

Determining the Genotoxicity of Certain Chemicals
Through the Somatic Mutation and Recombination
Test in *Drosophila melanogaster*

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Abstract:

Through the $w / w+$ somatic mutation and recombination test (SMART), I hope to determine the genotoxicity — the ability to produce mutations — of certain chemicals that are suspected to be carcinogenic. The SMART determines genotoxicity by exposing larvae to different concentrations of the chemicals being tested. The assay takes advantage of the opportunity to expose the imaginal discs of the larvae to the chemical, and in doing so, hopefully mutate at least one cell. The imaginal discs are groups of cells that reproduce through mitosis and eventually differentiate into the different parts of the fly's body. If even one cell is successfully mutated, its daughter cells will carry the same mutation, eventually growing into a region of mutated cells among normal cells.¹ The chemicals that I hope to test are potassium chromate (K_2CrO_4), a chemical proven to be genotoxic through the wing-spot test²; juglone (5-hydroxy-2 methyl-1,4 naphthoquinone), a natural chemical proven to be genotoxic in the eye-spot test³; acetaldehyde, a suspected mutagen with many uses, from manufacturing disinfectants and explosives, to flavoring foods such as desserts and soft drinks⁴; and ethidium bromide, a dye used in gel electrophoresis that is also an expected mutagen. Last term, I decided to test these chemicals using the 48-hour feeding method. This method requires separating three-day old larvae from the media they are in and putting them in vials containing chemical-treated media. This

¹ Graf *et al*, *An efficient tool for the detection of genotoxic activity of pure compounds or complex mixtures as well as for studies on antigenotoxicity*, African Newsletter on Occupational Health and Safety [Electronic source]

² Spano *et al*, *Recombinagenic activity of four compounds in the standard and high bioactivation crosses of *Drosophila melanogaster* in the wing spot test*, *Mutagenesis* 385.

³ Gaivao *et al*, *The $w/w+$ SMART assay of *Drosophila melanogaster* detects the genotoxic effects of reactive oxygen species inducing compounds*, *Mut. Res.* 139.

⁴ US Dept. of Health, *9th Report on Carcinogens* (2001), [Electronic Source].

proved to be very difficult, however, so I tried the chronic feeding method this term. For this method, I allowed the flies to mate for a few days in vials containing untreated media, then transferred them to vials containing chemical-treated media. Once the new flies emerge, I examined their eyes for the presence of white spots, which should occur if the chemicals are mutagenic. I performed the assay three times, but each time I failed to obtain any results. Next term, however, I plan on continuing my attempts to test potassium chromate, and hopefully also the other chemicals.

Introduction:

In the year 2001, 1,268,000 people in the United States were expected to be diagnosed with cancer, and 553,400 people were expected to die from cancer. It is the second leading cause of death in the United States, causing 25% of all deaths in the country.⁵ For this reason prevention and early detection of cancer is a common goal. It has been proven that many people are genetically predisposed to have a greater susceptibility for developing cancer, which makes prevention more difficult. Other factors, however, are also known to cause cancer, such as exposure to certain natural and artificial substances. Determining what these substances are is crucial to avoid exposure to them. For this reason, many tests have been developed to determine the mutagenicity of chemicals. The mutagenicity of chemicals is pertinent to cancer studies, because it has been shown through years of testing and experiments that

⁵ American Cancer Society, *Cancer Facts and Figures* (2001), 4-5.

compounds that are proven to be mutagens are usually also carcinogens, substances that lead to the development of cancer cells from normal cells.⁶

In the past, genetic studies that determined mutagenicity were focused primarily on screening for genetic alterations such as chromosome aberrations, gene mutations, and aneuploidy. Since recombinagenic activity is considered to be a way to measure indirectly general damage to DNA, newer tests have been developed that test for mitotic recombination and mitotic gene conversion. These tests also screen for mutations such as substitutions and frameshifts. The mutations caused by substitutions are known as transitions and transversions. A transition occurs when the wrong purine is paired with a pyrimidine, or when the wrong pyrimidine is paired with a purine. A transversion occurs if a purine was changed to a pyrimidine, or vice-versa, by the substitution. A frameshift is when a single base is inserted into or deleted from the DNA strand. This changes the order of the codons, which can change the function of the DNA.⁷ Presently many tests are available for determining the genotoxicity of chemicals in bacteria, yeast, *Drosophila*, and mammal cells, both in vivo and in vitro.⁸ A simple test that uses bacteria is the Ames test. Developed by microbiologist Bruce Ames, the Ames test is the original short-term test for detecting carcinogens. Ames and his colleagues developed several strains of *Salmonella typhimurium* with a mutation that prevents these cells from generating the amino acid histidine. These mutants, known as histidine-minus mutants, grow well in media containing

⁶Varmus *et al*, *Genes and the Biology of Cancer* (New York, 1993), 61.

⁷ Department of Biology and Biochemistry, *Ames Test: Testing Mutagens' Effects on a Biochemical Pathway*, University of Houston [Electronic Source]

⁸ Spano *et al*, Recombinagenic activity, 386

histidine, but not in media lacking histidine. In the Ames test, histidine-minus *S. typhimurium* is plated on media without histidine and the test chemical is added. If the chemical is mutagenic, it should back-mutate the *Salmonella*. In other words, the chemical should mutate the bacteria so that it regains the ability to produce histidine. If this happens, there will be colonies of back-mutated bacteria scattered across the plate. If the chemical does not cause any mutations, there should be little to no growth. The mutation that causes *S. typhimurium*'s inability to make histidine can be any of the three mentioned earlier, and the back mutation that revives this ability does not always completely revert the mutated material back to its original form.⁹ Not only does the Ames test assess mutagenic ability, but it also tests a substance's level of mutagenic activity. The number of back-mutated colonies present on the chemical-treated plates will reflect the chemical's mutagenicity.¹⁰ It is incapable, however, of directly determining the mutagenicity of chemicals that need to first be metabolized by the body, known as promutagens and procarcinogens. To test these chemicals with the Ames test, a mixture of liver enzymes must be added to the bacteria.¹¹ A more accurate assay is the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. Several factors make the assay in *Drosophila* advantageous over other tests. Fruit flies are eukaryotic organisms that are capable of enzymatically activating promutagens and procarcinogens. Culture media for *Drosophila* is inexpensive and allows the

⁹ *Ibid.*

¹⁰ Prescott *et al*, *Cancer: The Misguided Cell* (Sunderland, 1986), 130-131.

¹¹ Varmus *et al*, *Genes*, 63-64

breeding of large numbers of flies.¹² Also, *Drosophila* has four pairs of chromosomes and approximately 14,000 genes, in comparison to the estimated 70,000 genes found in the human genome. *Drosophila*'s manageable genome size and the fact that its mutations are well-known are two more factors that make it a likely candidate for the assay.¹³

There are two versions of the somatic mutation and recombination test, one known as the wing-spot test, and another known as the eye-spot test. Both versions are based on the fact that during the larval stage of development in *Drosophila*, groups of cells known as imaginal discs that are found throughout the body proliferate mitotically and then differentiate into distinct structures of the adult fly's body, such as the eyes and wings. To perform the test, *Drosophila* larvae must be exposed to the test compound. This can be done chronically by adding the chemical to the flies' medium and allowing them to feed for 48 hours, or it can be done acutely by feeding the flies the test chemical for a period of 6 hours.¹⁴ If the test compound is mutagenic, it can alter the genotype of any one of the cells. That cell will then produce more altered cells through mitosis. This group of altered cells will be visible among the unaltered cells if the new genotype has a new, visible phenotype. These patches that exhibit a different phenotype are the result of the mother cell's loss of heterozygosity. This induced homozygous condition will be either dominant or recessive. A change to homozygous dominant will not provide a contrasting phenotype with heterozygous dominant, but a change to homozygous recessive will allow the

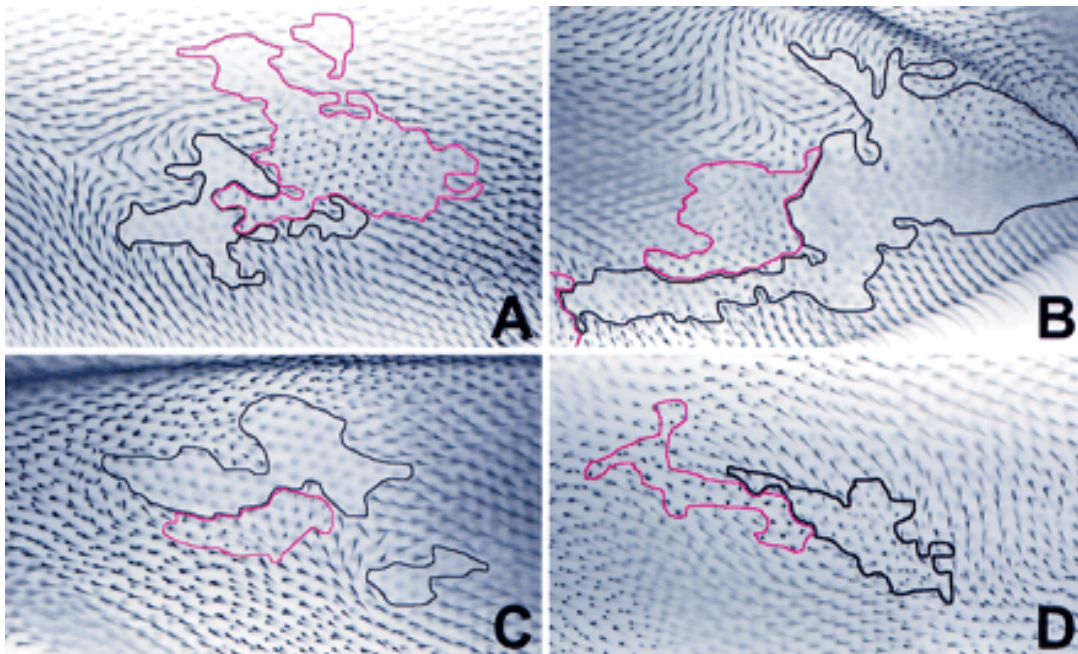
¹² Graf *et al*, An efficient tool

¹³ <http://www.ceolas.org/fly/intro.htm>

¹⁴ Graf *et al*, An efficient tool

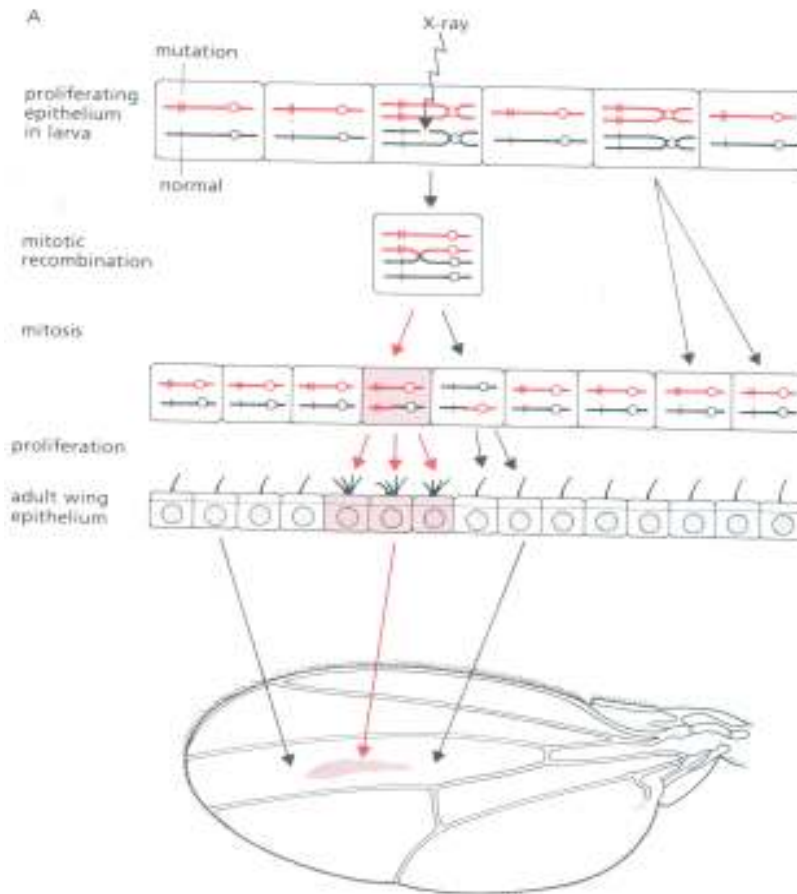
recessive phenotype to appear among the dominant phenotype present in surrounding cells. The mutant flies can then be detected in comparison to normal flies by spots consisting of altered cells in the wings or eyes.¹⁵

Figure 1: Twin spots occurring in wings of flies produced in cross between flies with *Vang* wing-hair genes and *frizzled* (*fz*) mutants.



¹⁵ *Ibid.*

Figure 2: Model of mitotic recombination leading to spots in wing.



The *mwh / flr* wing-spot version of the test uses flies with wing-hair mutations and focuses on two different crosses: the standard (ST) cross and a high bioactivation (HB) cross. The HB cross is more sensitive than the ST cross to many procarcinogens and promutagens, due to a high level of expressed cytochromes P450.¹⁶ Both crosses produce two types of progeny: marker-heterozygous and balancer-heterozygous. Both types of flies are checked for the presence of spots in their wings in which the wing hairs are different from the expected phenotype. In the marker-heterozygous flies, the spots can be the effect of either mitotic recombination or mutation. In the balancer-heterozygous

¹⁶ Spano *et al*, Recombinagenic activity, 386

flies, the spots can be due to mutation but not to recombination.¹⁷ This distinction is necessary to help determine how much of the genetic alterations to the fly's DNA is caused by classical mutations that may not be chemically induced. Examples of the spots that should result from the assay are present in Figure 1. Figure 2 demonstrates the principle of mitotic recombination that leads to the formation of the spots. Although recombination presented in the figure is brought about by irradiation rather than exposure to chemicals, the same sequence of events takes place in the cell during the wing-spot assay. The irradiation of the proliferating cells can lead to chromosome breaks that cause an exchange between the two chromatids as shown in the figure.¹⁸ The first two-thirds of Figure 3 provide another example of standard recombinagenic activity, along with an example of the kind of activity that causes twin spots. If there are two mutations arranged as shown in the figure, mitotic recombination can give rise to two sister clones, one from each mutant cell.

¹⁷ *Ibid*, 385.

¹⁸ Peter A. Lawrence, *The Making of a Fly: The Genetics of Animal Design* (Oxford, 1995), 82-83.

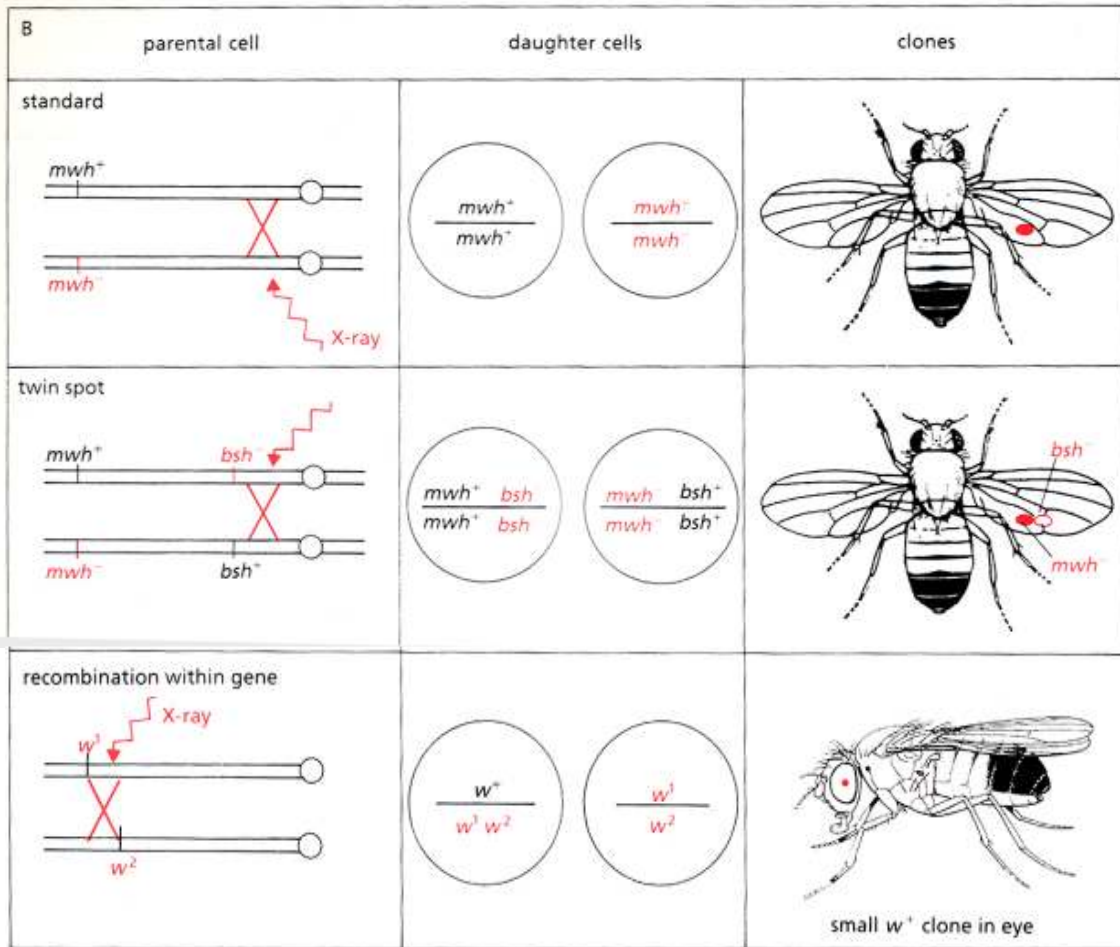


Figure 3: Diagram of the recombinagenic activity that is necessary for the SMART wing and eye assay.

In the w / w^+ eye-spot test, flies with mutations for eye-color are crossed. The last one-third of Figure 3 demonstrates what kind of recombinagenic activity leads to formation of the spots in the eyes. Mitotic recombination between two differently located mutations can regenerate the wild-type w^+ gene that makes pigment.¹⁹ This version of SMART is much simpler than the wing-spot version. However, it is not performed as often as the wing-spot test. As it is used more often, it will probably be developed further so that it is as accurate as the wing-spot test. This assay is useful because it is an effective in vivo test in a eukaryotic animal and a good way to screen potentially dangerous chemicals.

¹⁹*Ibid*, 83.

The results of these assays are likely to prove genotoxicity in most other eukaryotes.

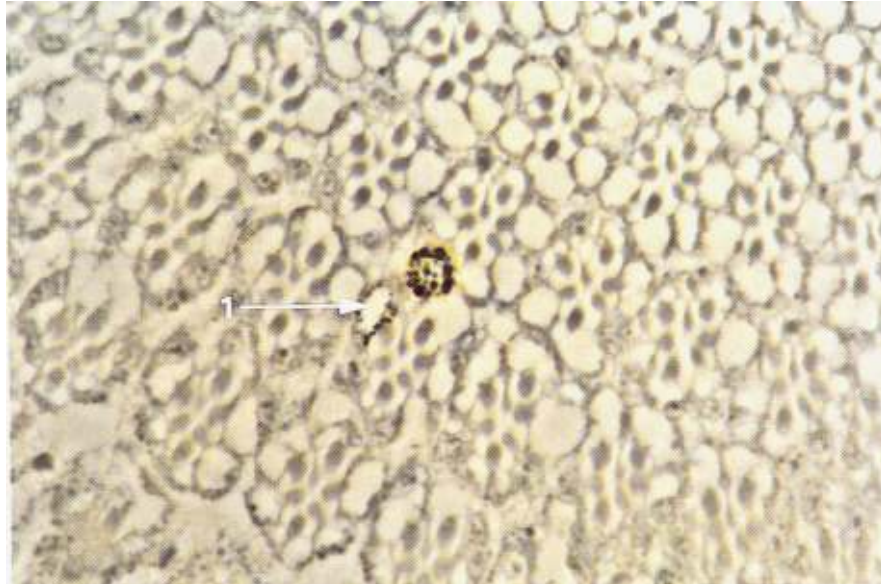


Figure 4: A *white+* clone in a section of the eye.

In this assay, I will cross white-eyed males (X^wY) and wild-type females ($X^{wt}X^{wt}$) and feed them with various chemicals. I will not be looking for recombination. Instead, I will be screening for a somatic point mutation (frameshift) that will revert the wild-type allele to a white-eye allele. Since the white-eye gene is recessive, the expected phenotype of the cross is wild-type:

	X^{wt}	X^{wt}
X^w	$X^{wt}X^w$	$X^{wt}X^w$
Y	$X^{wt}Y$	$X^{wt}Y$

If the chemicals that are fed to the flies are mutagenic, however, the flies should have white spots in their eyes. These spots are the result of a chemically-induced mutation of the wild-type allele that will allow the recessive trait to show among the dominant trait.

I tried to choose chemicals that either humans are exposed to regularly or come in contact with in the lab. I decided on the chemicals potassium chromate, juglone, ethidium bromide, and acetaldehyde.

- Potassium chromate (K_2CrO_4) is a yellow crystalline substance. In an aqueous solution, it is used in titrations with silver nitrate ($AgNO_3$). Together they form a red precipitate of Ag_2CrO_4 .²⁰ Potassium chromate is also used to make dyes, inks, pigments, and enamels.²¹
- Juglone (5-hydroxy-2 methyl-1,4 naphthoquinone) is a brown dye found in various consumer goods, such as hair dye and walnut oil stain. It is derived from walnut shells and is also an active ingredient found in dietary supplements.²²
- Ethidium bromide is used in the preparation of agarose gels for gel electrophoresis. It is a blood-red dye that binds in between the base-pairs of DNA. Although by itself it will not fluoresce when exposed to ultraviolet light, when it is bound to DNA, it fluoresces under ultraviolet light, emitting a red-orange light. This is used to detect the fragments of DNA that have traveled through the gel.²³
- Acetaldehyde is used primarily as a chemical intermediate in the production of acetic acid, pyridine and pyridine bases, peracetic acid, pentaerythritol, butylene glycol, and chloral. It is also used in the production of many household goods, such as disinfectants, drugs,

²⁰ <http://www.squ.edu.om/agr/OnlineCourses/SalinityTitration/Titration01.html>

²¹ <http://www.earthsystems.org/gopher/toxics/Potassium%20Chromate>

²² <http://ntp-server.niehs.nih.gov/htdocs/Chem-Background/ExSumPdf/Juglone.pdf>

²³ Jeremiah Hagler, *Biology 600 Lab Supplement* (Unpublished Data, 1999), 33.

perfumes, lacquers, and varnishes. Acetaldehyde is also an important component of food flavorings that are added to dairy products, candy, desserts, and soft drinks, among other things, and it is especially useful for providing artificial orange, apple, and butter flavors.²⁴

Method:

Preparing Media

To prepare fresh media, rinse vials with isopropyl alcohol and let dry overnight. This is meant to kill molds and bacteria present in the vial that would otherwise contaminate the media. Once the vials have been allowed to dry, add equal parts water and Formula 4-24[®] Instant Drosophila Medium to the vial. Immediately after adding the water, swirl the vial so that the media at the bottom can dissolve. Do this for a few seconds, and let the vial sit for a few minutes so that the media can become firm. Repeat this method for each vial. As soon as the media becomes firm, the vial will be ready for use.

If the media is old, it will probably be dry and hardened. To refresh the media, add a few drops of water to it. The media should now be ready for use.

Selecting Virgin Flies

Since females can store the sperm from a single insemination and use it for a large part of their reproduction, virgin females must be used in the cross. If not, there will be no way to insure that the progeny are all crosses between the wild-type and the white-eyed flies. The males, however, need not be virgin. To obtain virgin females, allow wild type flies to breed for 3-4 days, then remove the

²⁴ US Dept. of Health, *9th Report on Carcinogens* (2001), [Electronic Source].

adults from the vial and allow the larvae to pupate. When the flies emerge, anesthetize them and separate by sex before the flies are 12 hours old. Leave the females in their separate vial for six days. If larvae are visible in the medium, then the flies were not virgin. If the medium is free of larvae, however, the flies are virgins and can be used for the cross.²⁵

Crossing Flies & Chemical Feeding of the Larvae

The flies used in this assay are *Drosophila* wild-type females ($X^{wt}X^{wt}$) and white-eyed males (X^wY), as mentioned previously. For the cross, vials containing fresh media were used. There are two possible methods for feeding the larvae. In fall term, I attempted to use the second method but was unsuccessful. This term, I used the first method, which proved to be much easier.

Method 1 (Chronic Feeding): Anesthetize the flies with FlyNap[®] and place an equal amount of males and females in vials of fresh media. Since fresh media is usually sticky, keep the vials on their sides so that the flies will not get stuck. Place the vials in an incubator and leave the flies together for three days. Prepare the test media by mixing 0.5 oz of dry media with 0.5 oz (approximately 15 mL) of the chemical at the desired concentration. After three days, remove the parent flies from the first set of vials and place them in the vials containing chemicals. Allow the flies to lay eggs in these vials for the next three days. Remove the parent flies before new flies have a chance to hatch. Once new flies do emerge, count them and separate the females from the males and place the

²⁵ Raymond Flagg, Carolina *Drosophila* Manual (Burlington, 1988), 8.

females in fresh media. Doing this will ensure that any flies that emerge in the vials containing chemicals are the result of the first cross, and not the offspring of any of the flies that were produced in the first cross. 2-6 days after the flies are separated, check their eyes for any white spots. Record the number of spots present in the eyes and the number of flies with eye spots.²⁶

Method 2 (48-hour Feeding): Anesthetize the flies and place an equal amount of white-eyed males and wild-type females in a vial containing fresh media. Leave them together for twenty-four hours. This will give the flies the opportunity to regain consciousness, mate, and lay eggs. After twenty-four hours, remove the parent flies from the vials containing their eggs. Allow the eggs to hatch, which should occur the day after they are laid. When the larvae are three days old, prepare the test media by mixing 0.5 oz of dry media with 0.5 oz (approximately 15 mL) of the chemical at the desired concentration, then wash the larvae out of the vials using tap water and collect them in a metal strainer. Put an equal amount of 3-day old larvae in each test vial, and allow them to feed for the rest of their larval life (approximately 48 hours). Leave them to then pupate and hatch into adult flies.²⁷ Once they have hatched, count the flies and separate the male from the females. Examine their eyes for white spots 2-6 days after they are separated. Record the number of spots present in the eyes and the number of flies with eye spots.

²⁶ Gaivao *et al*, The *w/w+* SMART, 140

²⁷ Spano *et al*, Recombinagenic activity, 388

Discussion:

Last term, I attempted to use the 48-hour method for feeding the larvae. I allowed the flies to mate and lay eggs for twenty-four hours, then left the vials in an incubator for four days rather than three. Since I had left the flies to lay eggs for twenty-four hours, I left the larvae for four days so that the larvae that hatched last would have the chance to reach three days of age before being treated with the chemicals. It might have been better, however, for the flies to have been removed after three days. This way, all the flies would have been fed for at least 48 hours and at most for 72 hours. Ideally, egg-laying would have been allowed for only eight hours so that the age difference between the oldest and the youngest larvae would not be too great. However, because of the limited amount of time I had in the lab and the small amount of flies that I had in each vial, I left the flies together overnight. After four days I attempted to collect the larvae by washing the media into metal netting over filter paper. This method proved to be inefficient, however, as I failed to collect any larvae. The larvae may have been too small to be collected in the metal netting, or the flies may have been too old to lay eggs. Since almost all of the parent flies died after they were transferred to new vials, I think the latter may be true.

Ames Test

I did not have enough time to perform the assay last term because I spent much of the term trying to perform the Ames test with *Escherichia coli* in place of *Salmonella typhimurium*. In order to find which of the strains available in the lab would be best for the test, I plated the *E. coli* strains XL1-Blue and DH5 α . I used

LB plates and minimal media plates supplemented with thiamine, and I kept them in the incubator at 37°C for several days. Since I believed that the XL1-Blue and DH5 α were thiamine-negative (requires thiamine-supplemented food supply to survive) I expected both strains to grow on both the LB plate and on the minimal media plate with thiamine. Instead, DH5 α did grow on the minimal media while XL1-Blue did not. This test was meant to determine which strain had the thiamine-negative genotype. I planned on using this strain to test the ability of the chemicals to back mutate the strain into one that had a thiamine-positive genotype. To test the possibility that the cells might grow better in liquid minimal media than in minimal media plates, I tried to grow the *E. coli* strains XL1-Blue, DH5 α and DH10 α in test tubes containing the media, three with thiamine and three without thiamine. I placed them for a few days in the orbital shaker located in the roll-in incubator. After a few days in the incubator, the bacteria exhibited very little growth, so I decided to start the assay with *Drosophila*.

* * * * *

Since the 48-hour method did not work last term, I switched to the chronic-feeding method – method 1 – although I did not follow the procedure exactly. My first few tries were unsuccessful. I prepared media with varying concentrations of potassium chromate and added approximately eighteen flies – nine wild-type females and nine white-eyed males. In my first trial, I prepared one vial of 0.5 mM potassium chromate, and another of 1.0 mM potassium chromate. I set up the vials on Friday, February 7th, and by the following Monday, all of the flies were dead and had not laid eggs. For the second trial, I prepared another two

vials on February 14th, one with 1 μM potassium chromate and another with 100 μM of potassium chromate. Although many of the flies in this trial survived longer than the flies in the first trial, they eventually died within a week. I feel that many of the flies that appeared to die very quickly really never revived after being anesthetized. In the vial containing 1 μM potassium chromate the flies appeared to have mated. At least one larva and four pupae were spotted in the vial, all of which never finished the development into adult flies. I believe that the concentration of the chemical was not strong enough to kill them right away, but enough to kill them over time. For my third trial, I followed the procedure described in the “Methods” section. On February 21st, I left thirty males and thirty females to mate in a vial of untreated media. Once larvae were visible, I transferred the flies that were still alive – approximately 37 flies – to a vial containing 1 μM potassium chromate. I also added 500 μL of 0.1 mM potassium chromate to the vial containing the larvae. Since I had to mix the chemical into the media, I do not believe that the chemical was distributed evenly.

Approximately five to six days later, new flies began to emerge from the vial that the original sixty flies were kept in. Surprisingly, several of the flies had completely white eyes. This was unexpected because the cross should have produced only heterozygous red-eyed flies, and the best result that I could have hoped for were flies with large white spots in their eyes. Although I hoped that I may have been successful in my attempt to mutate the flies, I suspected that I had probably made an error. A careful check of the parent generation confirmed my suspicions. I had accidentally placed one white female in with the rest of the

flies. Although this meant that the flies' white eyes were not caused by the chemicals, I was still able to test the wild type flies. After examining them under a microscope, I came to the conclusion that the chemicals had no effect on them. There is still one more vial to be checked, but the flies have not yet emerged. I feel that these flies may have also died during the pupal stage.

Mites

A few weeks into the term, I discovered that many of the vials that were in the environmental chamber were infested with mites. The longer the vials had been in the chamber, the more mites they held. After noting this, I threw out one vial and soaked the other, older vials in bleach before washing them. I also made it a point to remove vials from the environmental chamber after a period of no more than two weeks. Although the mites do not appear to have any adverse effect on the adult flies, I did notice that in the first vial in which I found them, many of the new pupae were not finishing their development. Further inspection showed that the mites were clustered around the pupae and were probably feeding on them. For this reason, the mites had to be controlled and eventually eliminated before they could infest vials that might yield mutated offspring. However, I could not throw away all of the infested vials because all of the vials were infested, and that would have meant the end of my project. To help prevent further infestation of fresh vials, I wiped down the chamber shelves and walls with a solution of one part benzyl benzoate to five parts isopropanol, as suggested in the *Carolina Drosophila Manual*. I also sprayed a piece of LabMat with the solution and used it to line the shelves to keep the mites from walking

from one vial to the next. I did this very recently, so only time will tell if the solution is effective in controlling the mites.

Conclusion:

After using both methods, I see that the chronic-feeding method is much more efficient. It is easier to let the flies lay eggs into the treated media, rather than trying to separate larvae from the media, a process that has the potential to destroy all of the larvae. Although I failed to obtain results thus far, I did learn which concentrations of potassium chromate are too high for any offspring to develop. I hope to continue my research next term, probably testing lower concentrations of potassium chromate. In the beginning of this term, I was under the impression that I would have enough time to test many different chemicals and get results, but I was mistaken. I have learned that the trials take a lot of time, even if you have a plan. The trials take such a long time to prepare because virgin females must be collected. Since a few days must pass before flies can be deemed virgin, I usually only collect late Monday, all day Tuesday, and on Wednesday. The flies collected early on Monday are usually not virgins, as they emerged over the weekend and had enough time to begin mating. I do not collect flies on Thursday or Friday because I try not to screen them for more or less than three days. This leaves me with little time to collect many flies. Some also die after being anesthetized, further decreasing my numbers. I hope to find a more efficient way to collect flies so that I can perform more trials.

Hopefully, I will be able to test some of the other chemicals, even if I can only test one more.

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14. US Dept. of Health, *9th Report on Carcinogens* (2001), [Electronic Source].
15. Varmus, Harold and Weinberg, Robert, *Genes and the Biology of Cancer* (New York, 1993).

Pictures:

1. Figure 1: <http://www.genetics.org/cgi/content/full/150/1/199>
2. Figure 2: Lawrence, Peter, *The Making of a Fly: The Genetics of Animal Design* (Oxford, 1995), 83.
3. Figure 3: Lawrence, Peter, *The Making of a Fly: The Genetics of Animal Design* (Oxford, 1995), 84.
4. Figure 4: Lawrence, Peter, *The Making of a Fly: The Genetics of Animal Design* (Oxford, 1995), plate 8.1 (between pp.108 & 109).

Appendix A

Mutations commonly found in *Drosophila melanogaster*.



a) Recessive sex-linked white



b) Recessive autosomal eyeless



c) Dominant wild-type



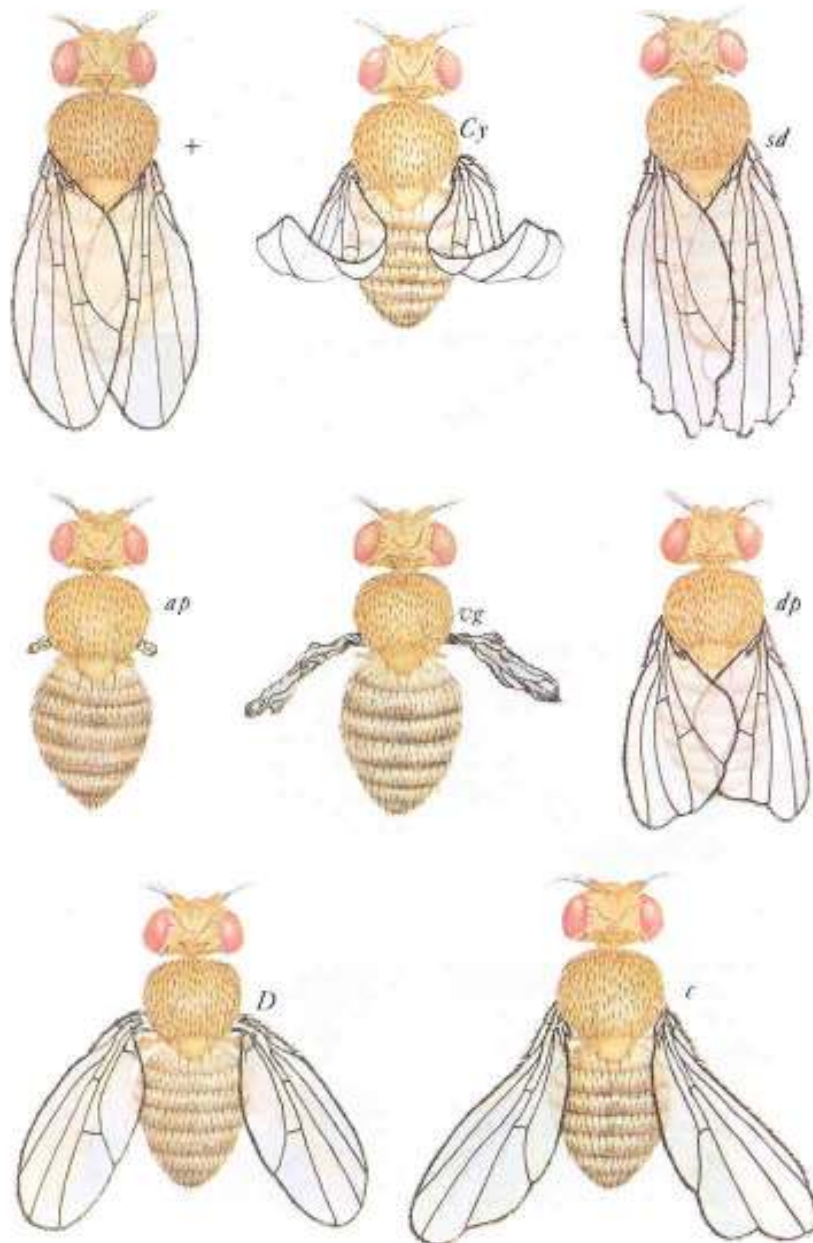
d) Recessive autosomal sepia



e) Dominant sex-linked Bar



f) Dominant autosomal Lobe



Wing mutations: +, wild; *CY*, Curly; *sd*, scalloped; *ap*, apterous; *vg*, vestigial; *dp*, dumpy; *D*, Dichaete; *c*, curved

- All photos and captions taken directly from *Carolina Drosophila Manual*.

Appendix B

Calculations used to determine amount of potassium chromate to add to vials to obtain certain concentrations.

0.1 mM K_2CrO_4

1) Add one part 0.1 M K_2CrO_4 to nine parts H_2O to get 0.01 M K_2CrO_4 .

2) Add one part 0.01 M K_2CrO_4 to nine parts H_2O to get 0.001 M K_2CrO_4 .

3) Add one part 0.001 M K_2CrO_4 to nine parts H_2O to get 0.0001 M K_2CrO_4 (which equals 0.1 mM K_2CrO_4).

0.5 mM K_2CrO_4

$$(0.5 \text{ mM/L}) \times (10^{-3} \text{ M/mM}) = 0.5 \times 10^{-3} \text{ M/L}$$

$$(0.015 \text{ L } H_2O) \times (0.5 \times 10^{-3} \text{ M/L}) = 7.5 \times 10^{-6} \text{ moles}$$

$$(0.1 \text{ moles/L}) \times L = 7.5 \times 10^{-6} \text{ moles}$$
$$X = 7.5 \times 10^{-5} \text{ L} = 75 \mu\text{L}$$

1.0 mM K_2CrO_4

$$(1.0 \text{ mM/L}) \times (10^{-3} \text{ M/mM}) = 1.0 \times 10^{-3} \text{ M/L}$$

$$(0.015 \text{ L } H_2O) \times (1.0 \times 10^{-3} \text{ M/L}) = 1.5 \times 10^{-5} \text{ moles}$$

$$(0.1 \text{ moles/L}) \times L = 1.5 \times 10^{-5} \text{ moles}$$
$$X = 1.5 \times 10^{-4} \text{ L} = 150 \mu\text{L}$$

1 μM K_2CrO_4

1) Add one part 0.1 M K_2CrO_4 to nine parts H_2O to get 0.01 M K_2CrO_4 .

$$2) [(0.015 \text{ L } H_2O) \times (10^{-6} \mu\text{M/M})] / (0.01 \text{ M/L}) = X \text{ L}$$
$$X = 1.5 \times 10^{-6} \text{ L} = 1.5 \mu\text{L}$$

100 μM K_2CrO_4

$$(100 \mu\text{M} / \text{L}) \times (10^{-6} \text{ M}/\mu\text{M}) = 1.0 \times 10^{-4} \text{ M/L}$$

$$(0.015 \text{ L } H_2O) \times (1.0 \times 10^{-4} \text{ M/L}) = 1.5 \times 10^{-6} \text{ moles}$$

$$(0.1 \text{ moles/L}) \times L = 1.5 \times 10^{-6} \text{ moles}$$
$$X = 1.5 \times 10^{-5} \text{ L} = 15 \mu\text{L}$$