype Progeny







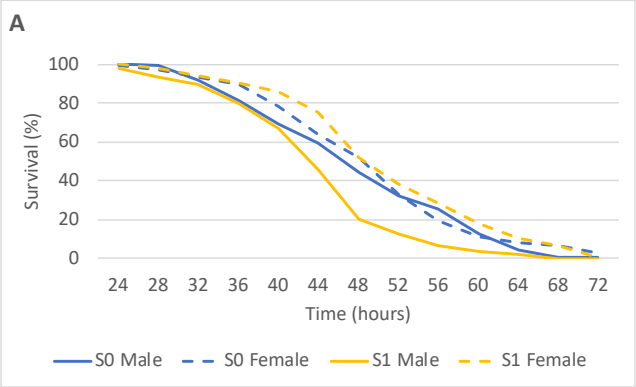
D

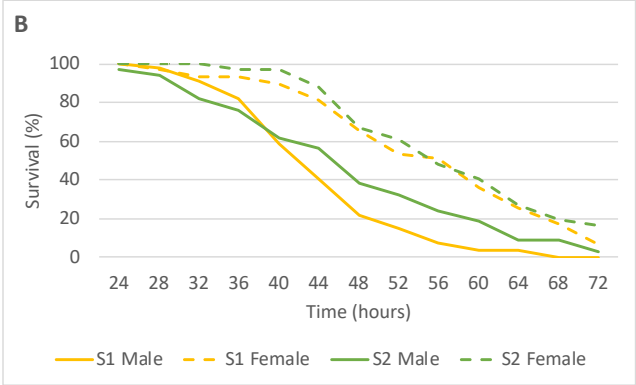
Conditions	Sex	z-score	p-value	Advantage
S0 to S1	♂	1.99	0.047	S0
	♀	2.38	0.0171	S0
S1 to S2	♂	1.29	0.2	N/A
	♀	4.52	<0.001	S1
S0 to S2	♂	1.52	0.13	N/A
	♀	4.52	<0.001	S0

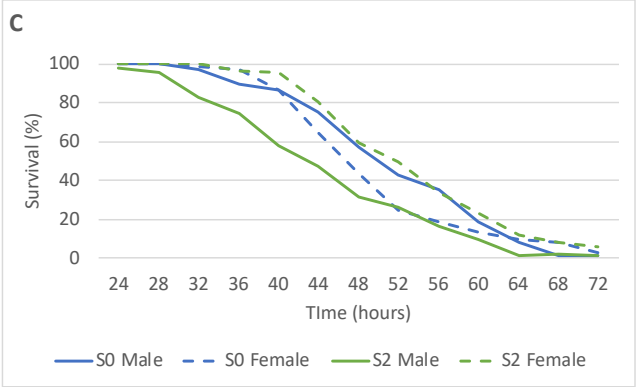
Figure 4) Starvation assay of wildtype (w^{1118}) *D. melanogaster.* Survival percentages were determined from totals of three trials. Key: S=starvation; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males with two exposures. **(A)** S0 vs. S1. **(B)** S1 vs. S2. **(C)** S0 vs. S2. **(D)** Mantel-Cox analyses were used for comparison of survival distributions. 'Advantage' indicates the progeny group that best withstood the stress (blue: higher resistance before paternal stress; red: higher resistance after paternal stress).

Analyses of survival distributions of DILP deficient progeny under starvation were more varied than those of the other three strain-stress subgroups; they showed an advantage towards paternally stressed progeny. As seen in the S0 and S1 survival distributions, S0 males endured starvation longer than S1 males, whereas no significant effect was detected in females (**Figure 5A**). Further, males from the S2 group had higher stress resistance than those from S1, and no significant effect was found for the females in this comparison (**Figure 5B**). In the S0 to S2 comparison, S0 males survived longer than S2 males and S2 females survived longer than their S0 counterparts (**Figure 5C**). The log-rank tests still indicate altered progeny resistance from paternal stress and suggest that stress resistance of DILP deficient female progeny heightens after two paternal stresses while stress resistance of male progeny lessens after one paternal stress and then heightens after a second (**Figure 5D**).

Stress Resistance Varies from Paternal Starvation in DILP Deficient Progeny







D

Conditions	Sex	z-score	p-value	Advantage
S0 to S1	♂	3.64	<0.001	S0
	♀	0.53	0.59	N/A
S1 to S2	♂	2.54	0.011	S2
	♀	1.09	0.28	N/A
S0 to S2	♂	3.26	0.0011	S0
	♀	2.36	0.0182	S2

Figure 5) Starvation assay of DILP Deficient (53197) *D. melanogaster.* Survival percentages were determined from totals of two trials. Key: S=starvation; 0=progeny of unstressed males; 1=progeny of males with one exposure; 2=progeny of males with two exposures. **(A)** S0 vs. S1. **(B)** S1 vs. S2. **(C)** S0 vs. S2. **(D)** Mantel-Cox analyses were used for comparison of survival distributions. 'Advantage' indicates the progeny group that best withstood the stress (blue: higher resistance before paternal stress; red: higher resistance after paternal stress).

DISCUSSION

The purpose of this study was to develop a method of quantifying transgenerational transferal of environment induced epigenetics through the paternal germline. Most literature exploring environment-induced epigenetic inheritance do so by inflicting a single stress on the P generation, neglecting the potential effects of multiple instances of exposure on the F1 generation. This present study assesses fitness of progeny with multiple paternal exposures to thermal and dietary stresses. This study is also the only work of literature that explicitly quantifies transgenerational effects in progeny through assays of the paternally inflicted stress. According to majority of the results, resistance to stress deteriorated with increasing instances of paternal stress in heat shock and starvation (**Figure 2-4**). While the transgenerational effects remained consistent in the S wildtype group and the HS groups, inconsistent levels of resistance were observed in the S DILP deficient group (**Figure 5**). As a whole, the results provide evidence for the transgenerational inheritance of epigenetic information, supporting the original hypothesis.

While the vehicle of epigenetic inheritance observed in this study is largely unknown, possible mechanisms can be differentiated through various molecular analyses. The mechanistic candidates for transgenerational epigenetic modifications are DNA methylation and chromatin remodeling (Rutherford, 2003; Sharma *et al.*, 2015; Wang *et al.*, 2017). DNA methylation occurs minimally in *Drosophila* and is thus an unlikely mechanism for the transgenerational effects reported in this present study (Kunert, 2003; Capuano *et al.*, 2014; Messerschmidt *et al*., 2014; Zhang *et al.*, 2015). Alteration to the chromatin states is a more feasible epigenetic mechanism as its occurrence is widely documented in *Drosophila* (Bouazoune & Brehm, 2006). One study notes the loosening of polytene chromatin structures of the *ashsp70* gene in *Drosophila* as a result of heat exposure (Tulin, 2003). Likewise, Seong *et al.* observed defective heterochromatin defined by the phosphorylation of the previously silenced dAFT-2 gene in response to heat shock and osmotic stress (Seong *et al.,* 2013). Seong *et al*., among others, also shows that the altered parental heterochromatin states are transferred to subsequent generations, providing a potential mechanism for the inheritance observed in the present study (Déjardin, 2005; Ciabrelli *et al.*, 2017). Position effect variegation (PEV) is a genetic phenomenon that shows phenotypic patterns in gene expression through juxtaposition of euchromatin and heterochromatin, and it can be used to confirm the theory of heritable chromatin states (Wallrath & Elgin, 1995; Elgin & Reuter, 2013). PEV is commonly utilized to detect the presence of changes to chromatin states. The most common reporter gene for observing chromatin remodeling in *Drosophila* is *white* (Vogel *et al.,* 2009). Öst *et al.* examines addition PEV reporter genes to explore transgenerational responses of paternal diet, a method that could be used to

explore chromatin remodeling in progeny sired by stressed fathers as seen in this present study (Öst *et al.*, 2014).

A less likely, non-epigenetic hypothesis offers the possibility that the physiological stress endured by the P generation resulted in damaged germline DNA that was inherited by the F1 generation. This mechanism for the transgenerational effects observed here pertain mostly to the thermally stressed flies as increased temperature has been shown to cause somatic mutation in *Drosophila* while dietary restriction has not (Suzuki & Procunier, 1969; Edman *et al.*, 2009; Garcia *et al.*, 2010). Although not yet documented, stress-induced haploid mutation would provide a semi-feasible mechanism for the transgenerational effects of paternal stress observed in this study. To differentiate this theory from that of chromatin modeling in terms of verity, methodological reproduction of this study on a large scale is required with progeny sired by stressed and unstressed fathers. Phenotypic abnormalities can be quantified to determine the frequency of mutation in paternally stressed progeny as an indication of genomic alteration in spermatozoa.

Another model for nongenetic inheritance is RNA transferal. However, RNA inheritance is unlikely to have occurred in this study as only the paternal germline was altered; it is widely understood that RNA is transferred to progeny through maternal germline cells (Blumenstiel & Hartl, 2005; Brennecke *et al*., 2008; Soni *et al.*, 2013).

If the reduced stress resistance of progeny sired by stressed fathers as observed in the results is a phenomenon that occurs commonly in nature, there are likely evolutionary implications that suggest fitness. In this study, progeny sired by fathers with an abiotically stressed environment survived that stress for a shorter period of time. In the wild, flies must produce more offspring in times of environmental stress in order to ensure the survival of their genes. Thus, an increase in reproductive fitness at the cost of physical fitness to the environmental stress could be advantageous. Male *Drosophila* exhibit a decrease in lifespan but increase in early-life reproductive output with the presence of male mate rivals, possibly associating increased fecundity with reduced longevity (Bretman *et al.*, 2013). It is also well documented that both male and female *Drosophila* with higher reproductive activity have lower longevity than their virgin counterparts (Partridge & Andrews, 1985; Prowse & Partridge, 1997; Flatt, 2011). These observations can be legitimized by the antagonistic pleiotropy hypothesis, which outlines a model that explains senescence through the antagonistic evolution between early-life and late-life traits controlled by the same gene (Schnebel & Grossfield, 1988). In the case of a thermally stressed environment, evolution would favor fly generations that have lower longevity but higher fecundity as less

eggs are likely to survive into adulthood under that stress. In a dietary restricted environment, selection would likely favor lower longevity and lower fecundity as less progeny consume less resources, ultimately limiting food scarcity. Both scenarios model likely obstacles of ancestral and present-day *Drosophila*. Although fecundity would have to be quantified to confer this evolutionary advantage, the results of this study indicate an epigenetic mechanism underlying this type of tradeoff evolution, which is largely unknown (Wayne *et al.*, 2006).

There a several explanations that can justify the inconsistences seen in the DILP deficient starvation subgroup. Many comparisons (24) were conducted in this study, increasing the chance of data discrepancies as the probability of getting an anomalous outcome in nonzero. The magnitudes of significance were less prevalent in the DILP deficient starvation subgroup than others; significant p-values supporting higher resistance after paternal stress were all above 0.01, on the cusp of insignificance as compared to the p-values of the opposing advantage from other subgroups, which were mostly less than 0.001 (**Figure 5D**). This could be explained by the loss of the third trial for this subgroup due to a threat at the laboratory's campus; this prevented the termination of a stress-inducing starvation, resulting in the death of all P generation fathers. Additionally, the progeny in S groups likely experienced temperature fluctuations as flies were taken out of the laboratory during data collection, possibly altering stress resistance as *Drosophila* are ectotherms. To conclude the validity of this trend in the DILP deficient starvation group, assessing its persistence by reproduction of the experiment is required. Despite this discrepancy, it is apparent that the expression and functionality of the seven DILP genes play a minimal role in stress-related transgenerational responses (Fontana *et al.*, 2010); transgenerational effects in both strains were largely the same, indicating that the insulin genetic pathway is not critical for the occurrence of the phenomenon observed. Although direct comparisons between the strains were not conducted due their genotypic differences that could alter resistance, it can be inferred that a component of mTOR pathway other than insulin is likely involved in the reduction of resistance from paternal stress.

It can be concluded that the speculated alteration to the epigenome through chromatin remodeling was one of non-binary nature as transgenerational effects were significantly greater with each successive paternal stress. Also, this study provides evidence for transgenerational transferal of epigenetic information that contradicts the purely genetic mechanism for Darwin's evolution as proposed by Mendel. However, future steps can be taken to explore the extent to which this phenomenon holds true. Because stress resistance of the F1 generation was only tested in this present study, it would be of interest to investigate the effect of paternal stress on the F2 generation. Finally, conducting the molecular analysis as described above would be useful to confirm the epigenetic nature of the relationship observed.

REFERENCES

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

Blumenstiel, J. P., & Hartl, D. L. (2005). Evidence for maternally transmitted small interfering RNA in the repression of transposition in Drosophila virilis. *Proceedings of the National Academy of Sciences*, *102*(44), 15965–15970.

Bouazoune, K., & Brehm, A. (2006). ATP-dependent chromatin remodeling complexes in Drosophila. *Chromosome Research*, *14*(4), 433–449.

Brennecke, J., Malone, C. D., Aravin, A. A., Sachidanandam, R., Stark, A., & Hannon, G. J. (2008). An Epigenetic Role for Maternally Inherited piRNAs in Transposon Silencing. *Science*, *322*(5906), 1387–1392.

Bretman, A., Westmancoat, J. D., Gage, M. J. G., & Chapman, T. (2013). Costs And Benefits Of Lifetime Exposure To Mating Rivals In Maledrosophila Melanogaster. *Evolution*, *67*(8), 2413–2422.

Bygren, L., Tinghög, P., Carstensen, J., Edvinsson, S., Kaati, G., Pembrey, M. E., & Sjöström, M. (2014). Change in paternal grandmothers´ early food supply influenced cardiovascular mortality of the female grandchildren. *BMC Genetics, 15*(1), 12.

Calabrese, E. J. (2008). Hormesis and medicine. *British journal of clinical pharmacology*, *66*(5), 594-617.

Capuano, F., Mülleder, M., Kok, R., Blom, H. J., & Ralser, M. (2014). Cytosine DNA Methylation Is Found in Drosophila melanogaster but Absent in Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Other Yeast Species. *Analytical Chemistry*, *86*(8), 3697–3702.

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

Ciabrelli, F., Comoglio, F., Fellous, S., Bonev, B., Ninova, M., Szabo, Q., … Cavalli, G. (2017). Stable Polycomb-dependent transgenerational inheritance of chromatin states in Drosophila. *Nature Genetics*, 49(6), 876–886.

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.

Déjardin, J., & Cavalli, G. (2005). Epigenetic Inheritance of Chromatin States Mediated by Polycomb and Trithorax Group Proteins in Drosophila. *Epigenetics and Chromatin Progress in Molecular and Subcellular Biology*, 31–63.

Edman, U., Garcia, A. M., Busuttil, R. A., Sorensen, D., Lundell, M., Kapahi, P., & Vijg, J. (2009). Lifespan extension by dietary restriction is not linked to protection against somatic DNA damage in Drosophila melanogaster. *Aging Cell*, *8*(3), 331–338.

Elgin, S. C. R., & Reuter, G. (2013). Position-Effect Variegation, Heterochromatin Formation, and Gene Silencing in Drosophila. *Cold Spring Harbor Perspectives in Biology*, *5*(8).

Flatt, T. (2011). Survival costs of reproduction in Drosophila. *Experimental Gerontology*, *46*(5), 369–375.

Fontana, L., Partridge, L., & Longo, V. D. (2010). Extending Healthy Life Span--From Yeast to Humans. *Science*, *328*(5976), 321–326.

Garcia, A. M., Calder, R. B., Dollé, M. E. T., Lundell, M., Kapahi, P., & Vijg, J. (2010). Age- and Temperature-Dependent Somatic Mutation Accumulation in Drosophila melanogaster. *PLoS Genetics*, *6*(5).

Hercus, M. J., Loeschcke, V., & Rattan, S. I. S. (2003). Lifespan extension of Drosophila melanogaster through hormesis by repeated mild heat stress. *Biogerontology*, *4*, 149–156.

Kouda, K., & Iki, M. (2010). Beneficial Effects of Mild Stress (Hormetic Effects): Dietary Restriction and Health. *Journal of Physiological Anthropology*, 29(4), 127–132.

Kunert, N. (2003). A Dnmt2-like protein mediates DNA methylation in Drosophila. *Development*, *130*(21), 5083–5090.

Kral, J. G., Biron, S., Simard, S., Hould, F., Lebel, S., Marceau, S., & Marceau, P. (2007). Large Maternal Weight Loss From Obesity Surgery Prevents Transmission of Obesity to Children Who Were Followed for 2 to 18 Years. *Obstetrical & Gynecological Survey, 62*(4), 233-235

Laplante, M., & Sabatini, D. M. (2012). mTOR Signaling in Growth Control and Disease. *Cell*, *149*(2), 274–293.

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.

Öst, A., Lempradl, A., Casas, E., Weigert, M., Tiko, T., Deniz, M., … Pospisilik, J. A. (2014). Paternal Diet Defines Offspring Chromatin State and Intergenerational Obesity. *Cell*, 159(6), 1352–1364.

Partridge, L., & Andrews, R. (1985). The effect of reproductive activity on the longevity of male Drosophila melanogaster is not caused by an acceleration of ageing. *Journal of Insect Physiology*, *31*(5), 393–395.

Prowse, N., & Partridge, L. (1997). The effects of reproduction on longevity and fertility in male Drosophila melanogaster. *Journal of Insect Physiology*, *43*(6), 501–512.

Ricoult, S. J. H., & Manning, B. D. (2012). The multifaceted role of mTORC1 in the control of

lipid metabolism. *EMBO Reports*, 14(3), 242–251.
Rutherford, S. L., & Henikoff, S. (2003). Quantitative epigenetics. *Nature Genetics*, *33*(1), 6–8.
Sancak, Y., Thoreen, C. C., Peterson, T. R., Lindquist, R. A., Kang, S. A., Spooner, E., … Sabatini, D. M. (2007). PRAS40 Is an Insulin-Regulated Inhibitor of the mTORC1 Protein Kinase. *Molecular Cell*, *25*(6), 903–915
Schnebel, E. M., & Grossfield, J. (1988). Antagonistic Pleiotropy: An Interspecific Drosophila Comparison. *Evolution*, *42*(2), 306.
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.
Sharma, U., Conine, C. C., Shea, J. M., Boskovic, A., Derr, A. G., Bing, X. Y., … Rando, O. J. (2015). Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science*, *351*(6271), 391–396.
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.
Soni, K., Choudhary, A., Patowary, A., Singh, A. R., Bhatia, S., Sivasubbu, S., … Pillai, B. (2013). miR-34 is maternally inherited in Drosophila melanogaster and Danio rerio. *Nucleic Acids Research*, *41*(8), 4470–4480.
Strome, S., & Lehmann, R. (2007). Germ Versus Soma Decisions: Lessons from Flies and Worms. Science, 316(5823), 392–393.
Suzuki, D. T., & Procunier, D. T. (1969). Temperature-sensitive Mutations in Drosophila melanogaster, III. Dominant Lethals and Semilethals on Chromosome 2. *Proceedings of the National Academy of Sciences*, *62*(2), 369–376.
Tulin, A. (2003). Chromatin Loosening by Poly(ADP)-Ribose Polymerase (PARP) at Drosophila Puff Loci. *Science*, *299*(5606), 560–562.
Valtonen, T. M., Kangassalo, K., Pölkki, M., & Rantala, M. J. (2012). Transgenerational Effects of Parental Larval Diet on Offspring Development Time, Adult Body Size and Pathogen Resistance in Drosophila melanogaster. *PLoS ONE*, *7*(2).
Villamor, E., & Cnattingius, S. (2006). Interpregnancy weight change and risk of adverse pregnancy outcomes: A population-based study. *The Lancet, 368*(9542), 1164-1170.
Vogel, M. J., Pagie, L., Talhout, W., Nieuwland, M., Kerkhoven, R. M., & Steensel, B. V. (2009). High-resolution mapping of heterochromatin redistribution in a Drosophila position-effect variegation model. *Epigenetics & Chromatin*, *2*(1), 1.
Wallrath, L. L., & Elgin, S. C. (1995). Position effect variegation in Drosophila is associated with an altered chromatin structure. *Genes & Development*, *9*(10), 1263–1277.
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.
Waterland, R. A., Travisano, M., Tahiliani, K. G., Rached, M. T., & Mirza, S. (2008). Methyl donor supplementation prevents transgenerational amplification of obesity. *International Journal of Obesity, 32*(9), 1373-1379.
Wayne, M. L., Soundararajan, U., & Harshman, L. G. (2006). *BMC Evolutionary Biology*, *6*(1), 57.
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.
Yoon, M.-S. (2017). The Role of Mammalian Target of Rapamycin (mTOR) in Insulin Signaling. *Nutrients*, *9*(11), 1176.
Zhang, G., Huang, H., Liu, D., Cheng, Y., Liu, X., Zhang, W., … Chen, D. (2015). N6-Methyladenine DNA Modification in Drosophila. *Cell*, *161*(4), 893–906.