Effects of High Sugar or Low Protein Parental Diets on Developmental Time of Offspring in *Drosophila melanogaster*

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Submitted in Partial Fulfillment of the Requirements for Graduation

from the Malone University Honor's Program

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November 1, 2019

Abstract

A variety of environmental factors can affect the growth and development of animals. In fact, some studies have shown that the environmental conditions of parents can affect their children. The goal of this project is to determine how the parental diet of *Drosophila melanogaster* impacts developmental time of their progeny. Studies have shown that offspring with one parent (either maternal or paternal) reared on a low protein diet with the other parent on a standard diet have a faster developmental time compared to offspring with both parents on standard and both parents on low protein diets. Building on the previous studies, this project investigates the effects of both a low protein diet and a high sugar diet. This study also explores whether separate strains react differently to alterations of parental diet. Two different strains of *D. melanogaster* are examined to determine if the different strains responded to altered parental diets similarly or not. Transgenerational effects on parental diet in offspring are found in certain diet crosses, mostly different from previous studies. Strain difference is also found between the two strains examined. Overall, additional progeny would need to be analyzed to more precisely determine the exact transgenerational effects a parental diet has on offspring developmental timing in *D. melanogaster*.

Acknowledgements

I would like to acknowledge everyone who played a role in this academic accomplishment. First, my family and friends who constantly supplied me with encouragement and love that I needed to get through this long process. Thank you for teaching me to dream big, and to push through to reach those dreams. I love you all.

Secondly, I'd like to thank Nicole Chirambanegomo and Mayah Jones for spending countless hours counting and separating flies for this project. I could have never dreamed of accomplishing this task without both of your help, so I truly thank you both.

Thirdly, I'd like to acknowledge and thank each of my committee members, who have demonstrated patience and have given helpful advice throughout this research process. I always appreciate your guidance and unwavering support.

Lastly, I'd like to acknowledge and give a huge thank you for Kate Huisinga. Thank you for taking me under your wing my freshman year and for giving me the support, encouragement, patience, understanding, and invaluable instruction throughout these years. I absolutely could not have done this without your constant help. Thank you so much.

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Introduction

Negative health conditions related to diet can include heart disease, obesity, and diabetes in humans [1]. The CDC reports that the prevalence of obesity was 18.5% and affected about 13.7 million children and adolescents in the United States, and the prevalence of obesity was 39.8% and affected about 93.3 million of US adults in 2015-2016 [15]. Because such a high percentage of our population is impacted by obesity, it is important to have the clearest understanding of what is causing these rates. Evidence for male line effects of nutrient availability on future generations in humans comes from the Overkalix cohort [16]. In this Swedish town, historical crop records were used to infer nutritional access among the ancestors of the current population. Food supply in grandparents was linked to mortality rates two generations later in a gender-specific manner. Paternal grandfathers' food supply was associated with altered metabolism in grandsons, whereas paternal grandmothers' food supply was linked to outcomes in their granddaughters. In both cases increased risk of metabolic disease was dependent on the age of grandparental exposure to inadequate diet. Specifically, inadequate diet in early adolescence (ages 9–13) was correlated with decreased risk of mortality in grandchildren, whereas the same exposure experienced by young adults (ages 18–22) was associated with increased risk. These types of studies indicate a link between parental diet and offspring development in humans, but this analysis is limited by the inability to control all aspects of a diet. However, by using flies as a model system, we can study how the diets influence the health and development of their offspring which may assist us in answering questions about the growing problem of human health.

Drosophila melanogaster is a good model organism to use for studying human disease because the relationship between fruit fly and human genes is so close that often the sequences of

newly discovered human genes, including disease genes, can be matched with equivalent genes in the fly [14]. Another benefit is that *D. melanogaster* have a short and simple reproduction cycle. It is normally about 8-14 days, depending on the environmental conditions. This allows researchers to cultivate several generations in a matter of months, allowing for relatively quick experiments. *Drosophila* is also easily and inexpensively maintained in a laboratory.

D. melanogaster provides opportunities to study impacts of nutrition and metabolism because they share metabolic organs and processes with many vertebrates, including humans. These metabolic factors that make them so valuable to study include analogous insulin, insulin-like-growth-factor, target of rapamycin (TOR) signaling pathways, regulation of circulating sugars, energy storage, and energy mobilization [6]. Multiple studies have explored the direct relationship between nutritional environment, gene expression and metabolism in *Drosophila melanogaster* [6]. For example, a study found that *Drosophila* larvae ate fewer calories on high-sugar compared with high fat and protein diets, but developed more severe hyperglycemia, or elevated hemolymph glucose, compared with other high-calorie diets [8]. Furthermore, several studies have investigated the effect of parental diets on the development and metabolic profile of their progeny, however, the results of these studies, as outlined below, on transgenerational effects are not as straightforward.

Various studies using *Drosophila melanogaster* have demonstrated that parental diet can impact certain phenotypes of their offspring. In particular, scientists have examined developmental time [1-5], carbohydrate levels [6-8], and triglyceride levels [6] in offspring of parents with altered diets. Carbohydrates studied include glucose, trehalose, and glycogen. Research suggests that to do this, one must first obtain an isogenic line of *D. melanogaster* [3]. This prevents genetic differences from affecting developmental time or metabolic profiles.

Multiple diet variations have been tested for transgenerational differences in phenotype. For example, Valtonen tested transgenerational impacts by reducing the amount of yeast by one eighth of the standard diet [3]. Buescher created a high sugar diet by increasing the sucrose by 6.7 fold [6]. Dew-Budd induced a high fat diet by adding 3% coconut oil to their standard diet [10].

While most public studies affect metabolic change through diet, it is also possible to create transgenerational effects through genetic mutation. This has been demonstrated using Adipokinetic Hormone Receptor (AKHR) loss-of-function mutations, which induce obesity in the parental generation. Once the parental generation is crossed and bred, the following generations are no longer AKHR homozygous recessive mutants. Therefore, the F1 generation shouldn't be impacted by their own AKHR loss-of-function, rather due to the transgenerational effect. In the F1 generation of AKHR heterozygous offspring, a consistent and slight increase of glycogen levels was observed. It was also observed that triglyceride levels were significantly lower in F2 progeny descended from genetically mutated grandfathers and heterozygous mothers [11]. While the experiment in this study doesn't use genetic mutation to alter the parental generation, it is important to note that sex may play an important role in the observed transgenerational impacts. It is also important to establish that while this study uses diet to alter the parental generation, it is possible to alter them with alternative methods such as genetic mutation, which impacts the metabolites of F1 and F2 progeny.

Developmental time can be observed multiple ways in *D. melanogaster.* Two sources record developmental time in F1 progeny by examining the vials daily and noting when pupation was first observed and when adults eventually emerged [1-2]. Another source measured developmental time as the length of time between oviposition and adult eclosion by counting the

emerged adults twice daily until eclosion ceased [4]. Valtonen records a different study exploring transgenerational impacts by collecting the emerged adults three times a day until eclosion ceased [3]. A study which looked at progeny with parents who were reared on four times more sucrose than the standard diet reported that the offspring had a significantly longer metamorphic stage of about half a day (0.48 days) than the control [1]. A separate study exploring the transgenerational effects of progeny whose parents were reared on low protein levels (one-eighth of the control level) report mixed growth rate results compared to the control flies [3].

Factors that have shown to have an impact on transgenerational studies are strain differences and which parental gender receives the experimental diet (i.e. male, female, or both parents) [1]. Studies have shown that the same experiment conducted on multiple strains of *D. melanogaster* have recorded completely different results. Wild type strains have genetic diversity within themselves but have no identified mutations that would easily explain differences in results. In one paper, multiple isogenic lines derived from single females in a wild population, referred to as iso-female lines, were exposed to either low or high protein relative to sugar diets. The F1 progeny were subsequently reared on a standard diet to expose transgenerational effects. In most of the lines, progeny whose parents were exposed to the low protein diet had increased glycogen and decreased triglyceride levels compared to the offspring of parents were reared on the high protein diet. Interestingly, these changes vary greatly per strain, from nearly nonexistent to a two or three-fold difference, with a change in the opposite direction in one strain [1]. To test if there are strain-to-strain differences in how parental diet affects developmental time, this study examines the effects of three separate strains.

Additional studies indicate that which parental sex experiences the altered diet may have a significant impact on the results in transgenerational metabolism studies. In one study, they fed

only the maternal parent a high sugar diet, while they left the paternal parent on a control diet [6]. Offspring demonstrated mild elevation of whole-body glucose and trehalose. Additionally, only the male offspring showed a significant reduction in cholesterol compared to the control. To further study, they found that the glucose and trehalose levels were increased in the males only. These metabolic changes persisted throughout the F2 generation as well, with the exception being that the TAG levels in the male F2 generation were slightly increased [6]. As of now there is no explanation as to why it impacts males differently from females, or if there would have been different results had the paternal parent been fed the high sugar diet rather than the maternal parent. Regardless, it is important to note that which parental sex experiences an altered diet may impact the effect of the altered diet on future generations. Because of this observed difference, I chose in this study to test both maternal and paternal effects as well as the effect of both parents being on an altered diet.

Most interestingly, Valtonen [3] reports that when both maternal and paternal parents are reared on the low protein diets, their offspring have the longest growth rate. This would seem to make sense since the parents are deprived of protein in their diet. The data also shows that when one parent, either the maternal or paternal, is on the low protein diet, while the other parent is on the standard diet, the offspring develop the fastest. This is a very intriguing result because one might hypothesize that when both parents are on control diet that their offspring would develop the quickest, yet that is not what is observed.

The experiments presented here aim to detect any transgenerational effects of parental nutrition on the developmental time of offspring using *D. melanogaster.* Establishing that transgenerational effects occur demonstrates the contributions each parent's diet has on progeny as well as the combined effect that the two parents' diets may have on offspring.

Materials and Methods

Part 1

Three different strains of flies (*D. melanogaster*) were used in the experiments conducted in this study. The Oregon R strain used in the experiment were collected from a laboratory base population of 400,000 flies that had been maintained in the laboratory for approximately 25 years. Stock and adult flies were reared on what will be referred to as standard food (Table 1). The y^1w^{67C23} strain used in the experiment were collected from a laboratory base population that had been maintained in the laboratory for approximately 15 years. This strain has mutations in eye color which led the stock to have white eyes rather than red. The strain also had a body color mutation leading it to have a yellow body rather than a dark tan body. Stock and adult flies were reared on standard food. The DGRP-307 strain originates from the Drosophila melanogaster Genetic Reference Panel (DGRP), which is a community resource of 205 sequenced inbred lines. This strain was obtained from the Bloomington Drosophila Stock Center at Indiana University.

Ingredients	*Control*	*High Sugar*	*Low Protein*
Water (ml)	1000	1000	1000
Agar (g)	10	10	10
Yeast 500 (g)	80	80	10
Yeast Extract (g)	20	20	2.5
Peptone (g)	20	20	2.5
D-(+)-Glucose (g)	60	228	60
Sucrose (g)	30	114	30
1M MgSO4 (mL)	2	2	2
CaCl2 x 2H2O (g)	0.5	0.5	0.5
Propionic acid	6	6	6
Tegosept (ml)	10	10	10

Table 1. Standard, high sugar, and low protein diets used for Part 1. Diets were modelled after a previous study [3] that researched how diet impacts developmental time transgenerationally.

For the study 400 males and 400 virgin females per each strain (OR, y^1w^{67C23}, and DGRP-307) were collected from the stock. At the age of 4–5 days post eclosion the flies were

released in a bottle and allowed to mate and lay eggs for 24 hours. The following day eggs were harvested and transferred either into 'standard food' or 'high sugar' or 'low protein' vials at a density of 20 eggs per vial (altogether 50 vials per condition for a total of 1000 eggs). To transfer the eggs, a clean blunt dissecting needle was used to carefully remove the eggs under the microscope and then the eggs were gently laid on top of the food in the vials. The 'standard food' vials contained 15 mL standard food for the larvae. The 'low protein' vials contained the same volume of food but the amount of yeast 500 was reduced to one-eighth of the amount relative to the standard diet. The 'high sugar' diet increased the D-(+)-Glucose to 5.3 times the amount in the standard food, and the sucrose was increased 3.8 times more than standard (Table 1). The vials were maintained at 25°C in an approximate 12 hours:12 hours light:dark regime.

Part 2

Due to difficulties obtaining progeny using the method described in Part 1, an alternative growth protocol was employed. In the second method, males and virgin females were collected from stock bottles that were 10-20 days old. At the age of 4–5 days post eclosion the flies were placed (15 males and 15 females per vial) in vials either containing 'standard', 'low

Ingredients	*Standard Diet*	*Low Protein Diet*	*High Sugar Diet*
Water (ml)	1000	1000	1000
Agar (g)	5.3	5.3	5.3
Yeast 500 (g)	18.24	2.28	18.24
D-(+)-Glucose (g)	60.71	60.71	230.7
Sucrose (g)	30.35	30.35	115.33
Cornmeal (g)	100.94	100.94	100.94
Acid A	11.76	11.76	11.76
Tegosept (ml)	8.24	8.24	8.24

Table 2. Standard, low protein, and high sugar diets used for Part 2. Diets were modelled from Malone University's standard diet for *Drosophila melanogaster*.

protein' or 'high sugar' food and allowed to mate and lay eggs for 24 hours. This method allowed the larvae to consume only the specific diet they were laid on. The 'standard food' vials contained 15 mL of standard food for the larvae. The 'low protein' vials contained the same amount of food but the yeast 500 was reduced to one-eighth of the amount [3]. The 'high sugar' vials contained the same amount of food but the amount of D-(+)-Glucose was increased to be 5.3 times more than the standard diet, and the sucrose was increased to be 3.8 times more than the standard diet (Table 2). The vials were maintained at 25°C with approximately

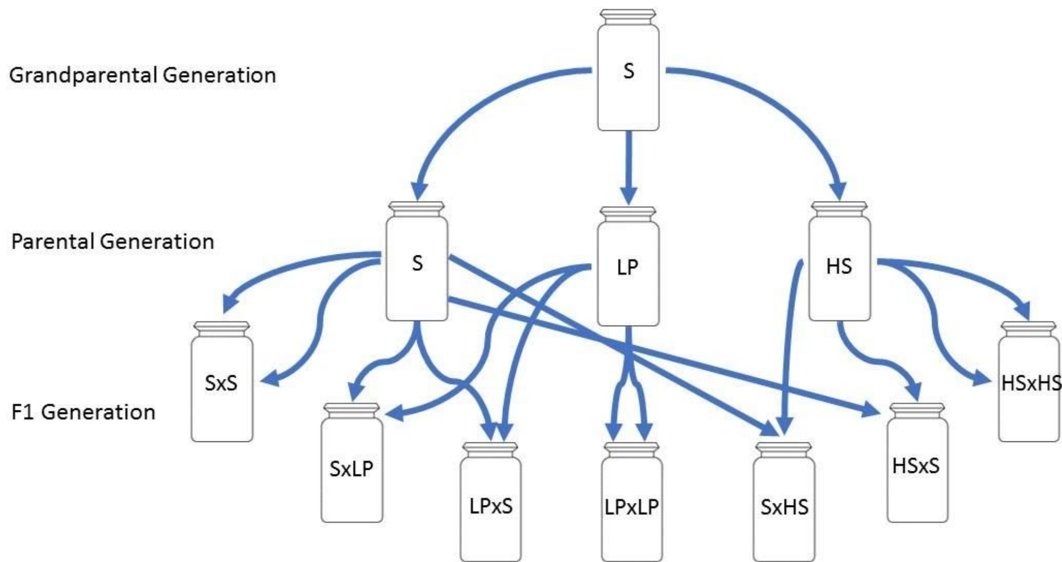

Figure 1. Experimental Cross Design. 100 males and 100 virgin females mated on standard food for 24 hours in 4 bottles of 25 males and 25 females per bottle. Their progeny was hatched and reared on the same standard food. After separating males and virgin females and allowing them to age 4-5 days, they were transferred into vials consisting of 15 females and 15 males per vial on either standard, low protein, or high sugar diet. The grandparental generation could mate 24 hours before being transferred to a new vial of the same diet. The progeny, considered the parental generation, was separated into males and virgin females and aged 4-5 days before being crossed as shown above in pairs. The time it took the F1 generation to reach adulthood (egg-laying to eclosion) was monitored 3 times daily

12 hours:12 hours light:dark regime. As adults emerged (parental generation) they were collected as virgins and were housed in same sex groups of about 15 flies per vial. At the age of 4–5 days post eclosion the virgins and males were crossed in the four possible sex-by-developmental nutrition combinations per strain and diet:

1. males on standard diet with females on standard diet (S-S)
2. males on test diet with females on test diet (P-P)
3. males on standard diet with females on test diet (S-P)
4. males on test diet with females on standard diet (P-S)

For each strain, the low protein and high sugar diets were tested with three different parental combinations each for a total of six test crosses. Furthermore, one control cross was performed which both parents were on the standard diet (Figure 1):

1. standard female x standard male (SF x SM)
2. standard female x high sugar male (SF x HSM)
3. high sugar female x standard male (HSF x SM)
4. high sugar female x high sugar male (HSF x HSM)
5. standard female x low protein male (SF x LPM)
6. low protein female x standard male (LPF x SM)
7. low protein female x low protein male (LPF x LPM)

The parents (4 males and 4 females per vial) could interact and lay eggs for 24 hours in vials containing standard food, so that when progeny were laid, they would only consume standard food. Development time of the next generation flies was measured as the length of time between oviposition and adult eclosion. To measure the developmental time the emerged adults were collected three times a day until eclosion ceased. CO_2 was used in handling the flies.

Chi-Squared analysis was used to determine if the developmental time of each dietary combination was significantly different from the developmental time of the flies whose parents were raised on the standard food (Table 2). To compare the standard control diet to each poor diet cross for each strain, the total number of individuals which eclosed in certain time frames (233 hours, 240 hours, 247 hours, 258 hours, 265 hours, 272 hours, 282 hours, 289 hours) for a given diet cross were summed with the total individuals which eclosed in that time from the control diet cross (SF x SM). This served as the observed values. Table 3 shows the observed times of eclosion for the control diet crosses for both the y^1w^{67C23} and OR strains. To obtain expected values, the total number of individuals which eclosed at all time periods was multiplied by the fraction of individuals which eclosed at that certain time period relative to the total number of flies in both control and that test diet, and was then divided by the total of all individuals from the control and diet cross. This was repeated for both the expected value for the control and the expected value for the diet cross. The sum of every *D* value was the Chi-Squared value. Then a Chi-Squared analysis using the appropriate degrees of freedom was performed to give the *P* value. If the *P* value is under 5%, it is considered significant.

Results

Part 1

While growing the fly stock, many flies died from sticking to the standard food (Table 1). This delayed the process of collecting the correct number of flies needed to start the crosses. Most eggs that were moved onto the different test diets from the crosses never hatched into larvae. Instead, an orange fungus grew in the vial over the eggs (Figure 2). To determine the cause of the orange fungus, a control vial with just standard food in it was placed in the

incubator. A standard food vial smeared with the clean blunt dissecting needle used to relocate the eggs was also placed in the incubator. After four days the control vial had no growth, and the vial rubbed with the blunt dissecting needle had the same orange growth seen with the eggs. This

Figure 2. Picture of vial when using a blunt dissecting needle to relocate eggs. As pictured, the food when using diets in Table 1 quickly grew an orange tinted fungus after using a blunt dissecting needle to relocate the eggs. The fungus easily killed the flies and didn't allow them to produce progeny.

indicated that the cause of the fungus was likely coming from the dissecting needle used to transfer the eggs, although the needle had been cleaned between transfers. Because this method of rearing the flies on different diets was not successful, we altered our growth scheme. This also allowed the removal of the blunt dissecting needle from the process which caused the orange fungus. The results using that approach are explained in Part 2.

Part 2

Using a different standard diet (Table 2) allowed the fly stock to expand more rapidly. The control diet in Table 2 has 61.76 fewer grams of yeast 500 than the control in Table 1.

Cornmeal is present in the control diet in Table 2, whereas there is no cornmeal in the control diet in Table 1. Finally, the control diet in Table 1 included 2 mL 1M $MgSO_4$ and 0.5 g $CaCl_2 \times 2H_2O$, whereas the control diet in Table 2 doesn't include those ingredients. The proportions of change for the 'low protein' and 'high sugar' diets remained the same in Tables 1 and 2. Less flies died from getting stuck in the food using the diet in Table 2, which allowed for more flies to eclose and grants comparison of the effect of diet on the developmental time. However, even with the altered growth protocol some of the crosses had more progeny eclose than others. For example, in the OR strain the SF x HSM experimental cross produced 523 progeny, while the HSF x SM experimental cross only produced 6 progeny (Table 4). The effect of the different diet on each strain individually will be examined first, followed by comparing y^1w^{67C23} developmental times to OR developmental times.

Hours until eclosion	***# eclosed y^1w^{67C23}***	***# eclosed OR***
223	2	4
233	84	124
240	134	96
247	40	74
258	142	189
265	35	55
272	7	17
282	18	19
289	4	8
296	2	2
306	3	0
313	0	0
320	1	0
408	1	0

Table 3. Number of progeny eclosed at specific time frames in both the y^1w^{67C23} strain and the OR strain for the control (SF x SM) cross. This data was used to compare developmental time of different dietary crosses to test for significance.

y^1w^{67C23}	n	df	χ^2	P
Control vs SF x HSM	417	7	12.2	0.0935
Control vs HSF x SM	84	5	15.2	0.00935
Control vs HSF x HSM	139	6	39.6	0
Control vs SF x LPM	551	7	65.8	0.00000753
Control vs LPF x SM	17	1	0.0340	0.854
Control vs LPF x LPM	41	5	24.1	0.000206
OR				
Control vs SF x HSM	523	7	13.0	0.0728
Control vs HSF x SM	6	N/A	N/A	N/A
Control vs HSF x HSM	14	1	20.3	0.00000655
Control vs SF x LPM	159	6	88.0	0
Control vs LPF x SM	13	1	3.88	0.0490
Control vs LPF x LPM	15	N/A	N/A	N/A

Table 4. Chi squared analysis data for the y^1w^{67C23} and OR strains of *Drosophila melanogaster*. This table shows the *n, df,* χ^2, and *P* values obtained using Chi-Squared analysis. '*n*' defines the number of progeny that eclosed per cross. '*df*' defines the degrees of freedom used in each Chi-Squared analysis. This analysis demonstrates which developmental times differed significantly from the control (SF x SM) cross. The highlighted values are the *P* values under 0.05, which are crosses with developmental times significantly different from the control cross. Crosses labelled N/A did not have enough data to conclude whether the differences in developmental time was statistically significant or not.

y^1w^{67C23}

The time from oviposition until eclosion was recorded for the progeny from each cross. Table 3 shows the number of progeny eclosed from the control cross at specific times for both the y^1w^{67C23} strain and the OR strain. To determine if altering a parental diet affected this timing, Chi-Squared analysis was used to see if the developmental time was significantly different from that of the parents on the standard diet. In the y^1w^{67C23} strain, the following crosses were included in the table as statistically significant variables predicting development time (Table 4):

HSF x SM

HSF x HSM

SF x LPM

LPF x LPM

A significant interaction indicates that the development time of the experimental test crosses varied significantly from the control cross. It appears that the progeny from each experimental cross found statistically significant developed slower than the control cross (Table 5). The percentage of progeny eclosed at 9.3-10.3 days in the test crosses are all smaller than the control cross. Similarly, each test cross has much higher percentage of progeny eclosed at 11.5+ days than the control cross, except for the HSFxSM cross having the majority of progeny eclose at 10.4-11.4 days. This data concludes that in these crosses in the y^1w^{67C23} strain, transgenerational effects of developmental time can be seen, showing that the developmental time of offspring is slower than if the parental generation's diets weren't altered.

OR

The time from oviposition until eclosion was recorded for the progeny from each cross. In the OR strain two experimental crosses, the HSF x SM and the LPF x LPM did not produce

the amount of progeny needed to collect enough data for results. The following crosses were included in the table as statistically significant variables predicting development time (Table 4):

HSF x HSM

SF x LPM

LPF x SM

	% eclosed at 9.3-10.3 days	% eclosed at 10.4-11.4 days	% eclosed at 11.5+ days
y^1w^{67C23}			
SFxSM	55.20%	39.10%	5.73%
HSFxSM	34.50%	60.70%	4.76%
HSFxHSM	32.40%	54.70%	13.00%
SFxLPM	46.80%	43.20%	10.00%
LPFxLPM	34.10%	46.30%	19.50%
OR			
SFxSM	50.70%	44.40%	4.93%
HSFxHSM	42.90%	7.14%	50.00%
SFxLPM	62.30%	33.30%	4.40%
LPFxSM	76.90%	23.10%	0.00%

Table 5. Percentage of progeny eclosed at 9.3-10.3 days, 10.4-11.4 days, and 11.5+ days in the y^1w^{67C23} and OR strains in statistically significant crosses. This table allows for comparison of developmental time between crosses at certain time frames. The higher percentage of progeny eclosed at earlier time frames, the faster the overall developmental time for the cross. The higher percentage of progeny eclosed at later time frames, the slower the overall developmental time for the cross.

It appears that the progeny from the HSF x HSM experimental cross developed slower than the control cross. However, the progeny from the SF x LPM and LPF x SM experimental crosses appear to have developed faster than the control cross (Table 5).

DGRP-307

Hardly any progeny from the DGRP-307 strain eclosed from the control or experimental crosses. There was not enough data to determine developmental time for this strain.

y^1w^{67C23} vs OR

Not enough progeny eclosed to determine if there are strain-to-strain cross differences with the HSF x SM, LPF x SM, and LPF x LPM experimental crosses. Statistical significance was found in the SF x SM and the SF x LPM crosses (Table 6). The control cross (SF x SM) had significantly different developmental times between the y^1w^{67C23} and OR strains (Table 3 and Table 6). It appears that the progeny of the y^1w^{67C23} strain developed faster than the progeny of the OR strain on the SF x SM cross, while the y^1w^{67C23} strain developed slower than the progeny of the OR strain of the SF x LPM crosses (Table 7). It seems that the following crosses produced the most progeny within both strains (Table 6):

SF x SM (471 in y^1w^{67C23}, 588 in OR)

SF x HSM (417 in y^1w^{67C23}, 523 in OR)

SF x LPM (551 in y^1w^{67C23}, 159 in OR)

y^1w^{67C23} vs OR	*y^1w^{67C23} n, OR n*	*df*	χ^2	*P*
SF x SM	471, 588	7	27.4	0.000286
SF x HSM	417, 523	7	8.02	0.331
HSF x SM	84, 6	N/A	N/A	N/A
HSF x HSM	139, 14	1	3.21	0.731
SF x LPM	551, 159	7	133	0
LPF x SM	17, 13	N/A	N/A	N/A
LPF x LPM	41, 15	N/A	N/A	N/A

Table 6. Chi-Squared analysis data comparing the y^1w^{67C23} and OR strains of *Drosophila melanogaster.* This table shows the *n, df,* χ^2, and *P* values obtained using Chi-Squared analysis. This analysis demonstrated which developmental times differed significantly between strains in each cross. The highlighted values are the *P* values under 0.05, which are crosses with

developmental times significant strain to strain differences. Crosses with N/A did not have enough data to conclude whether the differences in developmental time was statistically significant or not.

	% eclosed at 9.3-10.3 days	% eclosed at 10.4-11.4 days	% eclosed at 11.5+ days
y^1w^{67C23} SFxSM	55.20%	39.10%	5.73%
OR SFxSM	50.70%	44.40%	4.93%
y^1w^{67C23} SFxLPM	46.80%	43.20%	10.00%
OR SFxLPM	62.30%	33.30%	4.40%

Table 7. Percentage of progeny eclosed at 9.3-10.3 days, 10.4-11.4 days, and 11.5+ days in the *y^1w^{67C23}* and OR strains in statistically significant crosses. This table allows for comparison of developmental time between crosses at certain time frames. The higher percentage of progeny eclosed at earlier time frames, the faster the overall developmental time for the cross. The higher percentage of progeny eclosed at later time frames, the slower the overall developmental time for the cross.

Discussion

Overall, the flies did not produce the expected amount of progeny in Part 1 of the experiment. This was because the flies continually got stuck in the food and died, which didn't allow them to reproduce (Figure 2). The flies responded well to the diet used in Part 2 (Table 2) and produced more progeny so that the experimental crosses were able to be made.

In general, I saw that crosses with females on standard diet produced more progeny, whereas most of the crosses where the maternal parents' diet was altered (high sugar or low protein) produced fewer progeny. Research demonstrates that female flies reared on a standard diet laid 40-50 eggs, whereas female flies reared on a poor diet reared 10-15 eggs [17]. This suggests that altering the females' diets may have decreased the amount of eggs they laid, which could explain why certain crosses didn't produce as many adult progeny. Yeast is also known to promote egg laying in female flies, but the flies in the experiment continued to stick to the yeast and die. Consequently, yeast was not sprinkled on the top of the food for the flies in this experiment, which also may have contributed to fewer eggs being laid. Unfortunately, because some crosses didn't produce a high number of progeny it is difficult to determine whether certain data is significant and how so. If the crosses had produced a higher number of progeny that eclosed, the study might have been able to determine with more certainty whether the parental diets had impacted developmental time in progeny.

Most of the parents in the DGRP-307 strain died, and therefore did not produce progeny that eclosed. As a result, data concerning developmental time could not be collected from this strain. This was the only strain recently purchased and brought to Malone University for this study, so it could be hypothesized that the new environment wasn't agreeable for the strain. Even

the standard diet didn't prove to be adequate to keep the DGRP-307 strain alive long enough to produce offspring.

In the y^1w^{67C23} strain, all crosses significantly different in developmental time from the control cross appeared to develop slower than the control (Table 5). This data concludes that transgenerational effects of developmental time can be seen in these crosses in the y^1w^{67C23} strain, showing that the developmental time of offspring is slower than if the parental generation's diets weren't altered. Both altered diets (high sugar or low protein) are not as ideal for the flies as the control diet. The high sugar diet can induce hyperglycemia and increased glycogen stores in adults [7] and can also cause obesity in flies [13]. The low protein diet is not as ideal as the control diet because there are less nutrients available to the flies [1]. Faster developmental time would be more environmentally fit, especially in the wild, where the larval food sources of *D. melanogaster* such as decaying fruit are likely to become unsuitable over time or used by competing larvae [3]. It seems reasonable that the transgenerational effects seen in the offspring showed slower developmental time considering that one or both of their parents weren't reared on the control (more ideal) food.

In the OR strain, the HSF x HSM cross appeared to have a slower development time, whereas the SF x LPM and the LPF x LPM crosses appeared to have a faster development time (Table 5). These results are more intriguing because in these crosses, either one or both parental diets were altered to not be as ideal, so it doesn't make sense that the offspring would develop faster than they would have in the control crosses. The conclusion that the SF x LPM cross does develop faster than the control SF x SM cross agrees with the data from Valtonen's study [3] showing that a combination of standard and test diet in the parents give fastest developmental time in offspring. This could suggest that the mechanisms of causing transgenerational effects in

offspring is a more complicated process that it appears. However, these crosses had very low numbers of progeny eclosed compared to other crosses (Table 4). With a higher number of progeny eclosed, the study would be able to more confidently conclude whether the developmental times were faster or slower than the controls.

The control cross had significantly different developmental times between the y^1w^{67C23} and OR strains. The difference in development times in the control crosses between the strains does demonstrate strain-to-strain differences regarding developmental time. The OR strain has a faster developmental time in the SF x LPM cross than the y^1w^{67C23} strain, showing higher percentages in early time frames and lower percentages in later time frames (Table 7). These differences show that developmental times may not be universal between strains of *Drosophila melanogaster*. Further research can be done to conclude what genetic differences in strains cause the variation in developmental time.

Overall, the study presents evidence that there are transgenerational effects of high sugar or low protein parental diets on the developmental time of offspring. There are also strain differences regarding developmental time, showing that some transgenerational effects of parental diet on the developmental time of offspring may not apply to all strains. The mechanism of passing down transgenerational effects seems to be more complicated than anticipated, with evidence that parents reared on poor diets don't always pass down negative transgenerational effects. With more time to grow more progeny in the crosses with low numbers of progeny, the study would be able to collect more data and be able to more confidently suggest the exact transgenerational impacts of parental diets on offspring. Further research can be conducted to determine how to alter parental diet while simultaneously maintaining high numbers of progeny, which would allow for overall clearer data.

Appendix

Raw Data from $y^{1}w^{67C23}$ strain

		Hours																		
Cross	Day	223	233	240	247	258	265	272	282	289	296	306	313	320	327	334	344	351	408	Total
SFxSM	1		13	35	3	61	16		6										1	
	2		20	32	1	39	9		2	1										
	3		10	22	9	25	3	2	1	1		2								
	4		14	18	11	3														
	5	2	15	10	13		2	3	5		1	1		1						
	6		12	7	3	14	5	2	4	2	1									
Total flies:		**2**	**84**	**134**	**40**	**142**	**35**	**7**	**18**	**4**	**2**	**3**		**1**					**1**	473
Total Vials:		56 Total																		
SFxHSM	1		11	24	5	59	10	4	1	1										
	2		10	11	5	24	3	2		1										
	3		12	18	9	9	9		1	1	1		1		1					
	4	1	9	20	3	17	8	1	1											
	5	1	17	8	3	2	6	4	8	2										
	6		20	7	8	19	5	3	4	4	3	1								
Total flies:		**2**	**79**	**88**	**33**	**130**	**41**	**14**	**15**	**9**	**4**	**1**	**1**		**1**					418
Total Vials:		42 Total																		
HSFxSM	1																			
	2		2	6		9	2	2												
	3			3		2														
	4		1	2	2	2														
	5		2	2	1	4	2	2	1											
	6		4	3	1	14	8	4	2	1										
Total flies:			**9**	**16**	**4**	**31**	**12**	**8**	**3**	**1**										84
Total Vials:		30 Total																		
HSFxHSM	1			1																
	2			2		7		2												
	3			5	2	23	12	3												
	4			2	6	1	3	9				2								
	5		2	7	2	3	2	4	9	2	1									
	6		6	4	6	5	1	1	1	3										

Total flies:			**8**	**21**	**16**	**39**	**18**	**19**	**10**	**5**	**1**	**2**								139
		Hours																		
Cross	Day	223	233	240	247	258	265	272	282	289	296	306	313	320	327	334	344	351	408	Total
Total Vials:		18 Total																		
SFxLPM	1		52	41	8	97	13	12	2	1		1							1	
	2		15	21	7	41	8	2	3											
	3		5	11	5	18		2	2											
	4		7	12	8	3	2		2	3	5	9		5	3	4	2	2		
	5	4	16	5	9	3	5	8	4											
	6		23	5	4	18	4	2		6	3	2								
Total flies:		**4**	**118**	**95**	**41**	**180**	**32**	**26**	**13**	**10**	**8**	**12**		**5**	**3**	**4**	**2**	**2**	**1**	556
Total Vials:		48 Total																		
LPFxSM	1			4		7														
	2																			
	3			4		1														
	4		1																	
	5																			
	6																			
Total flies:			**1**	**8**		**8**														17
Total Vials:		6 Total																		
LPFxLPM	1					1			2			1								
	2			2						1										
	3			6		2	1													
	4		1	2		2														
	5			2	1		4		2											
	6					4	3	2			2									
Total flies:			**1**	**12**	**1**	**9**	**8**	**2**	**4**	**1**	**2**	**1**								41
Total Vials:		6 Total																		

Raw Data from OR strain

		Hours																		
Cross	Day	223	233	240	247	258	265	272	282	289	296	306	313	320	327	334	344	351	408	Total
SFxSM	1		29	24	23	125	34	6	8	5	1									
	2		25	31	3	20	2	3												
	3		17	20	24	3	8	3			1									
	4	4	7	9	12	5	2		2											
	5		30	5	8	14	5	4	2											
	6		16	7	4	22	4	1	7	3										
Total flies:		**4**	**124**	**96**	**74**	**189**	**55**	**17**	**19**	**8**	**2**									588
Total Vials:		30 Total																		
SFxHSM	1		13	12	7	67	13	4	4	1	2	3	1	1						
	2		34	31	14	55	10	4	1	7	5									
	3		12	17	4	25	8	7		1	2	1								
	4	5	19	14	12	5		3	1											
	5	3	16	11	14	9	2			2	1		2		1					
	6	4	13	3	1	3	4		9	5	2	1	2							
Total flies:		**12**	**107**	**88**	**52**	**164**	**37**	**8**	**15**	**16**	**12**	**5**	**5**	**1**	**1**					523
Total Vials:		30 Total																		
HSFxSM	1												1							
	2											1								
	3																			
	4																			
	5																			
	6																			
Total flies:												**1**	**1**							2
Total Vials:		6 Total																		
HSFxHSM	1			5	1	1														
	2																			
	3																			
	4																			
	5																			
	6																			
Total flies:				**5**	**1**	**1**														7

		Hours																		
Cross	Day	223	233	240	247	258	265	272	282	289	296	306	313	320	327	334	344	351	408	Total
Total Vials:		4 Total																		
SFxLPM	1		16	15	6	23	4		1											
	2		6	10	5	11	2			1										
	3			6	1	3	2		1											
	4	3	5	5	5	3		1												
	5			5	3		1	1							1					
	6	2	3	2	1	1	1		1	2	1									
Total flies:		**5**	**30**	**43**	**21**	**41**	**10**	**2**	**3**	**3**	**1**				**1**					160
Total Vials:		18 Total																		
LPFxSM	1																			
	2		1																	
	3				1	1														
	4			1			1													
	5			2																
	6		2	1	2			1												
Total flies:			**3**	**4**	**3**	**1**	**1**	**1**												13
Total Vials:		6 Total																		
LPFxLPM	1				1		2													
	2								1		3									
	3									1										
	4								1											
	5				2															
	6							1			3		2							
Total flies:					**3**		**2**	**1**	**2**	**1**	**6**		**2**							17
Total Vials:		6 Total																		

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