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Dietary Analysis and Epigenetic Comparisons of Drosphila Melanogaster through Multiple Generations

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Recommended Citation
Zheng, Xu (Kevin) F., "Dietary Analysis and Epigenetic Comparisons of Drosphila Melanogaster through Multiple Generations" (2012). *Holster Scholar Projects*. 2.
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Dietary analysis and epigenetic comparisons of Drosophila melanogaster through multiple generations.

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Summary

Nutrition and the lack thereof has been a demanding issue in both the undeveloped and developed country. The unbalance diet between healthy and unhealthy diet has led to many of the problems presented in society such as obesity, heart disorders, and loss of longevity. It is now known that calorie may not provide the most accurate information in terms of the lifestyle and the well-being of humans. Rather the composition of the calorie is what becomes integral in people's understanding of the effect of food physiologically. With this knowledge, we seek to use a Drosophila melanogaster as a model organism to both confirm the current hypothesis on the role of yeast and sucrose and also to collect new data looking at the genetic changes that occurs at the epigenetic level. Our result supports both prior researches while also disproving others. We found that a yeast-rich diet produced flies fit flies that have higher fecundity rate while having reduced feeding rates. On the other hand, flies fed on high sugar have lower fecundity rate and have shortened lifespan. Our data also reveals that ab-libitum flies that tend to increase feeding rate when placed on high sugar concentration as oppose to high yeast concentration. These data will help future research in understanding the mechanism of the interaction between metabolism, health-related disorders, and longevity.

Introduction:

With the growing increase in the readiness and availability of food for the general public the amount the calories ingested has drastically increased. In the US population the easily access to excess and or unhealthy food has rose the obesity rate to 35.7% (Adult 2003). This has led to a drastic increase in heart attacks, strokes, diabetes, and other forms of illnesses. In many cases, the role of excess nutrients became detrimental to the actual well-being of the subject. It has shown, in previous studies of flies that by actual limiting the amount of calories and nutrients that it would benefit physiologically (Skorupa et al. 2008).

Originally, flies were thought to have no means of control in their feeding. However, recent studies shown that flies have the ability to regulate their feeding-ad libitum feeding (Min 2006). When compared between ad libitum and food deprived drosophila by using proboscis prints (amount of feeding marks on the food) it is found that ad libitum flies tend to eat small amounts of food at a time which allows them to have a smaller crop size. Food deprived drosophila when presented with a food stimulus tend to eat more food on average (Skorupa et al. 2008) And while ad libitum flies eat smaller amounts they eat more frequently as oppose to food deprived flies which eat large amounts of food but eat less frequent. In addition, it was found that food deprived flies when presented food of different yeast and sucrose concentration tend to eat ones with greater sucrose

concentration as oppose to yeast concentration (Skorupa et. al 2008). This exemplifies that when starved, flies tend to go for the food regime that gives the most amount of calories even though the nutritional value (low yeast) is much less. It also shows that smaller but frequent meals are favored (ad-libitum) in the Drosophila population over the large quantity but fewer frequencies (Skorupa et al.). Therefore ad libitum flies do not regular their feeding behavior in ways that food deprived flies do.

Drosophila's feeding mechanism changes base on the environment they are in. By utilizing this aspect, we can research the effects of different food regime on drosophila. Their energy intake is measured by the amount of triglyceride in form of lipid droplets. These triglyceride accumulations are modulated by increased dietary carbohydrate while at the same time is suppressed by yeast concentration. Thus, increasing the level of protein decreases the accumulation of fat (Edgecomb et al. 1994). New research have utilized this knowledge and have found that by increasing the yeast intake in drosophila it produces flies that are lean while increasing the sucrose concentration produced obese flies. The optimal ratio of yeast to sucrose is 10g/10g/ddl (Danielle et al.).

Thus, in our research we will use this knowledge and test the changes associated with drosophila behavior and as well as genetic changes upon feeding on different food regimes. In order to look for genetic changes we examined epigenetic histone modifications across different generations. Antibody H3k4 and H3K9 were picked due to their common appearance in chromosomes. Drosophila salivary glands are known to be associated with metabolism and feeding. In these glands, there contain polytene chromosome which are chromosome that goes thought many stages of replication without mitosis. Using these polytene chromosomes in immune-staining, we detected slight epigenetic changes that can be associated with the varying concentration of yeast and sugar.

Results:

We documented the changes that occur in the drosophila population when put under different food conditions. Below is the data table in which our food conditions were used.

Table 1: Treatment media

	Regime 1	Regime 2	Regime 3	Regime 4
Yeast	10g	10g	5g	15g
Sucrose	10g	5g	10g	15g
Agar	1g	1g	1g	1g
Water	100ml	100ml	100ml	100ml

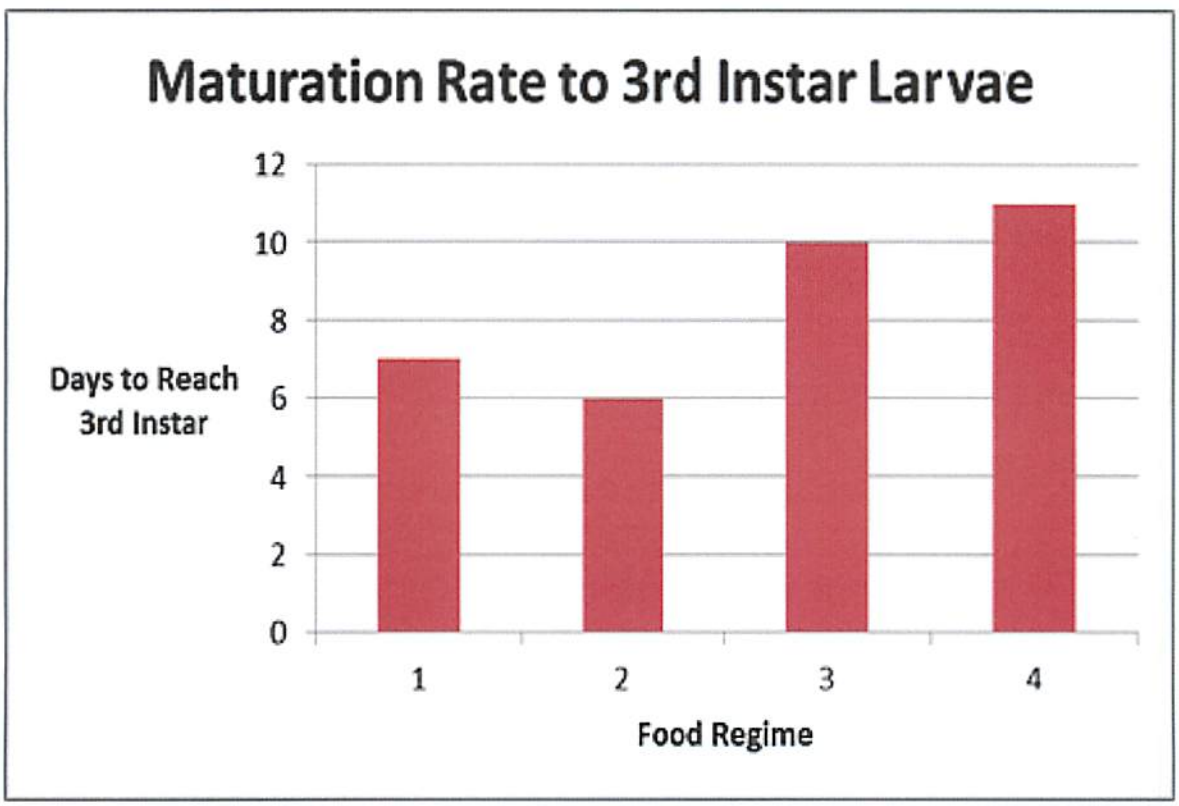
By using these four food regime, we raised Drosophila melanogaster in vials at 25°C and 50% humidity. From these flies, we collected data on their healthy and growth through the different larvae stages and as well as adulthood.

Flies tend to be leaner in high yeast concentration.

In food regime 2 the flies were on average 1/3 smaller than the flies raised in regime 3. Flies raised in regime 4 were on average the smallest. This correlates with the fact that regime 3 contains food with the highest sucrose concentration. Regime 2 while the flies were smaller, looked as fit, if not more fit than the flies raised in regime 3. This could be examined by the amount of movement and reaction speed when examining the flies in the vial. Flies in regime 2 had on average more mobile flies than flies raised in regime 3 which tend to be idler. In addition, the larvae of flies in regime 3 were almost twice the size of the larvae from food regime 2 at the 3rd instar phase.

Flies show maturation delay in high sucrose concentration.

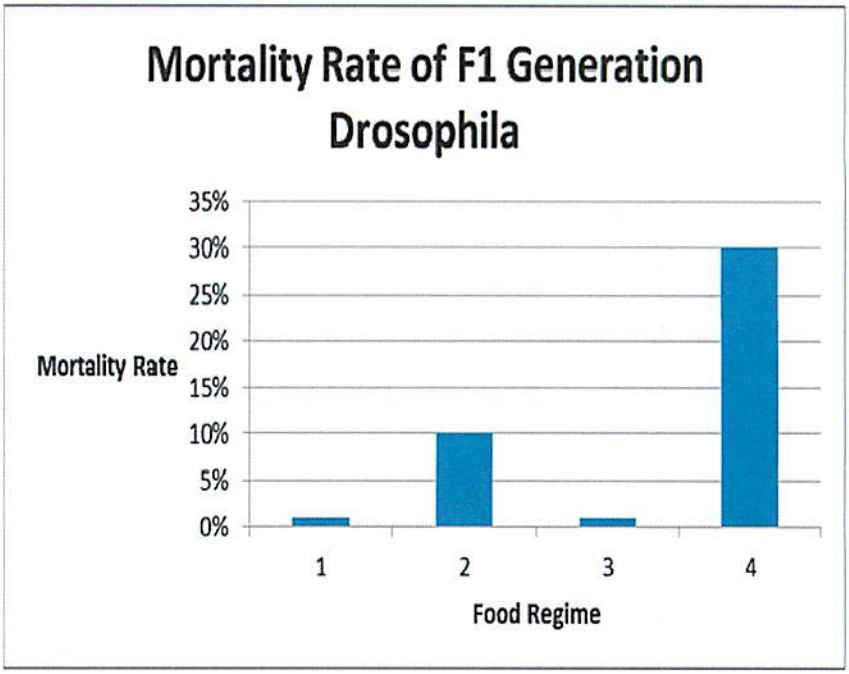
Flies raised in high yeast concentration matured to 3rd instar the quickest. In the normal condition (10% yeast, 10% sucrose) the time it takes flies to hatch form eggs and mature to 3rd instar larvae is about 7 days. Our research shows that by decreasing the sucrose slightly, the maturation rate of the flies increases by a day. On the other had when sucrose concentration is increased, we see that flies tend to feed more and do not reach 3rd instar until a much later period. When both sucrose and yeast are at high level, the low amount of water in the food is not adequate even for the flies. This produces stress that caused the maturation rate to be even longer than just increased sucrose concentration.



Graph 1: With increasing sucrose concentration, there is an increase maturation time.

Flies raised in high sugar concentration have lower fecundity rate than flies raised in high yeast concentration.

On average, the flies mated in high sucrose concentration now only show slower growth (larval stage) but also fewer larvae in general. When comparing larvae amount from 7th day of regime 2 and 10th day of regime 3, there is a greater amount of larvae on the walls of the vial in regime 2 as oppose to regime 3. Regime 1 had the highest concentration of larvae due to having the most optimal state. Regime 4 had the smallest concentration of larvae at 11th day due to being the least optimal for the flies.



Graph 2: Increasing yeast concentration coupled with decreasing sucrose concentration leads to high mortality rate.

Flies survivability decreases with either extreme concentration of yeast or sugar.

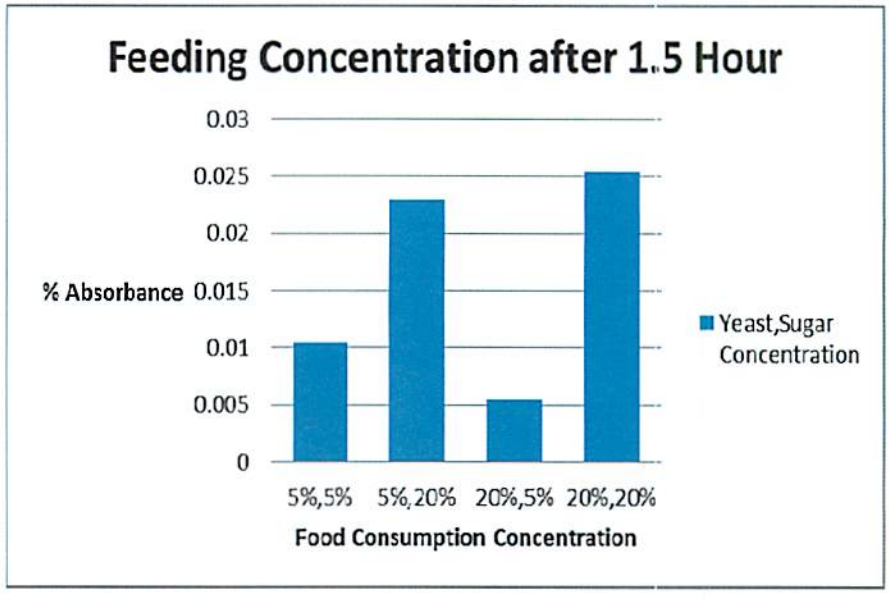
When the mortality rate is compared between the flies of the different regime there is a clear distinction between which ones is favored by the flies. Regime 2 which contains 10% yeast, 5% sugar has a mortality rate is 10%. This is tenfold bigger than regime 3 which has 5% yeast, 10% sugar concentration. Therefore flies in regime 3 are living longer than flies in regime 2. However, the highest mortality rate still resides in regime 4 where roughly 30% of the fly population dies before reaching adulthood. There was no different in the mortality rate of regime 1 and regime 3. Both show few to no larvae death and produce healthy adult flies. The graph of the mortality rate can be seen above on the right.

Flies raised in higher sugar concentration have enlarged salivary glands.

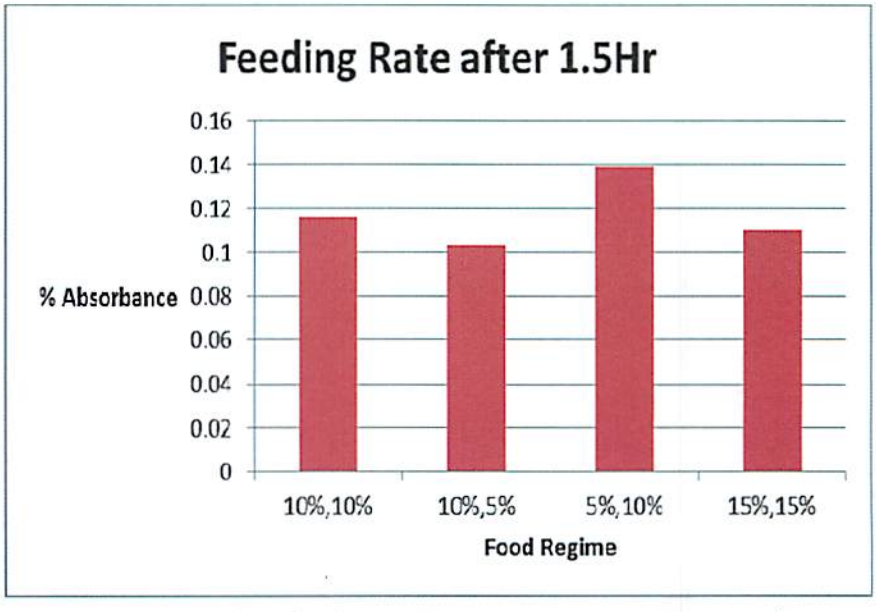
Upon dissection, the salivary glands from food regime 3 had the most distinct glands. The glands form regimes 3 were on average bigger than the glands from regime 2 and 1. Regime 4 glands were around the same size as regime 1 and 2. Having said this, the size of the glands correlated with the size of the larvae. Since regime 3 had the biggest size 3rd instar larvae the size of the glands were also the biggest. When dissecting smaller size larvae from regime 3, the glands were smaller also. Therefore there is a rough positive correlation between size of larvae and size of salivary glands.

Flies increase feeding rate when placed on high sugar low yeast food.

Flies tend to increase their feeding when placed on a higher sucrose (sugar) concentration. When difference combination of yeast and sucrose mixture were mixed with blue FRS dye a absorbance was measured used a spectrophotometer. A high absorbance reading means that the presence of the blue is high while a low absorbance means that the presence of the dye is low. High dye is a result of high intake of food while low dye would be a result of low intake of food. When comparing 5% Yeast, 20% sucrose with 20% yeast, 5% sucrose it is evident that the regime with the 20% sucrose has a consumption almost triple that of the 20% yeast, 5% sucrose one. The high absorbance reading from the 20% sucrose, 20% yeast also proves that high concentration of sucrose is favored through all cases. In another experiment using 5% and 10% combinations we see similar pattern. The one which had the highest concentration of sucrose and lowest concentration of yeast combination had the highest consumption rate while the one with the lowest sucrose had the lowest feeding rate. The smaller change in absorbance level correlates with the less drastic difference between the yeast and sucrose concentration.



Graph 3: A measure of absorbance of different combination of yeast and sucrose. Flies were fed in these regimes for 1.5hours before freezing.



Graph 4: A secondary food consumption assay comparing another combination of yeast and sucrose concentrations. Notation is : Yeast % Sucrose %

Increasing sucrose concentration decrease H3K4 signal.

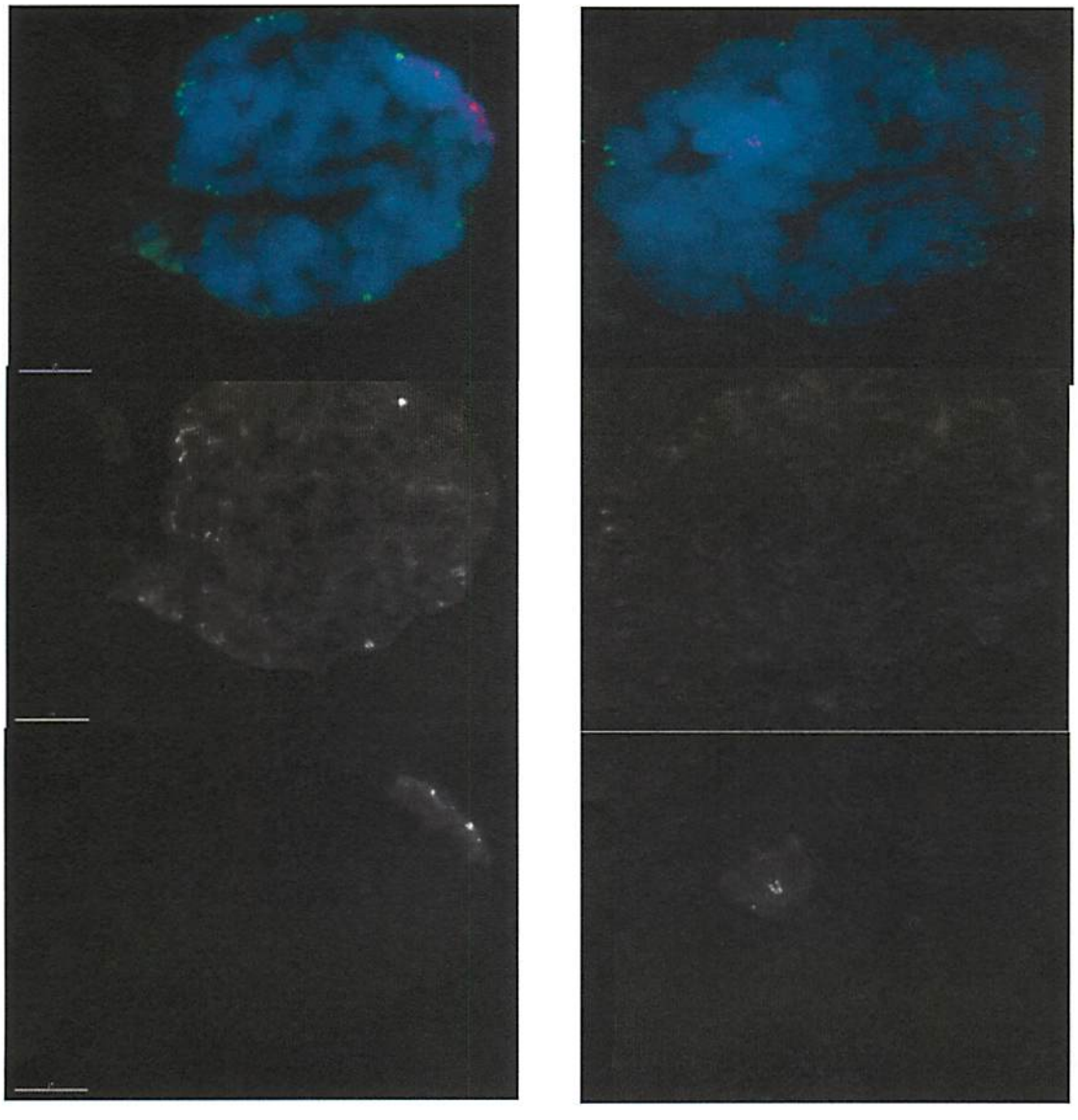


Image 1: On the left shows the immuno-staining of polytene chromosome of 5% Yeast 10% Sucrose food regime. On the right shows immune-staining of polytene chromosome of 10% Yeast 5% Sucrose.

Salivary glands were dissection from the respective drosophila in order to examine the polytene chromosomes for histone modification. Antibody H3K4 conjugated to mouse secondary and antibody H3K9 conjugated to rabbit secondary were used to examine epigenetic changes. In doing so we found that by increasing the sucrose and decrease the yeast concentration, we see a slight increase in the H3K4 (green) signal as seen in the bandings above. There was no different in the H3K9 signal which is chromo-center localized.

Increasing both sucrose and yeast concentration decreases H3K4 signal.

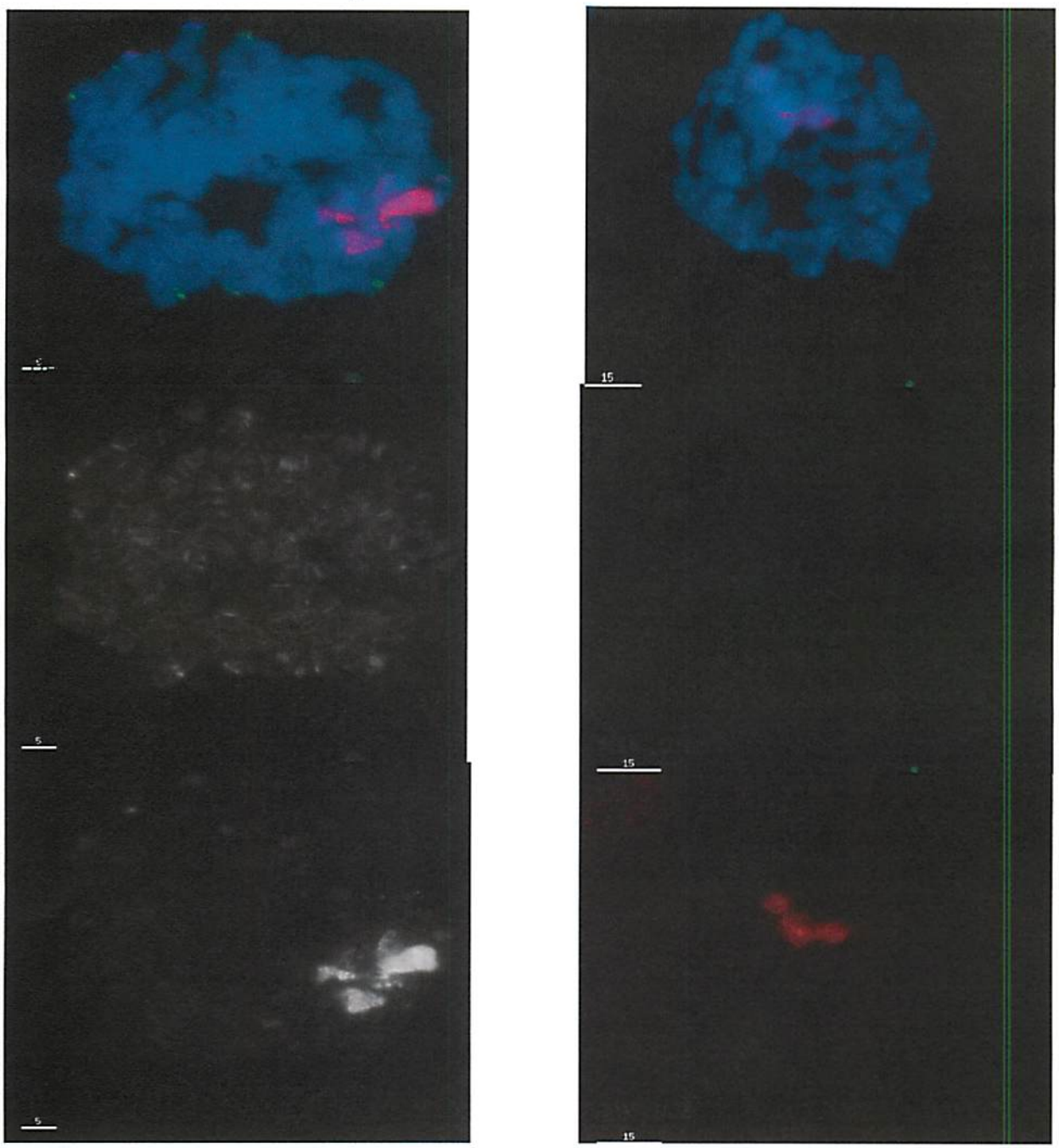


Image 2: On the left shows the immuno-staining of polytene chromosome of 10% Yeast 10% Sucrose food regime. On the right shows immune-staining of polytene chromosome of 15% Yeast 15% Sucrose.

In the image above when the sucrose concentration is increased and the yeast concentration is also increased there is a more drastic decrease in H3K4 signaling than when it is just an increase sucrose concentration. The red H3K9 signal remains unchanged. However, it is not known whether an increase in yeast along could decrease the H3K4 signal.

No reverse epigenetic changes associated with the different food regimes.

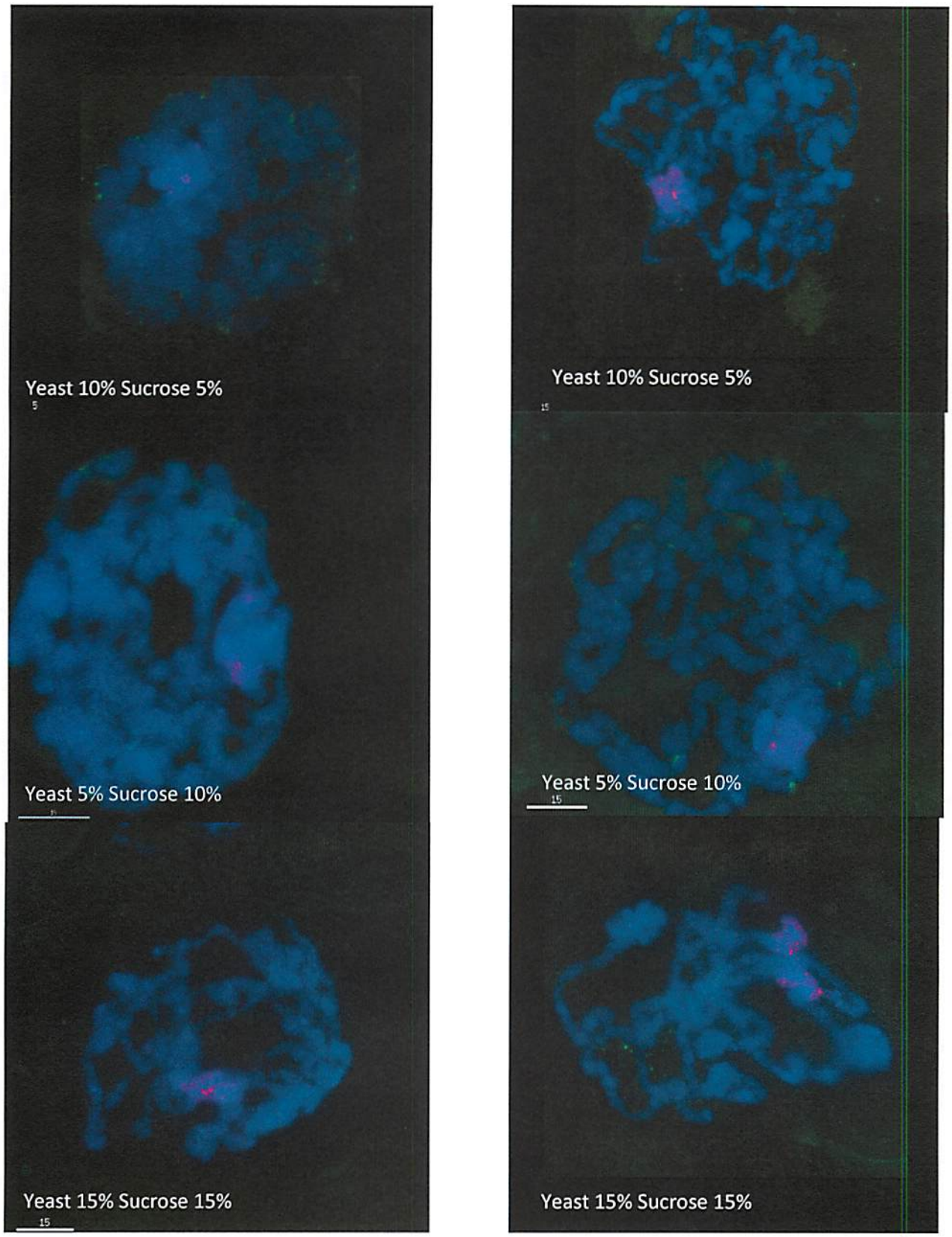


Image 3: On the left shows the immuno-staining of polytene chromosome of first generation drosophila. On the right shows the immune-staining of the polytene chromosome of third generation drosophila.

Adult flies were placed back into normal food condition (10% yeast, 10% sucrose) and are mated. We see in the larave salivary gland's polytene chromosome no significant change in signaling of H3K4 and H3K9. When comparing 1st generation larave polytene to 3rd generation larave polytene both the H3K4 signal and H3K9 signal are similar in intensity as seen above. The 15% yeast and 15% sucrose from both generation shows very minute H3K4 signal when observed. The H3K9 red signal remain unchanged across all food regimes. When comparing 3rd generaiton polytene chromosome we can see that the comparison between 10% yeast 5% sucrose and 5% yeast 10% sucrose remains as before with the latter having a slight decrease in green signal (some of it going into the background).

Discussion:

Our data suggests that by changes the concentration of yeast and sucrose diet we can alter the H3K4 signaling strength. H3K4 global signaling is reduced as sucrose concentration increased. In cases where both sucrose and yeast concentration is increase, it leads to a reduction in H3K4 signal as well. However, what cannot be stated from the data is whether or not increasing yeast concentration alone could cause the decrease in H3K4 signal. It could be that yeast concentration synergies with the sucrose concentration but when alone does not cause any changes by itself. In future, by using other marks beside H3K4 and H3K9, we can map out the different regions of the polytene chromosome which changes with sucrose and yeast concentration. Then comparing them by FACS sorting we could possible isolate an individual gene of interest that could be one of the multiple factors associated with diet and metabolism.

In addition, drosophila tends to increase their feeding rate when placed on a higher sucrose diet. This shows that drosophila, in order to try to compensate for the lack for the lack of protein in the low yeast diet by eating more of the food to get the nutrients. Likewise in a yeast rich diet, the drosophila reduces their feeding yeast due to the high protein rich diet available to them. Therefore drosophila has the ability to control the feeding rate much like many other organisms.

Furthermore drosophila lifestyle is affected by the diet they are placed in. When placed in a high sugar (sucrose) rich diet, the drosophila size was enlarged. This had a detrimental effect as the time it took the drosophila to grow is prolonged. However, while the maturation rate is prolonged, their mortality rate is smaller than that of the high yeast diet. While the high yeast diet drosophila had better fitness in the early stages of their lives, there was a higher rate of mortality for that drosophila which did not mature to adult hood. There we see a tradeoff between survivability and fitness. It is not known why there is such a distinction and why elevated levels of protein lead to higher mortality than elevated level of sucrose.

Experimental Procedures:

Treatment media for genetic analysis

Water, agar, sucrose, and yeast were mixed in a 1000mL Pryex 1003 beaker. The mixture is allowed to be heated to boiling on a hot plate. A stirred is used to homogenize the mixture. After a short cooling, 5mL of the mixture is pipetted into each of the fly vials. The vials are stored in 4° fridge after cooling overnight with a kimwipe covering. For the food consumption assays, 1% FRS Blue dye is added into the media also before allowing the media to cool.

Fly husbandry

Flies are kept in at 25°C and 50% humidity controlled environment. Flies are raised in 5mL treatment media until eggs are laid. Upon seen 1st instar larvae, the adult flies are discarded under light carbon dioxide anesthesia into 100% ethanol waste container. During third instar, a couple flies are to be removed for dissection purposes while the rest are allowed to grow. Upon reaching adulthood, the flies are transferred under light carbon dioxide anesthesia into new vials for a new mating cycle to begin.

Larvae dissection

Fly larvae are washed in 1X PBS and dissected in .7% saline solution on a dissection slide. To dissect, the mouth hooks are firmly grasp with one forceps. Using another forceps, grasp the body of the larvae approximately midway down. Then pull the larvae apart. Remove the mouth hooks form the salivary glands (which should look like two lobes). Remove any excess fats and debris from the glands.

Fixation and Staining

Note: For a more detailed fixation and staining technique see Appendix A.

Dissected larvae are transferred and fixed on a siliconized microscope slide using a mounting media (in Appendix A). Slide is placed into liquid nitrogen to preserve the DNA molecule and the surrounding protein configuration. Slide are washed in PBS 1X, blocked with BSA, and then blocked with primary antibody overnight at 4°C. Slides are rewashed with PBS the next day and blocked with BSA and then soaked with secondary antibody at room temperature for 1 hour. Slides are rewashed with PBS and mounted with DAPI and coverslip.

Diet Assay

5 females are each placed into one of the different food regime that also contains FRS Blue dye. They are allowed to feed for 1.5 hour at room temperature. Afterwards, flies are flash freeze using dry ice for 3 minutes. The flies are then decapitated and homogenized in 1.5mL micro-centrifuge tubes with 200µL of distilled water. The homogenates are spun for 10 minutes and 80µL of the clear liquid is diluted 1.5 times. The diluted liquid is placed into a spectrophotometer to be red at 630nm.

Microscopy

The prepared slides are looked under a DeltaVision De-convulsion microscope. Using the DAPI (blue), TRITC (red) , and FITC (green) channels the different stains were examined. Exposure times were optimized for each slide due to the wide variation in signal strength.

Acknowledgments:

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Appendix A:

Antibody Staining Part 1:

1. Retrieve polytene chromosome from fridge 4°C
2. Prepare .1% PBST (1% Trition X) in 50mL falcon tube.
3. Pour PBST (1%) into copling jar
4. Place microscope slide in PBST for 15minutes
5. Prepare PBSTB
    a. .5g BSA
    b. 50mL PBS
    c. .05mL Trition X
6. Pour out PBST and pour in PBS-TB in new copling jar
7. Place microscope slide in PBSTB to block for 30 minutes
8. Retrieve Ice Box and Cap
9. Place H3K4 and H3K9 into ice box
10. Place 1:100 ratio of H3K9 and 1:50 ratio of H3K4 into centrifuge tubes. Then add the required PBSTB concentration.
11. Vortex and spin the mixture for 30 seconds
12. Spray distilled water on humid chamber.
13. Cut 2 pieces of parafilm.
14. Place 30µL of antibody mixture onto the slide and place parafilm over it.
15. Store the slide in the humid chamber at 4°C overnight.

Antibody Staining Part 2:

1. Remove microscope slide from humid chamber.
2. Place/retrieve a copling jar and fill it with 1M PBS
3. Place microscope slide in copling jar.
4. Repeat twice more.
5. Make PBSTB and place microscope slide into the solution and block for 30 minutes.
6. Retrieve secondary antibody and place in ice box.
7. Using a ratio of 1:250 of secondary to PBSTB, prepare the required mixture.
8. Retrieve the humid box.
9. Place the slid eon the humid box and pipet 30µL of the mixture onto the slide
10. Cover the slide with parafilm and allow it to incubate at room temperature for 1 hour.
11. Wash the slide in PBS 2X.
12. Place a 30µL drop of DAPI mounting solution on a coverslip.
13. Place the coverslip over the microscope slide.
14. Seal the edges of the coverslip with nail polish.
15. Store in 4°C fridge.