

The Effects of Nicotine on Histone Modifications in *Caenorhabditis Elegans*

By

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June 2017

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Early development stages have been known to be very susceptible to certain types of stress. Tobacco smoke is one of these major stresses that will affect them during this stage and can increase how likely they are to become smokers later. This is the problem that needs to be looked at as generations keep getting exposed to nicotine repeatedly potentially creating many epigenetic changes and creating a preference for nicotine over time. A lot of research has been done on how early nicotine exposure will affect you throughout the rest of your life and the transgenerational molecular mechanisms are still being researched today. In this study, we investigated the transgenerational effects of nicotine on H3K9 by testing the Spr-5 mutant and seeing it's chemotactic expressions compared to N2 (wild type) strain. The N2 strain expressed a preference for nicotine over multiple generations showing that nicotine preference or addiction can be passed down. When testing the Spr-5 strain which is missing an important histone in the germline dealing with epigenetic factors the preference is still passed down. This shows us that not all epigenetic activity is done in the germline and is done outside the germline as well. This

study is trying to provide how nicotine effects histones and how this will be passed down through multiple generations. From this data, *C. elegans* may be a useful organism in testing the epigenetic factors of nicotine over time and identifying the molecular pathways of how this happens.

The Effects of Nicotine on Histone Modifications in *Caenorhabditis Elegans*

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Master of Science in Molecular Biology and Biotechnology

by

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LIST OF SYMBOLS AND ABBREVIATIONS

<i>C. elegans</i>	Caenorhabditis Elegans
GLP	Good Laboratory Practice
EHMT2	Euchromatic histone-lysine N-methyltransferase 2
Setdb1	Set nuclear proto-oncogene domain bifurcated 1
H3K9me2	Dimethylation of histone 3 at lysine 9
H3K4me2	Di methylation of histone 3 at lysine 4
Spr-5	Probable lysine-specific histone demethylase 1
mRNA	Messenger RNA

Introduction:

Nicotine has been known to be a harmful drug for a long time and the physical side effects of it are still being discovered today. It's one of the most heavily used addictive drugs in the U.S. and the leading preventable cause of disease, disability, and death in the U.S. Cigarette smoking accounts for 90% of lung cancer cases in the U.S., and about 38,000 deaths per year can be attributed to secondhand smoke("Nicotine"). Even though smoking rates have declined over the years there are still other kinds of smoking such as hookah/waterpipe and vaping. People think this kind of smoking is better for them with no side effects but they are wrong. Smoking hookah or a single waterpipe smoking session has the equivalent of 1 or 50 cigarettes (Cobb, 2010). This in turn can lead to millions of people being exposed to nicotine when they think it's safe. If this trend keeps up then the types of diseases that are associated with it should arise as well. Some of these diseases associated with smoking are lung cancer, stroke, and bronchial disorders. Even smoking with a waterpipe during pregnancy has been known to cause birth defects. It can lead to low birth weights during pregnancy for women by them starting smoking during their first trimester. The babies on average weighed 100 grams less than that of the babies born to nonsmokers (Yamount, 1998).

Nicotine will one day be almost nonexistent due to all the research that is being done and my research tries to help understand more on how nicotine affects the histones and help create a better way to see how nicotine affects DNA. Research has already been done on how nicotine acts as a chromatin remodeling drug. It does this by inducing decreases in methyltransferases such as GLP, G9a, SETdb1, and levels of H3K9me2 (Kayla, 2013). Once nicotine was

administered to the subject and mRNA expression was tested a significant decrease in the levels of H3K9me2 was observed (Kayla, 2013). Looking at these results this makes me want to know how nicotine affects the methylation cycle and this is the main purpose of my research. Knowing how nicotine does this could help find out other answers and further the research in my lab by helping to find out more about microRNAs as well. Currently it is not known how nicotine affects the histone methylation on producing viable children in humans and if this affect can be passed down through multiple generations. Knowing this will help figure out if histone methylation will cause changes to embryos during childbirth allowing us to see what mechanism might cause certain birth defects or diseases.

C. elegans is a great model organism for studying the effects of nicotine on histones. *C. elegans* is fast and cheap as you would say but it can produce many generations over a short period and be easy to maintain with a food source called OP50. They also have many biological functions that are similar to that of humans which is why *C. elegans* have been used over the years. Their small size enables the researcher to be able to look at more than hundreds of animals on a 96 well plate (Nature Reviews Drug Discovery). Looking at that many subjects on a plate can give the researcher a lot of progeny to study. They are also good for transgenerational studies as well. With *C. elegans* being able to produce many generations in a short period of time this will shorten the time of the experiment and be less costly in the end. The life cycle of *C. elegans* is also very measurable. This is because *C. elegans* go through their life cycle in a timely manner going from one stage to the next and the rate of their life cycle can also be influenced. By changing the temperature at *C. elegans* develops it will change in how much time it takes to go from one stage to the next in the cycle. The transparency of the worm is also great for

studying *C. elegans* because the use of fluorescent markers to study biological processes in vivo is easier for this organism (Kayla L. Chase, 2013).

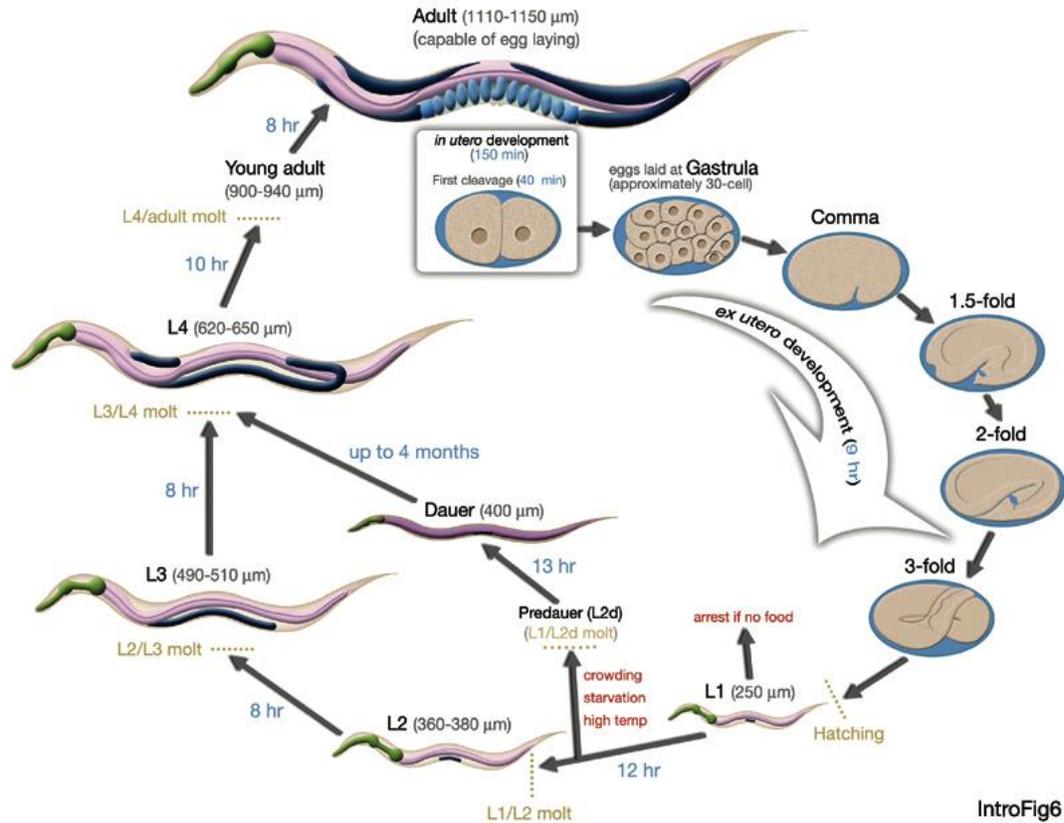


Figure 1. Life cycle of *C. elegans* at 22°C. 0 min is fertilization. Numbers in blue along the arrows indicate the length of time the animal spends at a certain stage. First cleavage occurs at about 40 min. post fertilization. Eggs are laid outside at about 150 min. post fertilization and during the gastrula stage. The length of the animal at each stage is marked next to the stage name in micrometers ("Caenorhabditis Elegans as a Genetic Organism." 2015).

Since *C. elegans* has a similar genome to that of humans it is a great organism to human disease genes and disease pathways (The *C. elegans* sequencing consortium, 1998). A more recent field of study that has been going on with *C. elegans* is epigenetics. This is what I'm interested in studying for my research. Epigenetics is mostly based on the experimental or environmental factors that are surrounding it. These experimental or environmental factors can turn the genes on or off and effect how cells read the genes instead of changing the DNA sequence. One

important epigenetic process is chromatin modification. The complex can be modified by substances such as acetyl groups (the process called acetylation), enzymes, and some forms of RNA such as microRNAs and small interfering RNAs (Bein Wheinhold, 2006). MicroRNAs are small RNA molecules, approximately 22 nucleotides long that can negatively control their target gene expression post transcriptionally (Chuang, 2007). MicroRNAs have been a relatively new discovery and play a role in how epigenetics works also. They are regulated by genetic and epigenetic mechanisms throughout the body but play an important role in the germline methylation of histones and proteins.

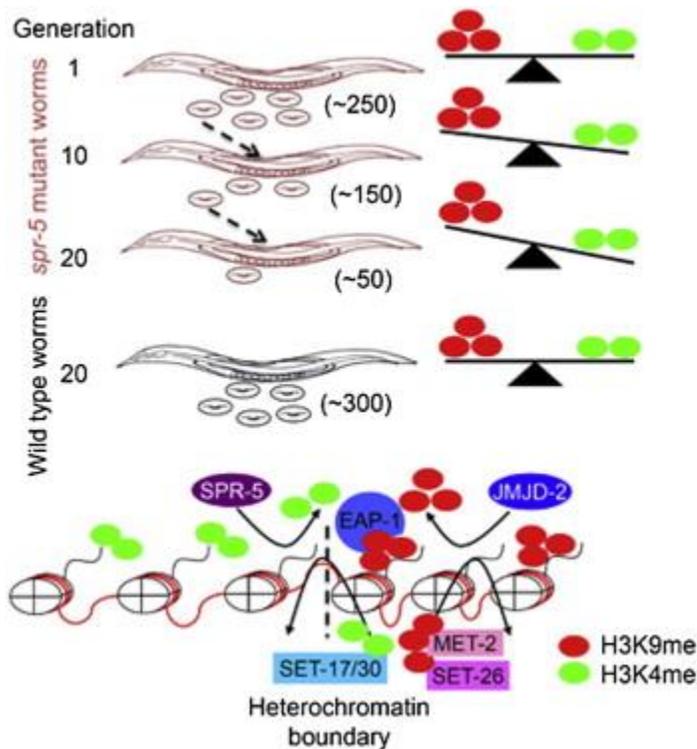


Figure 2. The Comparison of *spr-5* mutant worms to regular wild type worms. This figure represents how H3K9me and H3K4me effect chromatin remodeling and what happens when some of H3K4me is lost (Greer et al, 2014)

These effects of epigenetics can occur through multiple generations and vary on what factors are surrounding it. However, for epigenetics to be inherited through multiple

generations it must be initiated in the germline and stabilized in the germline as well (Kelly, 2014). There are multiple barriers to epigenetics lasting through multiple generations in sexually producing organisms. The aggressive epigenetic reprogramming mechanisms during gametogenesis and after gamete fusion happen in the germline which is where all heritable epigenetic alterations must pass through (Kelly, 2014). Chromatin structure is also important as it helps to regulate the transcriptional activity of the DNA to which is a component of regulation. Chromatin structure and its effects on genome regulation is also inheritable and this forms the basis of epigenetic regulation (Furuhashi, 2010). The zygote also plays a role in retaining epigenetic information but it's not known how this information is accessed in the zygote.

For my research, I want to know if nicotine affects the role of histone methylation through multiple generations. I hypothesize that nicotine affects generations through alterations by histone modifications. This would be able to tell us if the effects of nicotine can be passed down without the new generation smoking or using any type of nicotine. *C. elegans* has a histone methylation network consisting of H3K9me3 and H3K4me2 regulators of *spr-5* (Greer et al, 2014). These regulators are very important in the epigenetics of *C. elegans* as these controls what gets passed down from one generation to the next. When H3K4 methyltransferase is lost or knocked out the transgenerational sterility of *spr-5* mutants is suppressed (Greer et al, 2014). *Spr-5* is an important gene in regulating fertility in *C. elegans* and with *met-2* helps keep a normal life cycle of the worms. With the loss of *Spr-5* there will be a progressive loss of fertility and accumulation of histone H3K4 over generations (European Bioinformatics Institute, 2017). This gene is important in passing down the transgenerational epigenetic effects in the germline that leads to how nicotine is passed on and where these changes occur. This in turn

leads to the *egl-d* or *egl* phenotype mutant which refers to those mutants where an animal is defective in egg laying and retains its eggs (Herndon, L.A. et al. 2009). The germline is one of the most important parts of the epigenetic system when it comes to passing traits from one generation to another. Recent studies in *C. elegans* have shown that defective regulation of histone modifications, particularly histone methylation, correlates with heritable phenotypes (Kelly, 2014). Chemotaxis assays and *C. elegans* go great together as it's a great test to see if *C. elegans* is attracted to multiple substances across multiple generations.

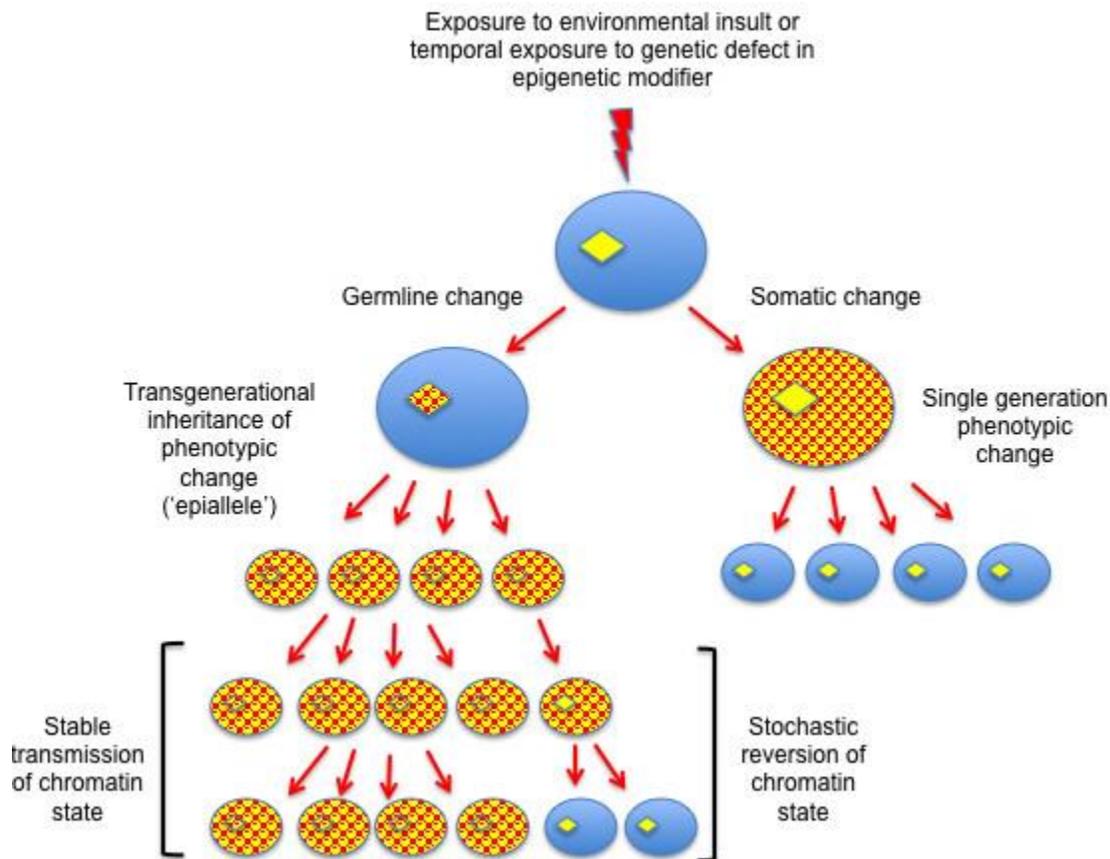


Figure 3. Transgenerational epigenetic memory. The soma (oval shapes) and germlines (diamonds) of a developing embryo are depicted. A transient environmental insult, or transient loss or ectopic activation of an epigenetic ‘modifier’ (lightning symbol) can create a change in the chromatin architecture in the genome (red pattern). If change in chromatin occurs in the developing soma (right side), it may be inherited though

cell division, but the change is (usually) limited to somatic lineages. If the chromatin is altered in the germline (left side), it may become stabilized, and a phenotypic alteration in the soma or germline may be observed in the offspring for multiple generations. The 'epiallele' often stochastically reverts to the original chromatin structure, resulting in a reversion to the original ancestral phenotype. (Kelly *Epigenetics & Chromatin* 2014 7:6 doi:10.1186/1756-8935-7-6)

Chemotaxis assays are used to test the sensory receptors of organisms and can help in determining what kind of chemical or substance the organism would be attracted to. This can help in determining the addiction of chemicals and if these chemicals cause the movement to change in the organism. *C. elegans* can detect hundreds of water-soluble and volatile molecules, which can evoke attraction, repulsion, feeding, egg-laying, mating, and developmental changes in the animal (Bargmann and Mori 1997). The *C. elegans* detects chemicals using a small number of chemosensory neurons whose morphology and synaptic connections are known through reconstruction of the entire nervous system from serial electron micrographs (White et al. 1986). These neurons are sensitive to multiple odorants and chemicals and depending on the concentration of it can be highly attractive to the worm. *C. elegans* contains eleven pairs of chemosensory neurons that help to detect a certain set of stimulants. These neurons use chemosensation to detect food and other sources of attraction. There has been lots of research done on *C. elegans* chemotaxis assays with nicotine exposure consisting from appetite suppression to response behaviors. Chemotaxis is a great way to test the transgenerational effects of nicotine because there is a wealth of data on nicotine and doing a chemotaxis assay can be done quickly and allow you to test many worms at once. Nicotine has been shown to be an appetite suppressant of *C. elegans* chemotaxis towards their regular food source which is *E. coli*. Nicotine has also been shown to be a chemotactic rewarding substance to *C. elegans*. Once their sensory neurons have been stimulated by nicotine the worms prefer this over other substances

because it stimulates their receptors making them feel good (Sellings et. al 2013). By using many *C. elegans* during chemotaxis it helps get better data because of the larger selection group to choose from and there will be more to compare to. Chemotaxis has also shown that the worms increase locomotion speed as well when exposed to a nicotine substance (Seth Wescott, 2015). Chemotactic memory has been shown to happen in *C. elegans* by them memorizing the changing attractant levels of the substance (Khang Tran). This further provides evidence that nicotine is a great chemotactic tool to use when testing *C. elegans*. Chemotaxis assays can be done in many ways but I am mainly focusing of the regular way to do chemotaxis but will do counting every twenty minutes for an hour to see how *C. elegans* are attracted to the test and control substance over time.

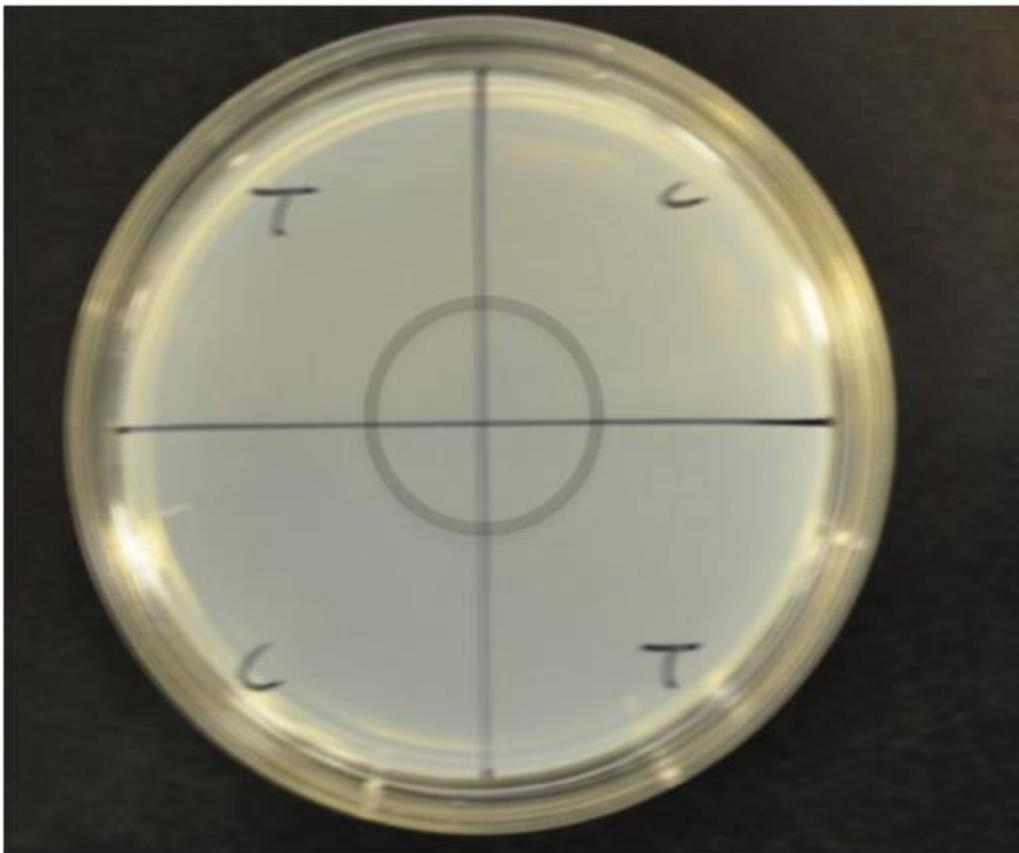


Figure 4. An example of a regular chemotaxis plate. This picture is what a standard chemotaxis plate will look like without the dots. You can see the origin is in the middle of the plate and is 0.5cms in diameter and each dot is 2 cm away for the middle of the plate (Smith, 2011)

Hypothesis and Objective:

The main question for my paper is “does nicotine affect histone methylation”? My hypothesis for this is that “nicotine impacts generations through alterations in histone modifications”. To test this hypothesis, I have three objectives.

- ▶ 1. Parent F0 nicotine exposure will alter the food preference of sensitized *C. elegans* strains.
- ▶ 2. Parent F0 nicotine exposure altered their phenotypic expression and these alterations will be inherited through generations.

From these three objectives, I designed my experiment to reflect how I can test the effects of nicotine across multiple generations using chemotaxis that will tell me how strong the effect is and what level of nicotine can cause the most change.

Experimental Design:

The wild type and *C. elegans* mutant are being tested and testing the histone H3K9 that works to produce a viable egg. Each mutant will be grown on regular NGM plates then synced so that every worm is in the same age. Then they will be grown on two test plates and one control consisting of five replicates. The test plates are low and high concentrations of nicotine; low concentration will be 1mM and the high concentration will be 1M. The nicotine is mixed in with the NGM plate mixture using the $C1V1=C2V2$ formula to make sure what is the correct volume of nicotine is included in mixture. The plates are seeded with 80ul of OP50 and then the synced worms are plated. The worms stay on the

test plates for 30 hours then washed off and put on regular NGM plates. Then the worms stay on each plate until the next morning checking frequently to see when they have reached young adult stage and chemotaxis starts. Chemotaxis plates were made fresh each week and are 6cm plates. Chemotaxis plates consist of the normal NGM regular plate mixture. Chemotaxis plates are made with a point of origin 0.5cm wide in the middle of the plate measured using a ruler and the test dots being placed 2cm from the origin in each quadrant. Regular food is on the control dots and a mixture of food with nicotine will be on the test dots. Start by using the control and low treatments first as the high lags in development. When starting chemotaxis, the worms are washed off their plates and put into the microcentrifuge tubes and is centrifuged down then the supernatant is pipetted and more M9 will be added this process is repeated two more times. After that 2ul of the test and control substance are pipetted onto the corresponding dots then 2ul of *C. elegans* and M9 are pipetted onto the middle of the plate. After the plate is done drying then the *C. elegans* are counted every twenty minutes to see how many have moved to each quadrant. The control is 2ul of OP50 on each dot then the test is 1ml of OP50 and 30ul of the corresponding nicotine concentration combined then 2ul of that dot is put on each test dot. five plates are made for each of the control and the test plates as well. The high treatment should be ready after the control and low are done. Wait two days and the worms are ready to sync again to test the second generation but these the worms will grow on regular NGM plates. For the second generation, the chemotaxis test is the same as the first and the third generation is the same also. While the chemotaxis experiment is going, the worms are counted every twenty minutes and seen how many have moved out of the circle into each quadrant and how far they have moved to each dot. How many that stay in the point of origin are counted also and compared with the others.

Results:

After doing Chemotaxis on the wild type *C. elegans* that had been grown on control, low, and high nicotine plates. Evidence was found that even the F2 generation of the worms still prefer the nicotine with food instead of nicotine without food. This is further exemplified when looking at the low and high dosed worms that have come from the test plates when observing how *C. elegans* move out of the point of origin over time to each quadrant. After twenty minutes, most of the worms are in the point of origin of the plate while the control contains some but the test quadrant usually have more. After counting the worms during the forty-minute mark the even more worms have moved out of the origin and into each quadrant. The worms that were already in each quadrant have begun to move closer to each dot and some have reached the dried test or control substance. The test quadrant still has more than the control quadrant by a good amount. After reaching the sixty-minute mark and counting then the experiment is over and can see the final number of worms in each quadrant. Almost all the worms have moved out of the origin and into each quadrant now. The number of worms that have moved into the test quadrant almost double the amount sometimes that is in the control quadrant. In the test quadrant, a lot of worms have made it into the test substance but a good amount remains outside the substance with some that are in the quadrant but are close to the origin. These worms are still attracted to the substance but not as strongly as the worms that have moved closer to the substance or are inside of it. When watching the treatment worms which have been pre-treated with nicotine before the assay move around on the plate they show a normal movement into each quadrant. At first, they were slow to move out of each quadrant but after twenty minutes is when the worms start to move at a faster pace towards the nicotine. This shows that the worms seem to remember the nicotine from when they were pre-treated and hurry over to get a taste of nicotine again. How

they moved towards the nicotine confirms that the control quadrant is the same way as most of have moved closer to the substance or are inside of it. These results show that the N2 test worms do prefer nicotine with food instead of just regular food. While measuring their reaction over time shows how long it takes for them to smell the substance and decide to what they prefer it helps us tell the amount of attraction that the worms have toward the substance. This pattern continues through all the generations for each showing that nicotine addiction can be passed down through multiple generations. The control plates for each test group gave results that were expected for each dose. Since the control worms were not grown on the high or low dose nicotine plates they were attracted to the regular food instead of the nicotine with food. Watching the worms move out of the origin towards the control quadrates they showed normal movement towards the dot and seem to be in no hurry to get to the regular OP50 E. Coli food. It seems that these worms have no extra movement problems and show normal behavior towards the normal food. When looking at how the control worms move over time compared to the low and high dose worms we see that the opposite has occurred. The control treatment worms have not been pre-treated with nicotine at a younger stage and have grown up on regular NGM plates. When starting at the twenty-minute mark most of the worms are still inside the origin but now more worms are in the control quadrant than the test quadrant. After forty minutes, more worms have moved out and still there are more in the control quadrant than the test quadrant and some have reached the substance in each. When reaching the sixty-minute mark almost all the worms have moved into each quadrant and the control quadrant still has more than the test. The number of worms in the control compared to the test quadrant is the almost same as the high and low dosed worms but the control quadrant has a lot more than the test has. This shows that since the control worms were not exposed to nicotine at a young age then they prefer to just eat the regular food.

When testing the mutant Spr-5 worms with chemotaxis we get the same result as the N2 worms. Across the multiple generations, the high and low dose nicotine worms prefer the nicotine with food while the control worms of each group still prefer the regular food. Looking at how the worms move over time they behave in the same way as the N2 worms. The low and high dose worms move across the plate during each twenty-minute mark the same way as the N2 did. Starting at twenty minutes most of the worms were in the origin but some have moved to each quadrant with the test quadrant having the most worms at the start. When the forty-minute mark had been reached, and counted more worms have moved out of the center and gone into each quadrant. The test quadrant still has more worms than the control and some have reached the test substance and some are getting closer. When the sixty-minute mark had been reached almost all of the worms have made it into each quadrant and the test quadrant still contains the most worms and a good bit has reached the substance. There are more located in the quadrant but not as close showing their rate of attraction. The control worms of Spr-5 are the same as the N2 worms as they prefer to eat the regular food instead of the nicotine with food. Their behavior over time is the same as N2 as well. This helps to confirm that nicotine does play a role in histone methylation and how the histones modify the chromatin across the generations. Since the mutant Spr-5 worm has lost some germline activity but the nicotine addiction is still passed down through multiple generations it means that the germline isn't the only way in how it is passed down based on histone methylation changes in the germline.

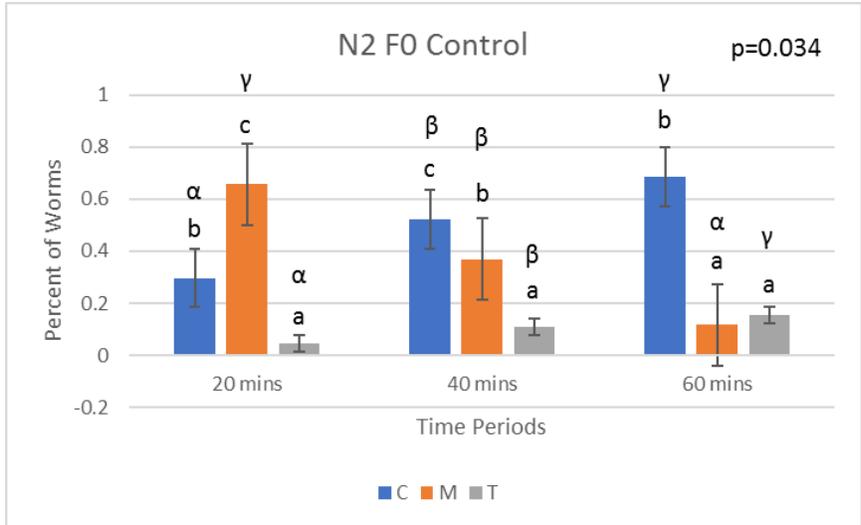


Figure 5. Wild type first generation control worms N2 is the wild type strain and F0 is the first generation. This graph shows the difference in control worm attraction over 60 minutes measuring at 20 min intervals.

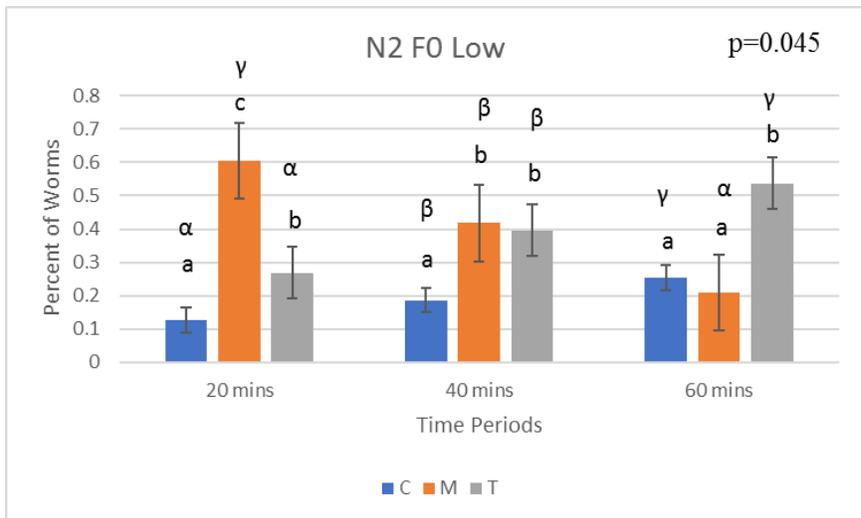


Figure 6. Wild type first generation low worms. This is the low dose worms from the F0 generation showing their attraction over 60 minutes measuring at 20 min intervals.

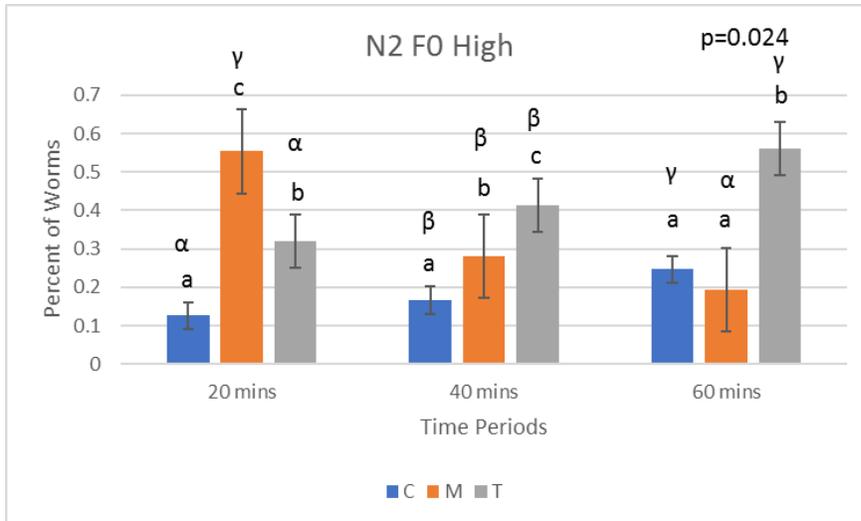


Figure 7. Wild type first generation high worms. This is the high dose worms from the F0 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

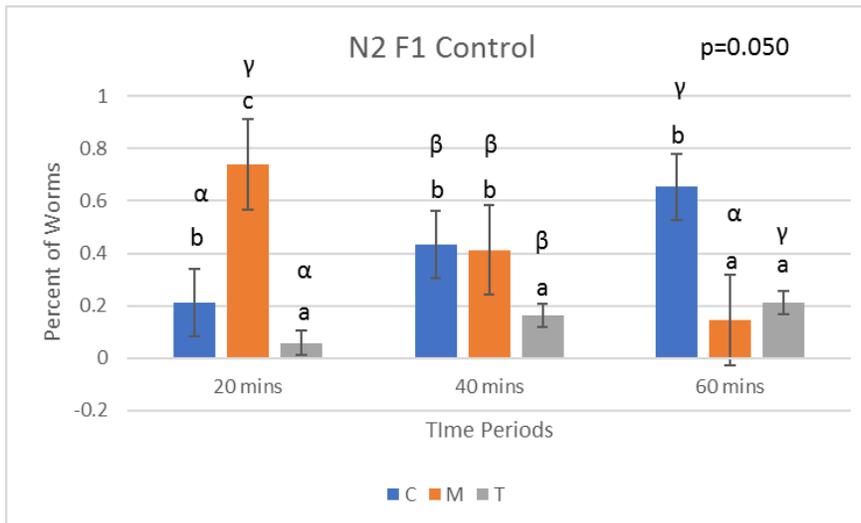


Figure 8. Wild type second generation control worms. This is the control worms from the F1 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

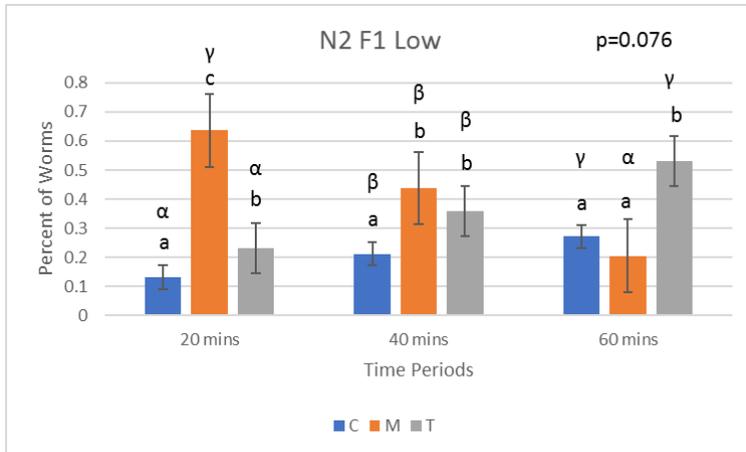


Figure 9. Wild type second generation low worms. This is the low dose worms from the F1 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

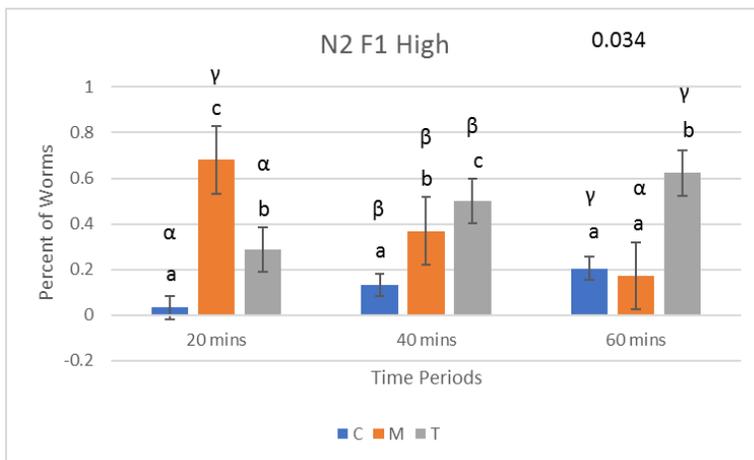


Figure 10. Wild type second generation high worms This is the high dose worms from the F1 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

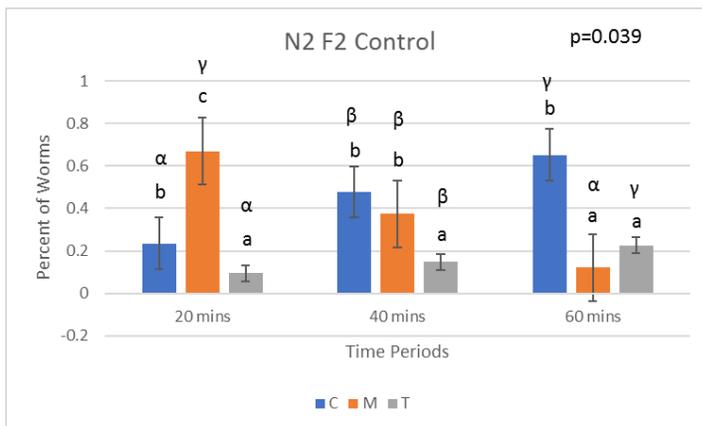


Figure 11. Wild type third generation control worms This is the control worms from the F2 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

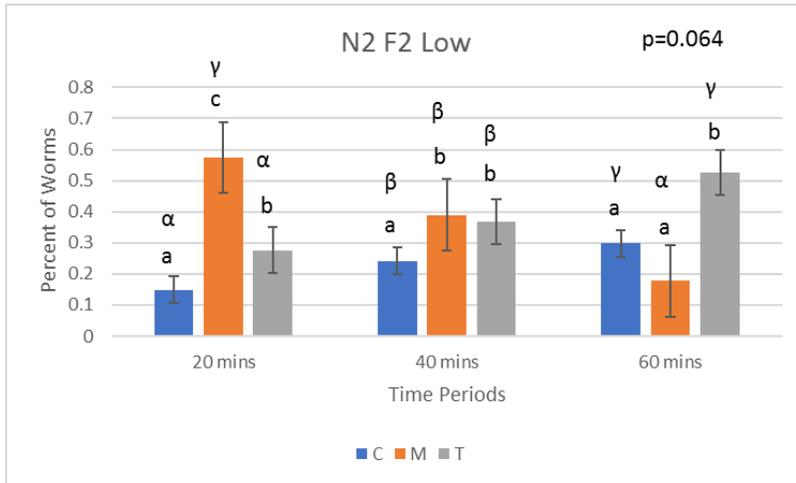


Figure 12. Wild type third generation low worms This is the high dose worms from the F2 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

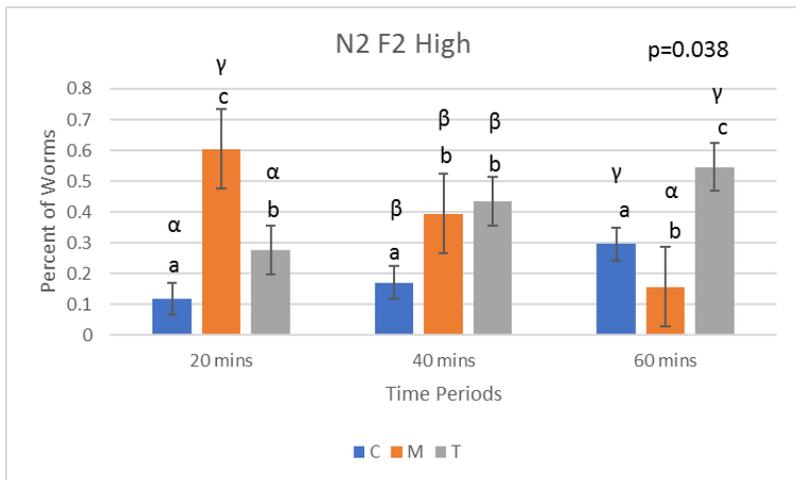


Figure 13. Wild type third generation high worms This is the high dose worms from the F2 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

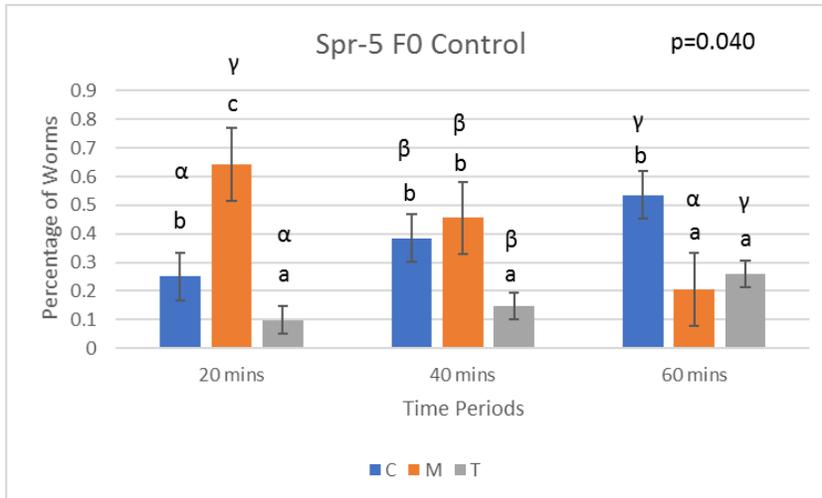


Figure 14. Mutant strain first generation control worms. Spr-5 is the mutant strain and F0 is the first generation. This is the control worms showing their attraction over 60 minutes measuring at 20 min intervals.

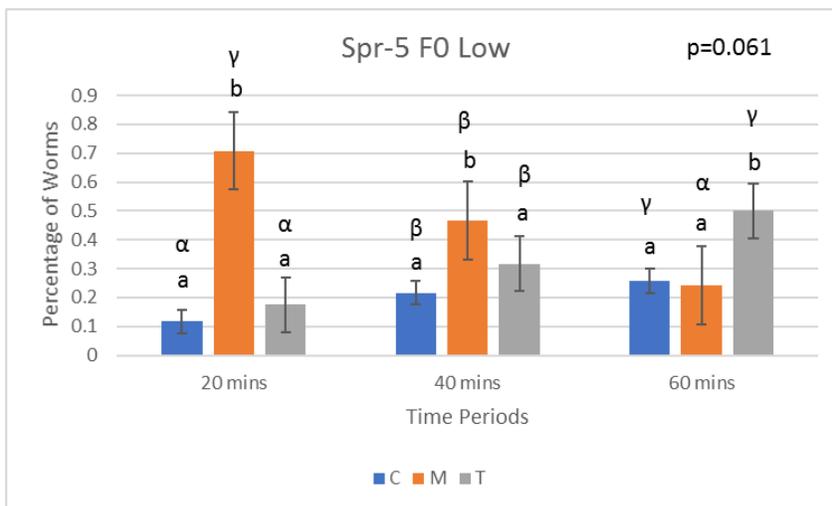


Figure 15. Mutant strain first generation low worms. This is the low dose worms from the F0 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

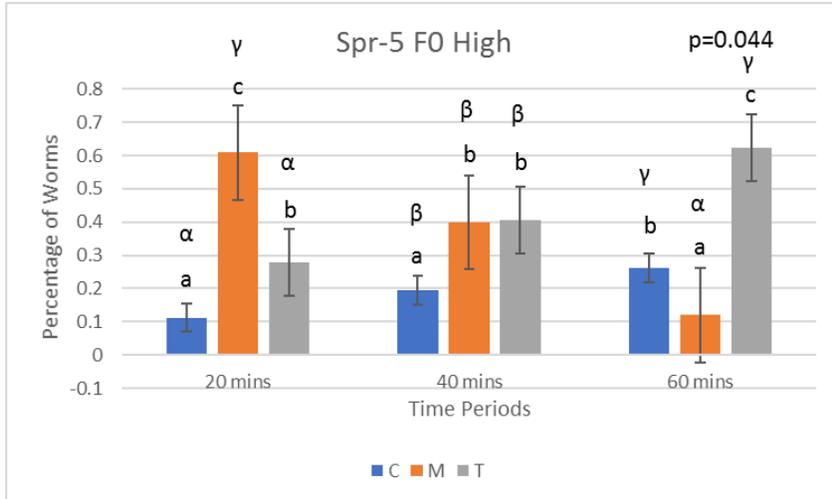


Figure 16. Mutant strain first generation high worms. This is the high dose worms from the F0 generation. Showing their attraction over 60 minutes measuring at 20 min intervals

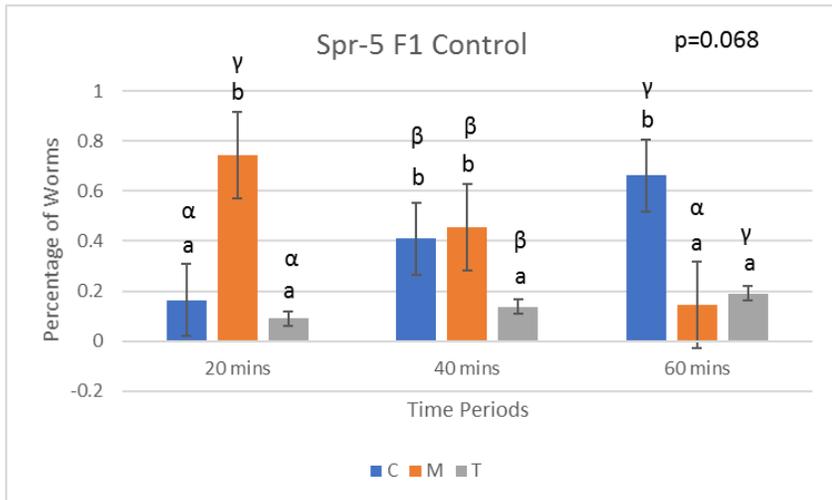


Figure 17. Mutant strain second generation control worms. This is the control worms from the F1 generation. Showing their attraction over 60 minutes measuring at 20 min intervals

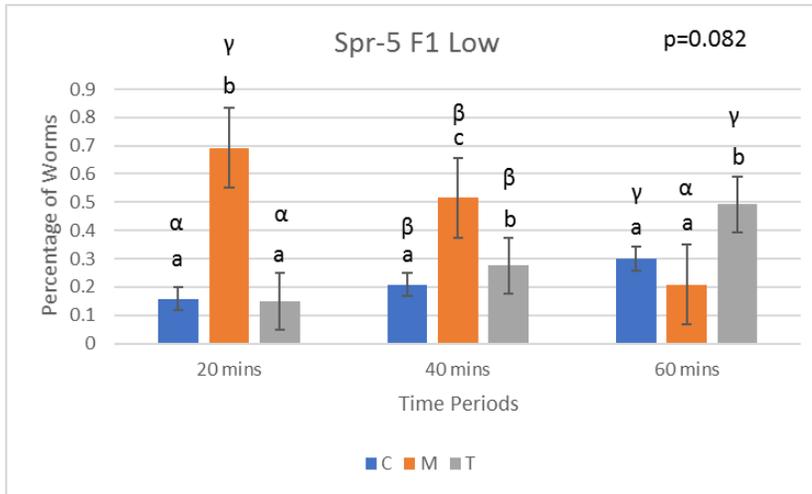


Figure 18. Mutant strain second generation low worms. This is the low dose worms from the F1 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

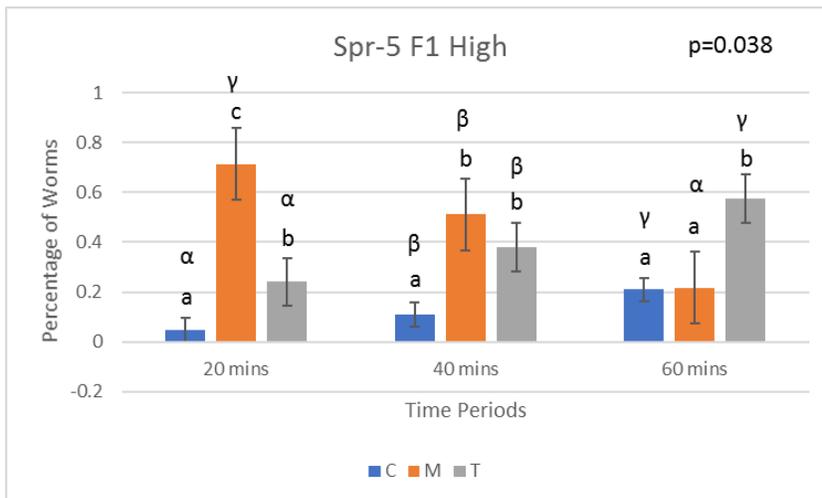


Figure 19. Mutant strain second generation High worms. This is the high dose worms from the F1 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

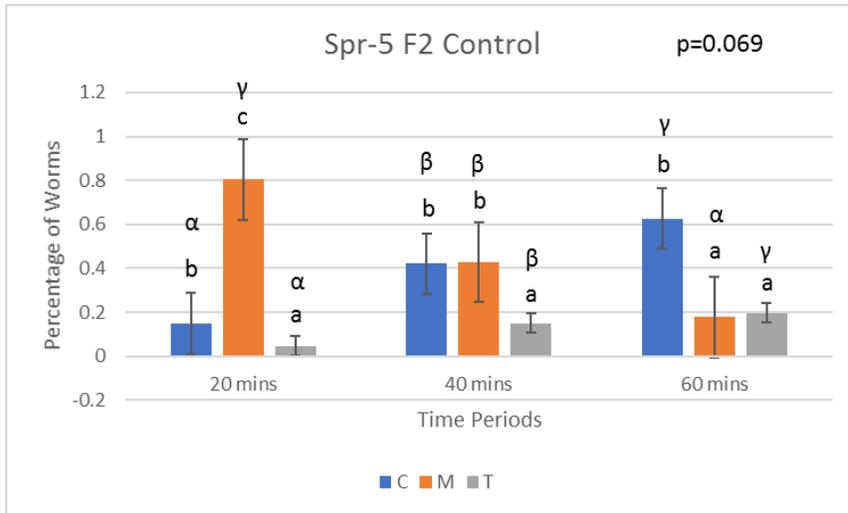


Figure 20. Mutant strain third generation control worms. This is the control worms from the F2 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

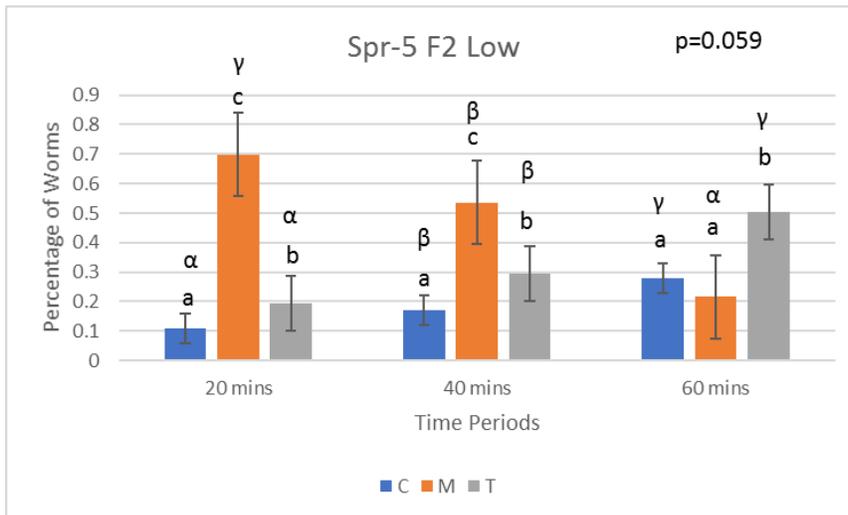


Figure 21. Mutant strain third generation low worms. This is the low dose worms from the F2 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

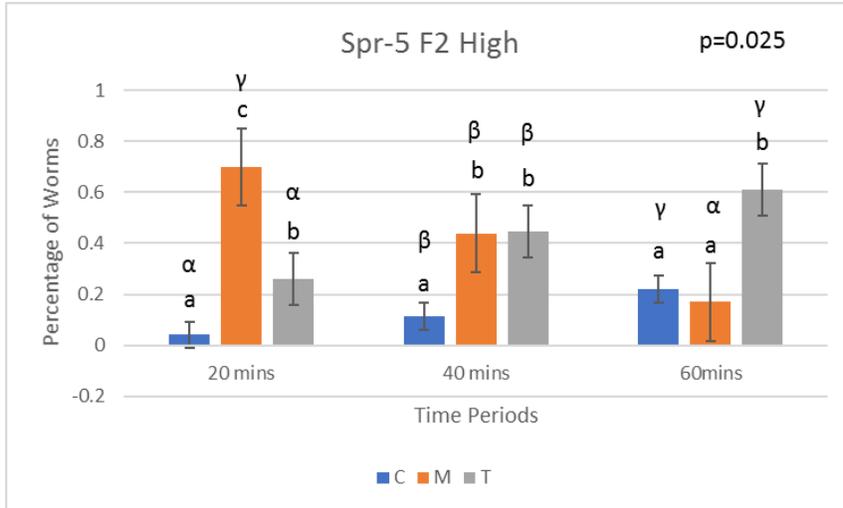


Figure 22. Mutant strain third generation high worms. This is the high dose worms from the F2 generation. Showing their attraction over 60 minutes measuring at 20 min intervals.

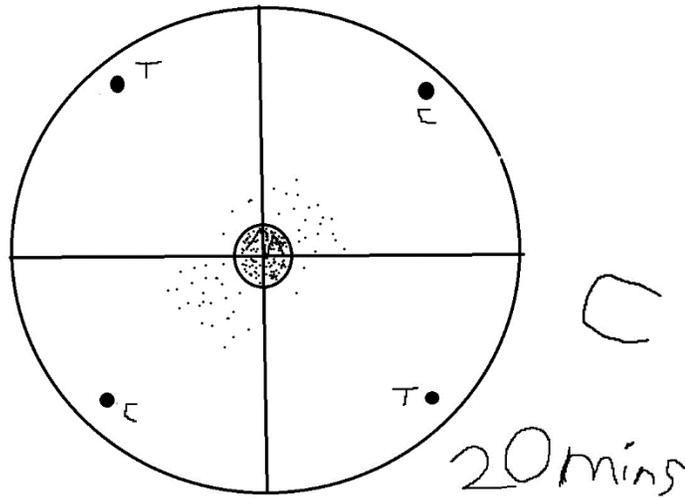


Figure 23. Control worms at 20 mins. This is an example of control chemotaxis plate movement at 20 mins.

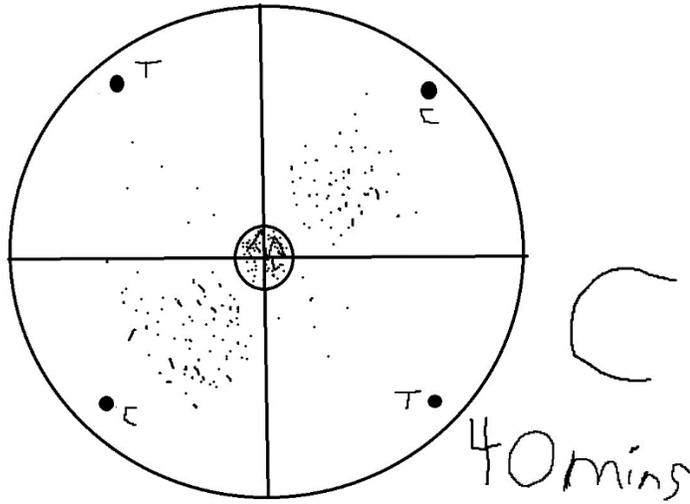


Figure 24. Control worms at 40 mins. This is an example of control chemotaxis plate movement at 40 mins.

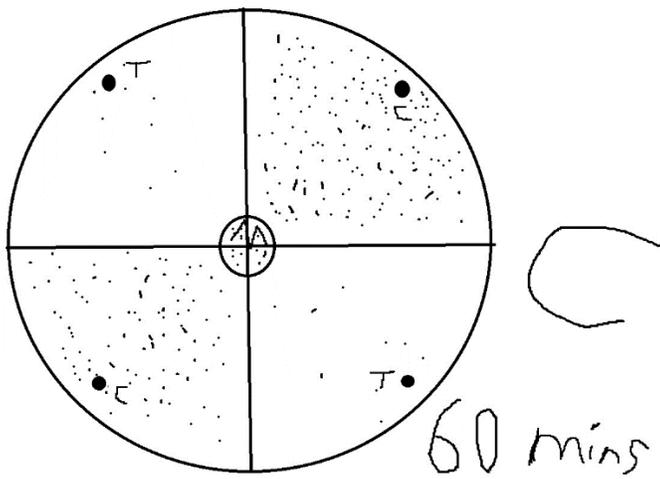


Figure 25. Control worms at 60 mins. This is an example of control chemotaxis plate movement at 60 mins.

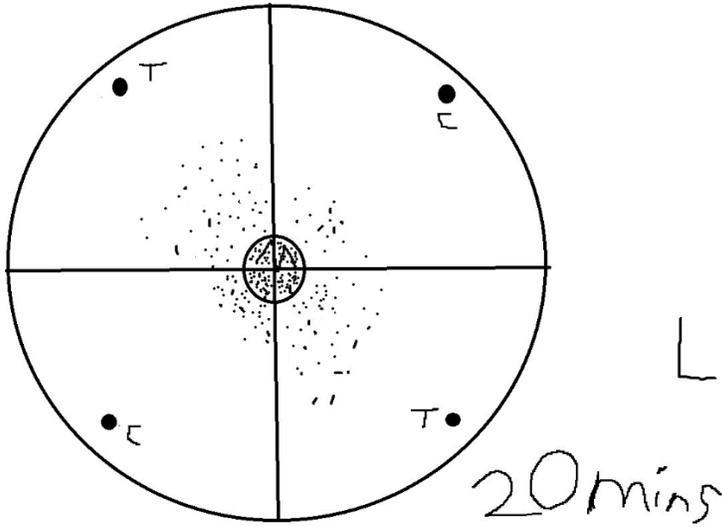


Figure 26. Low worms at 20 mins. This is an example of low dose chemotaxis plate movement at 20 mins.

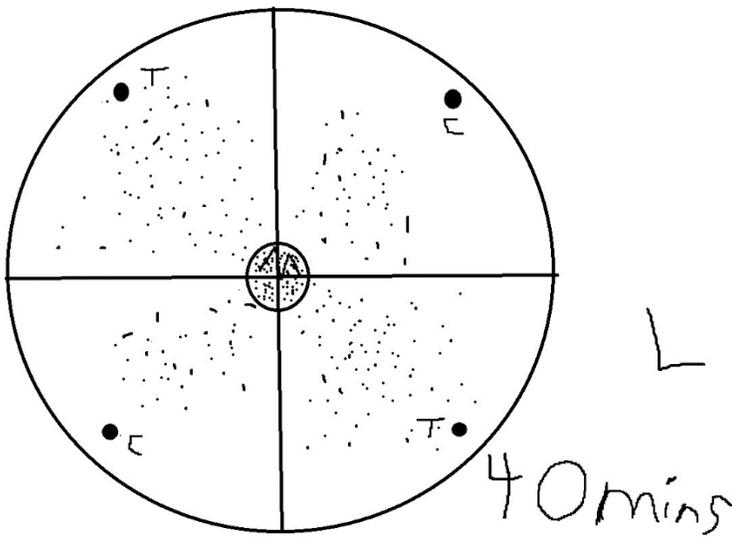


Figure 27. Low worms at 40 mins. This is an example of low dose chemotaxis plate movement at 40 mins.

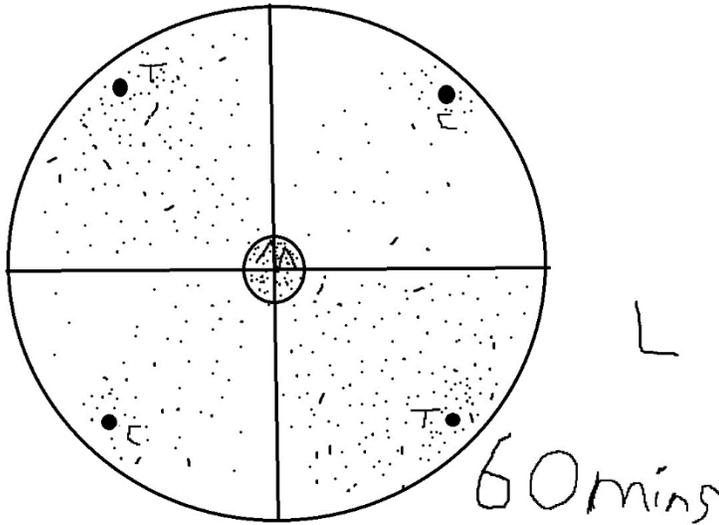


Figure 28. Low worms at 60 mins. This is an example of low dose chemotaxis plate movement at 60 mins.

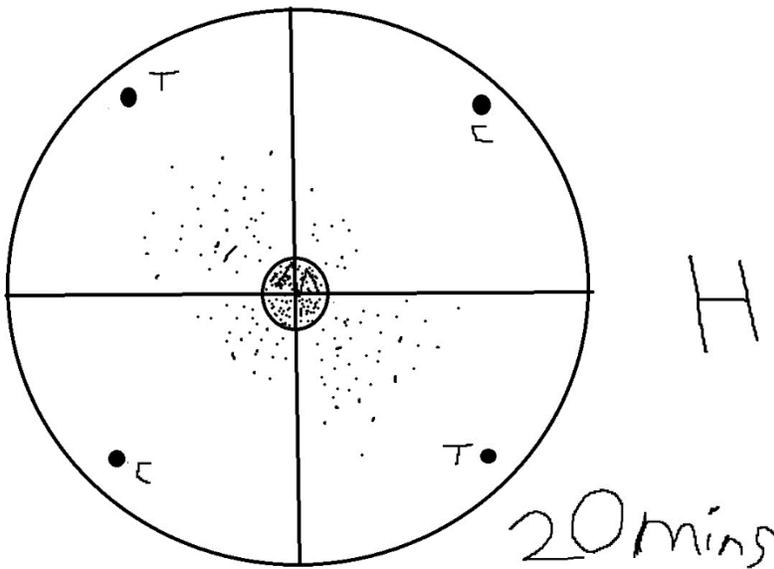


Figure 29. High worms at 20 mins. This is an example of high dose chemotaxis plate movement at 20 mins.

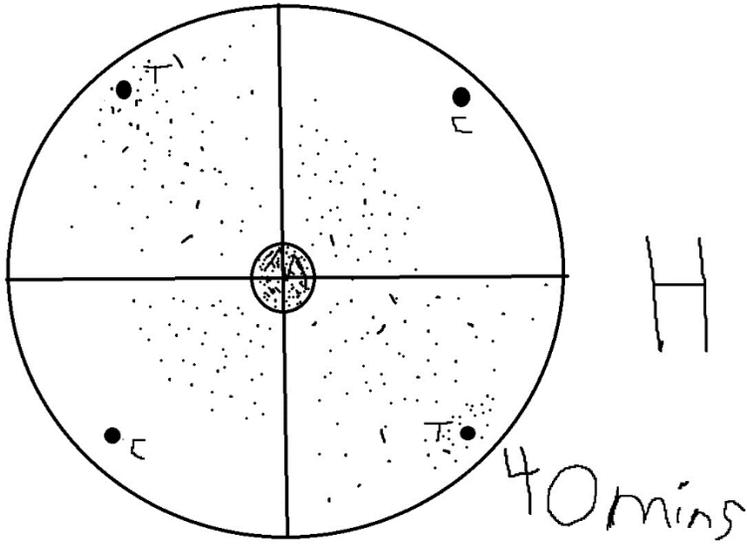


Figure 30. High worms at 40 mins. This is an example of high dose chemotaxis plate movement at 40 mins.

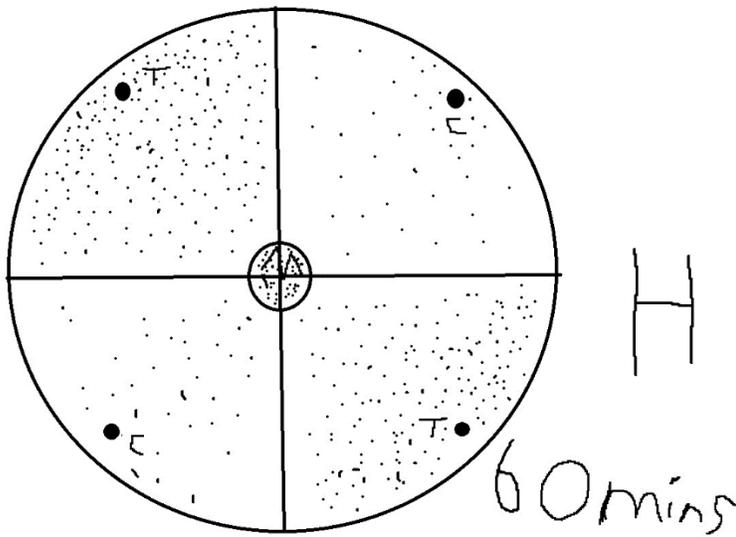


Figure 31. High worms at 60 mins. This is an example of high dose chemotaxis plate movement at 60 mins

Discussion:

My experiment shows that *C. elegans* can be addicted to nicotine and this addiction can be passed down through multiple generations. If the worm is exposed at an early stage then there is a high chance that it will become addicted and if given the choice later in its lifecycle it prefers nicotine. These results showed what I thought would happen as nicotine is a highly addictive substance and it's also very hard to get rid of this addiction. Humans try to quit it but usually end up going right back to it due to the drawback symptoms and the intense cravings. Therefore, nicotine is so addictive, because it makes your brain think that you need nicotine and without it your brain will try and find ways to have it again. *C. elegans* exhibits the same behavior during the chemotaxis experiment. After they were exposed at a young age they couldn't have nicotine again until the chemotaxis and most of the worms choose to have nicotine again. This might be because they are having withdraws and craving the smell or taste again. When looking at how fast some of the worms moved to each test quadrant shows just how much they wanted to have the nicotine and by the end of the test some of them were in the dot themselves getting the most exposed to it. Even the low dose worms were shown to be highly addicted to it as well. When compared to the high dose worms the results come out to be similar in how many end up in each quadrant and how fast they are attracted to the substance. There are still some worms who preferred just the regular food. Maybe these worms were more resilient to the effects of nicotine when they were younger or just choose to have normal food. When looking at the control results you will see that when they are not exposed when they are younger leads to them mostly just wanting the regular food but some do choose to go the nicotine with food. This could be because it is a new substance to them and they want to see what it is and the smell of nicotine can also make them want to try it as well. In both the control and test groups there are some worms who

decide to remain in the center of the plate also. There could be several facts for this such as not all food was washed off them during the washing period or neither substance seems to interest them right then.

When compared with other research that has done work with nicotine it has been shown as a reward substance for *C. elegans*. This would help prove the point that even after being exposed to it they still want the nicotine because it feels good to them and makes them feel happy after getting it. So when the worms are preexposed to nicotine they already know that is a rewarding substance and their sensory neurons remember what nicotine is like and when it senses nicotine again it remembers how good it was. This also helps with the control worms results as well and why all of them don't go toward regular food. Some of the worm's senses have taken a liking to nicotine without even having it before and start to develop an addiction for it. Other research tells why *C. elegans* prefers nicotine is because of the serotonin signaling pathway in the brain giving them this reward system for why they should prefer nicotine. This is one limitation in my research as I cannot see what is happening after they are exposed to nicotine and then after the chemotaxis experiment has occurred. This would help with the control worms as well as I would be able to see the before and after signaling of the serotonin pathway. This is important as humans almost have the same genome makeup and signaling pathway as *C. elegans*. Therefore, by studying this we can see just how humans can get addicted to nicotine and maybe prevent and stop addiction as well. We can also see that this addiction can be passed down through multiple generations and that those generations are more likely to get addicted if they were to come in contact to nicotine. For future experiments, I would like to dive more into the pathway of how this preference can be passed down and see what factors play a role in it. A great future experiment would be to what has happened to the pathway of the second and third

generations and see how it differs from the first. Another one would be see what has happened after those later generations have been exposed to nicotine and what if any changes have occurred. There are more experiments being done on this every day hopefully leading to a future where we can completely understand nicotine how to stop its affects.

Conclusions:

Nicotine turned out to be a very chemotactic substance as it showed the preference or addiction of nicotine to pretreated worms and that this can be passed down through generations. The control worms which were not pretreated did not appear to like the nicotine with food but prefer the regular OP50 E. Coli strain. It also showed that germline histone modifications are not the only way in how nicotine preference is passed down. The results also show that the effects of nicotine can be passed down to even the third generation of offspring. So, the effects of our grandparents smoking when they were young could have affected us and how are genes are behaving today. If we are easily swayed to try nicotine based on the smell of it or once we try it we are more easily addicted to the chemical is another possibility that our grandparents smoking is having on us. In future experiments, I would like to see what changes have occurred in the histone methylation and chromatin remodeling pathways and where else this happens besides the germline which has been a primary focus of how these changes are passed down. Nicotine is still all around us every day and we must be careful with our future generations and how it will affect them in the future.

References

"Nicotine." *Psychology Today*. Web. 6 Nov. 2015.

Greer, Eric L., Sara E. Beese-Sims, Emily Brookes, Ruggero Spadafora, Yun Zhu, Scott B. Rothbart, David Aristizábal-Corrales, Shuzhen Chen, Aimee I. Badeaux, Qiuye Jin, Wei Wang, Brian D. Strahl, Monica P. Colaiácovo, and Yang Shi. "A Histone Methylation Network Regulates Transgenerational Epigenetic Memory in *C. elegans*." *Cell Reports* 7.1: 113-26. 10 April 2015 Print.

Spr-5 (gene) - WormBase : Nematode Information Resource." *Spr-5 (gene) - WormBase : Nematode Information Resource*. N.p., n.d. Web. 02 Dec. 2015.

Caroline Cobb et al. *Am J Health Behav*. 2010 May-Jun; 34(3): 275–285.

Nuwayhid, I, Yamout, B., Ghassan, and Kambria, M. [Narghile \(Hubble-Bubble\) Smoking, Low Birth Weight and Other Pregnancy Outcomes](#) . *American Journal of Epidemiology* 1998;148:375–83 [accessed 2015 Sep 14].

Kayla A. Chase, Rajiv P. Sharma. DOI: <http://dx.doi.org/10.1017/S1461145712001101> 1129-1138 First published online: 1 June 2013

Kaletta, Titus, and Michael O. Hengartner. "Finding Function in Novel Targets: *C. Elegans* as a Model Organism." *Nature Reviews Drug Discovery Nat Rev Drug Discov* (2006): 387-99. Print.

"*Caenorhabditis Elegans* as a Genetic Organism." Web. 5 Nov. 2015.

The *C. elegans* Sequencing Consortium. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282, 2012–2018 (1998).

Bob Weinhold "Epigenetics: the science of change" *Environ Health Perspect*. 2006 Mar; 114(3): A160–A167.

Levine, A., Y. Huang, B. Drisaldi, E. A. Griffin, D. D. Pollak, S. Xu, D. Yin, C. Schaffran, D. B. Kandel, and E. R. Kandel. "Molecular Mechanism for a Gateway Drug: Epigenetic Changes Initiated by Nicotine Prime Gene Expression by Cocaine." *Science Translational Medicine* 3.107 (2011): 107-09. Print.

Herndon, L.A., Lints, R. and Hall, D.H. 2009. Glossary E. In *WormAtlas*. doi:[10.3908/wormatlas.6.5](https://doi.org/10.3908/wormatlas.6.5)

Kelly, William G. "Transgenerational Epigenetics in the Germline Cycle of *Caenorhabditis Elegans*." *Epigenetics & Chromatin* 2014 7.6: 6. Print.

Bargmann, C.I., and Mori, I. (1997). Chemotaxis and thermotaxis. In *C. elegans II*, T.B.D.L. Riddle, B.J. Meyer, and J.R. Priess, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 717–737.

Furuhashi, Hirofumi, Teruaki Takasaki, Andreas Rechtsteiner, Tengguo Li, Hiroshi Kimura, Paula M. Checchi, Susan Strome, and William G. Kelly. "Trans-generational epigenetic regulation of *C. elegans* primordial germ cells." *Epigenetics & Chromatin*. BioMed Central, 12 Aug. 2010. Web. 05 May 2017.

Smith, Allister. "Part:BBa_K631032." *Part:BBa K631032 - parts.igem.org*. IGEM11, 15 Sept. 2011. Web. 12 May 2017

Chuang, Jody C., and Peter A. Jones. "Epigenetics and MicroRNAs." *Pediatric Research* 61.5 Part 2 (2007): n. pag. Web.

Liu, Xiaolan, Xiaoyan Chen, Xinfang Yu, Yongguang Tao, Ann M. Bode, Zigang Dong, and Ya Cao. "Regulation of microRNAs by epigenetics and their interplay involved in cancer." *Journal of Experimental & Clinical Cancer Research* 32.1 (2013): 96. Web.

Sellings, Laurie, Schreiber Pereira, Cheng Qian, Thomas Dixon-Mcdougall, Christina Nowak, Bin Zhao, Rachel F. Tyndale, and Derek Van Der Kooy. "Nicotine-motivated behavior in *Caenorhabditis elegans* requires the nicotinic acetylcholine receptor subunitsacr-5andacr-15." *European Journal of Neuroscience* 37.5 (2013): 743-56. Web.

European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics. "Probable lysine-specific histone demethylase 1." *Spr-5 - Probable lysine-specific histone demethylase 1 - Caenorhabditis elegans - spr-5 gene & protein*. N.p., 10 May 2017. Web. 16 May 2017.

Tran, Khang, and Robert Grammer. *The Appetite Suppressing Effects of Nicotine on Caenorhabditis elegans Chemotaxis to E. coli*. N.p., n.d. Web.