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Biology 1481

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### Drosophila Lab Report

In an experiment conducted over the period of three weeks, fruit flies (*Drosophila melanogaster*) with specific traits were mated and evolved to better understand the principles of Mendelian inheritance. Through this experiment it was made possible to better understand the different types of genetic crosses such as monohybrid and dihybrid and how various selected traits and their properties could affect the outcome of crossover. It was also used to show how to interpret the results by analyzing phenotypic data and creating chi squared test to either accept the null hypothesis, or reject it. The test subjects, fruit flies, were selected due to being considered to have a very short life span and thus mating and evolving within a reasonable amount of time. It is possible to witness multiple generations of the species and visibly see the effects of crossover without the need to wait many centuries.

Gregor Mendel is well known for his discovery of the Principles of Inheritance. These principles were discovered through an experiment he carried out with pea plants. Mendel used seven specific characteristics to observe the variation of differentiating characters between generations of pea plants and their parents. He also aimed to essentially find out why certain differentiable traits were transmitted to offspring and how he could predict it and analyze the results. Through this experiment, the three principals were discovered as follows: 1. "Inheritance involves the passing of discrete units of inheritance, or genes, from parents to offspring," 2. "During reproduction, the inherited factors (now called alleles) that determine traits are separated

into reproductive cells by a process called meiosis and randomly reunite during fertilization,” 3.

“Genes located on different chromosomes will be inherited independently of each other.”

(“Mendelian Genetics.” 1) Mendel also stated that a trait must either be dominant or recessive.

Unfortunately, Mendel’s work was denied attention until thirty-four years after he published his book, *Experiments in Plant Hybridization*, when three independent botanists rediscovered Mendel’s work (Mendel 1). This experiment aims to recreate an instance of Mendel’s pea experiment using *Drosophila* fruit flies.

### Experiment A: Monohybrid Cross

#### *Hypothesis*

In assignment A, we are expecting to achieve a monohybrid cross with a 3:1 ratio in terms of the phenotypes Wild Type to Brown Eyes, this would prove that the flies are heterozygous for Brown Eye color and can be predicted by using a Punnett square (see fig. 1).

	A	a
A	AA	Aa
a	Aa	aa

Figure. 1. – This figure shows a Punnett square for a monohybrid cross. As shown it comes out to a 3:1 ratio

#### *Experimental Design*

In this experiment we chose the trait Brown Eyes due to a few important factors. The first being that Brown Eyes is not a lethal allele. Using a lethal allele in this experiment would skew our data and in return create a 2:1 ratio rather than the 3:1 that we are originally expecting it to

create. The trait is also not on a sex chromosome, if the trait had been on a sex chromosome than our data would have possibly been skewed as the sex of the fly would have been a dependent factor when crossover occurred and upon collection of the results, they would have been faulty. Only one trait for this experiment was chosen because this is a monohybrid cross, this means that the fly (or any organism, fly is said because we are using fruit flies in our experiment) is heterozygous for a single trait, be it recessive or dominant.

### *Materials and Methods*

First, we ordered fruit flies relative to the experiment shown in table 1 (Wild Type male and Brown Eye female). After ordering the appropriate flies we emptied the flies into a mating jar where they sat for approximately two weeks. In order to get the flies out of the jar and onto our microscope slide, we used a chemical called ether. We observed the flies underneath a microscope and proceeded to use a small brush to separate the flies into piles according to their phenotypes (see table 2) and collected the number of each fly with each phenotype. We then took one fly from each phenotype and mated them once more, using the exact same process, to achieve the F2 generation (see table 3). Upon finalization of the separation of the last generation (F2), we gathered results on how many Wild Type flies there were as well as how many Brown Eyed flies there were (ignoring sex). We counted this data and created an appropriate table (see table 4). After this we created a table using the data we had collected in the final cross and using this table we were able to create a chi squared analysis test to test wither our hypothesis was correct or not.

*Results and Discussion*

Table 1. – This table shows the starting phenotypes for both parental fruit flies of cross one in Experiment A.


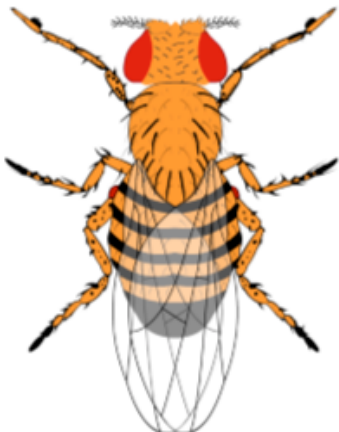
	<p><b>Parent for Cross #1</b></p> <p>Female; Brown Eyes</p> <p>Number with this phenotype: 1</p>
	<p><b>Parent for Cross #1</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 1</p>

Table 2. – Table two shows the two offspring and their population as well as phenotype after cross one.

These are also the parents of cross two offspring.

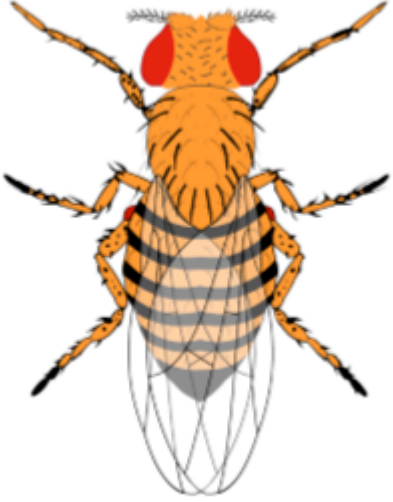
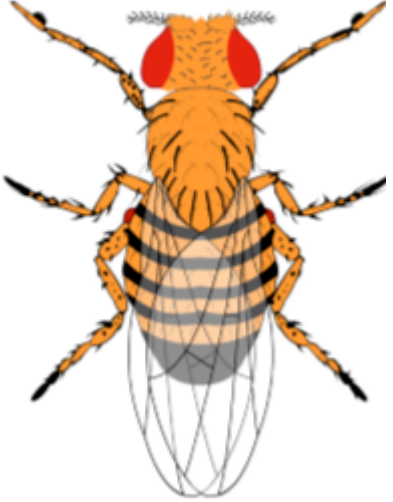

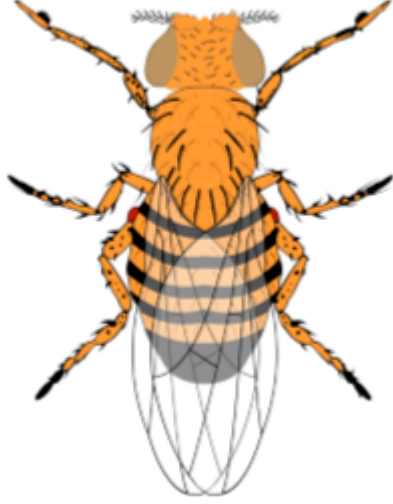
	<p><b>Offspring of Cross #1</b></p> <p>Female; Wild Type</p> <p>Number with this Phenotype: 590</p> <p><b>Parents:</b></p> <p>Female; Brown Eyes</p> <p>X</p> <p>Male; Wild Type</p>
	<p><b>Offspring of Cross #1</b></p> <p>Male; Wild Type</p> <p>Number with this Phenotype: 594</p> <p><b>Parents:</b></p> <p>Female; Brown Eyes</p> <p>X</p> <p>Male; Wild Type</p>

Table 3. – This table shows the population and phenotypes of all of the second generation fruit flies.

	<p><b>Offspring of Cross #2</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 474</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>
	<p><b>Offspring of Cross #2</b></p> <p>Male; Brown Eyes</p> <p>Number with this phenotype: 143</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>


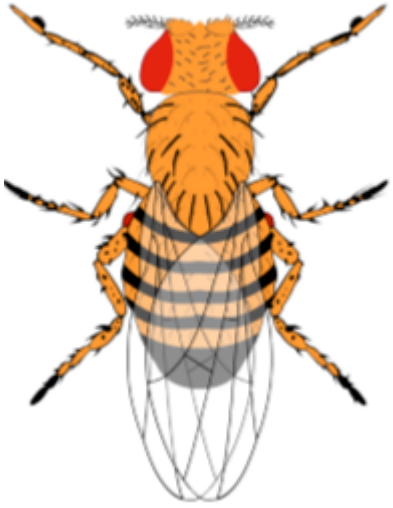
	<p><b>Offspring of Cross #2</b></p> <p>Female; Brown Eyes</p> <p>Number with this phenotype: 151</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>
	<p><b>Offspring of Cross #2</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 433</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>

Table 4. – This table shows the entire population (ignoring sex) of each phenotype in the second generation of Experiment A.

<p><b>Cross #2 – Parents:</b></p> <p>(Wild Type)</p> <p><b>X</b></p>
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(Wild Type)			
Sex Ignored			
<u>Phenotype</u>		<u>Number</u>	<u>Proportion</u>
Wild Type		907	0.7552
Brown Eyes		294	0.2448
	<b>Total</b>	1201	

Table 5 shows the number of observed flies per phenotype. For example, it shows us that we have counted nine-hundred and seven flies with the wild type phenotype, and two-hundred and ninety-four with brown eyes. We originally expected a ratio of about 3:1 wild type to brown eyes. With the total population that we have counted, doing that ratio within this population gives us the numbers shown in the expected column. As shown in the table it is very apparent there is a significant similarity between our observed results and the original expected results. Doing a chi-squared test analysis on this graph gives us a 0.1735 statistical value. This means that our hypothesis is almost exactly on point to the observed values. This reassures us that there is a significance between our observed and expected values. We have level of significance of around sixty-seven percent meaning that the values we have collected are directly significant of each other.

Table 5. – This table shows the amount of observed population per phenotype in comparison to our hypothesis and expected values, it also shows the chi-squared level of significance.

<u>Phenotype</u>		<u>Observed</u>	<u>Hypothesis</u>	<u>Expected</u>	<u>Chi-Square Term</u>
Wild Type		907	900	900.75	0.0434
Brown Eyes		294	300	300.25	0.01301
	<b>Total</b>	1201	1200	1201.00	0.1735
Chi-Squared Test Statistic: 0.1735					



Degrees of Freedom: 1			
Level of Significance: 0.677			

### *Conclusion*

In this experiment we tested whether or not the parents were heterozygous for the trait brown eyes. We expected to achieve a ratio of 3:1, wild type to brown eyes. With our tables and data collected above we can strongly conclude that the results we have acquired support that hypothesis directly. This is seen by performing a chi-squared test on our data, as performed in table five, and checking the level of significance as well as the test statistic. Both of them are relatively close to zero meaning that error was practically nonexistent within the experiment. Overall, our results highly accept the null-hypothesis.

### **Experiment B: Sex-linked Traits**

#### *Hypothesis*

In assignment B, we are attempting to show a reciprocal cross that we expect to represent a 3:1 ratio (ignoring sex). To achieve this expected ratio, we calculated the expected outcome with a Punnett square (see fig. 2).

	<b>A</b>	<b>a</b>
<b>A</b>	AA	Aa
<b>a</b>	Aa	aa

Figure. 2. – This figure shows a Punnett square for the sex-linked trait cross. As shown it comes out to a 3:1 phenotypic ratio (ignoring sex).

#### *Experimental Design*

In this experiment we are specifically testing the relationships with sex-linked traits. In order to do this, obviously, we must choose traits that are found on the X chromosome. Traits found on the X chromosomes are going to be considered x-linked traits or sex-linked traits. This is the primary reason as to why, in our experiment, we chose the trait white eyes. In theory we should be able to either observe crossover not happening to the opposite sex, or happening in such a way that the selected trait is not being crossed over but rather the original parent carrying the trait ends up a wild type.

### *Materials and Methods*

First, we ordered fruit flies relative to the experiment shown in table 6 (Wild Type female and white eye male). After ordering the appropriate flies we emptied the flies into a mating jar where they sat for approximately two weeks. In order to get the flies out of the jar and onto our microscope slide, we used a chemical called ether. We observed the flies underneath a microscope and proceeded to use a small brush to separate the flies into piles according to their phenotypes (see table 7) and collected the number of each fly with each phenotype. We then took one fly from each phenotype and mated them once more, using the exact same process, to achieve the F2 generation (see table 8). Upon finalization of the separation of the last generation (F2), we gathered results on how many female wild type flies there were as well as how many male wild type and white eyes there were. We counted this data and created an appropriate table (see table 9). After this we created a table using the data we had collected in the final cross and using this table we were able to create a chi squared analysis test to test whether our hypothesis was correct or not.

*Results and Discussion*

Table 6. – This table shows the original starting parental generation and its phenotypes.


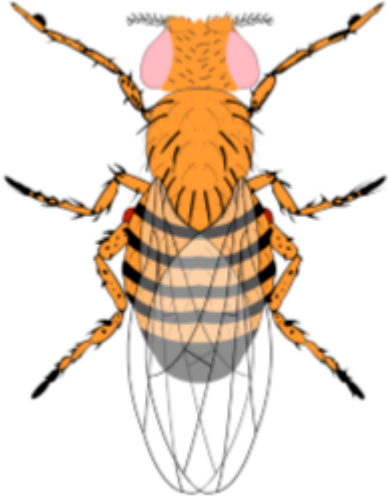
	<p><b>Parent for Cross #1</b></p> <p>Female; Wild Type</p>
	<p><b>Parent for Cross #1</b></p> <p>Male; White eyes</p>

Table 7. – This table shows the offspring of cross one in Experiment B and their phenotypes and population.





	<p><b>Offspring of Cross #1</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 626</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #1</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 575</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>

Table 8. – This table shows the offspring of the second cross in Experiment B with its phenotypes and populations.

	<p><b>Offspring of Cross #2</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 305</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 605</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>


	<p><b>Offspring of Cross #2</b></p> <p>Male; White eyes</p> <p>Number with this phenotype: 280</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
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Table 9. – This table shows all of the populations per phenotype and their proportion to the total.

<p><b>Cross #2 – Parents:</b></p> <p>(Female: Wild Type)</p> <p><b>X</b></p> <p>(Male: Wild Type)</p> <p>Sex Ignored</p>		
<u>Phenotype</u>	<u>Number</u>	<u>Proportion</u>
Wild Type	910	0.7647
White Eyes	280	0.2353
	<b>Total</b>	1190

Table 10 shows us that that due to our hypothesis of a 3:1 ratio, we should have some fairly similar expected values in comparison to our observed. Taking a look at the table, we can see that after a chi-squared test analysis we are left with the value of 1.3441. This in particular

shows us that the results we have gotten are almost identical to the observed results. There was some room for mistake there though as we are farther away from the zero point than in our last experiment. If we take a look at our significance value, we notice that there is a 0.558 meaning that we have about a fifty-five percent level of significance between our observed and expected values. This isn't the best, but it still accepts the null hypothesis. If we also observe the values we've collected in terms of crossover. It appears that crossover never happened. The males come out on top with white eyes, and there are absolutely no females to be found that have white eyes. This is a result of choosing the trait on a X chromosome and makes the trait sexually dependent. However, in the same sense that there are no females with white eyes, it's important to know that there are in fact males who are wild type.

Table 10. – This table shows us our chi-squared analysis between the phenotypes and populations.

<u>Phenotype</u>		<u>Observed</u>	<u>Hypothesis</u>	<u>Expected</u>	<u>Chi-Square Term</u>
Wild Type		910	892	892.50	0.3431
White Eyes		280	297	297.25	1.0010
	<b>Total</b>	1190	1189	1190.00	1.3441
Chi-Squared Test Statistic: 1.3441					
Degrees of Freedom: 1					
Level of Significance: 0.558					

### *Conclusion*

Due to the data collected in our tables we can conclude that our originally hypothesized ratio is correct and does accept the null hypothesis. This can be shown by the chi-squared analysis we had done in table 10. The level of significance is above the minimum five percentile of the null-hypothesis and therefore is in agreeance with it. What this means is that eye color in

the fruit flies doesn't necessarily have anything to do with sex determination. We can see genes that appear on the sex chromosome (X) that do not relate to sexual function.

### Experiment C: Dihybrid Cross

#### *Hypothesis*

We expect this experiment to have an outcome of a 9:3:3:1 Mendelian ratio, Wild Type to Shaven Bristles to Black Body to Shaven Bristles; Black body. This ratio was predicted with the use of the Punnett square shown below (see fig. 3).

	<b>AB</b>	<b>Ab</b>	<b>aB</b>	<b>ab</b>
<b>AB</b>	AABB	AABb	AaBB	AaBb
<b>Ab</b>	AABb	AAbb	AaBb	Aabb
<b>aB</b>	AaBB	AaBb	aaBB	aaBb
<b>ab</b>	AaBb	Aabb	aaBb	aabb

Figure. 3. – This figure shows a Punnett square for a dihybrid cross. As shown it comes out to a 9:3:3:1 ratio

#### *Experimental Design*

In this experiment we are using two traits as it is a di-hybrid cross. A dihybrid cross is a cross between two pure breeds that vary in two observed traits. It was absolutely necessary for this experiment not to choose traits that would be sex-linked or lethal as this would void our original hypothesis of a 9:3:3:1 ratio and create a problem that would either change the ratio completely or kill off an entire group of our flies. The traits chosen were both in compliance to this and theoretically the experiment should prove to be effective.



*Method and Materials*

First, we ordered fruit flies relative to the experiment shown in table 11 (Female with shaven bristles and black body and Male wild type). After ordering the appropriate flies we emptied the flies into a mating jar where they sat for approximately two weeks. In order to get the flies out of the jar and onto our microscope slide, we used a chemical called ether. We observed the flies underneath a microscope and proceeded to use a small brush to separate the flies into piles according to their phenotypes (see table 12) and collected the number of each fly with each phenotype. We then took one fly from each phenotype and mated them once more, using the exact same process, to achieve the F2 generation (see table 13). Upon finalization of the separation of the last generation (F2), we gathered results on how many wild type, shaven bristled, black body, and shaven bristles; black body flies there were (ignoring sex). We counted this data and created an appropriate table (see table 14). After this we created a table using the data we had collected in the final cross and using this table we were able to create a chi squared analysis test to test wither our hypothesis was correct or not.

*Results and Discussion*

Table 11. – This table shows the parental generation and phenotypes for cross one of Experiment C.



	<p><b>Parent for Cross #1</b></p> <p>Female; Shaven Bristles; Black Body</p>
	<p><b>Parent for Cross #1</b></p> <p>Male; Wild Type</p>

Table 12. – This table shows the offspring of cross one and their populations per phenotype.

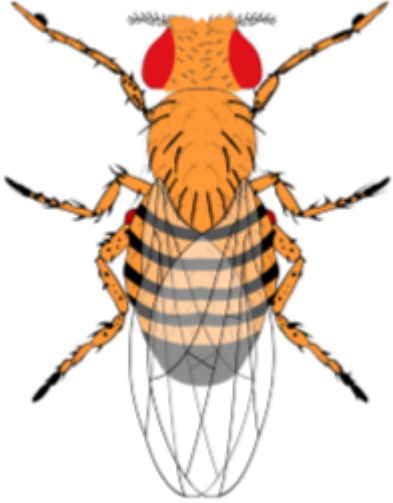







	<p><b>Offspring of Cross #1</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 594</p> <p><b>Parents:</b></p> <p>Female; Shaven Bristles; Black Body</p> <p><b>X</b></p> <p>Male; Wild type</p>
	<p><b>Offspring of Cross #1</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 584</p> <p><b>Parents:</b></p> <p>Female; Shaven Bristles; Black Body</p> <p><b>X</b></p> <p>Male; Wild type</p>

Table 13. – This table shows the offspring and phenotype of cross two in Experiment C.

	<p><b>Offspring of Cross #2</b></p> <p>Female; Shaven Bristles</p> <p>Number with this phenotype: 112</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 294</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>

	<p><b>Offspring of Cross #2</b></p> <p>Male; Shaven Bristles; Black Body</p> <p>Number with this phenotype: 37</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Male; Black Body</p> <p>Number with this phenotype: 104</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>

	<p><b>Offspring of Cross #2</b></p> <p>Male; Shaven Bristles</p> <p>Number with this phenotype: 125</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 339</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>



	<p><b>Offspring of Cross #2</b></p> <p>Female; Shaven Bristles; Black Body</p> <p>Number with this phenotype: 37</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Black Body</p> <p>Number with this phenotype: 104</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p> <p>Male; Wild Type</p>

Table 14. – This table shows all of the populations and proportions per phenotype, sex is ignored.

<p><b>Cross #2 – Parents:</b></p> <p>(Wild Type)</p>
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<p style="text-align: center;"><b>X</b></p> <p style="text-align: center;">(Wild Type)</p> <p style="text-align: center;">Sex Ignored</p>		
<u>Phenotype</u>	<u>Number</u>	<u>Proportion</u>
Wild Type	633	0.5495
Shaven Bristles	237	0.2057
Black Body	208	0.1806
Shaven Bristles; Black Body	74	0.0642
	<b>Total</b>	1152

Table 15 shows us a visual correlation between our observed values and the values that should have been expected through our hypothesis. It also shows the chi-squared calculation for each phenotype, as well as the data as a whole. Using this table we are able to conclude on the statistical value regarding how accurate our data is. As of now we are looking to find a 9:3:3:1 Medellin ratio as stated by our hypothesis. Our values do present themselves as having a direct natural ratio close to this, but our chi-squared level of significance proceeds to say otherwise. According to the table, our level of significance is about forty-three percent. This means that even though our values appear to have a correlation by the glance of an eye, the values have are hardly significant to each other. Our experiment still is able to fall under the null-hypothesis and we are able to accept that here.

Table 15. – This table shows the chi-squared analysis between the phenotypes in experiment C.

<u>Phenotype</u>	<u>Observed</u>	<u>Hypothesis</u>	<u>Expected</u>	<u>Chi-Square Term</u>
Wild Type	633	648	648.00	0.3472
Shaven Bristles	237	216	216.00	2.0417



Black Body	208	216	216.00	0.2963
Shaven Bristles; Black Body	74	72	72.00	0.0556
<b>Total</b>	1152	1152	1152.00	2.7407
Chi-Squared Test Statistic: 2.7407				
Degrees of Freedom: 3				
Level of Significance: 0.4333				

### *Conclusion*

Due to the table we have created in the previous step (see table 15) our experiment is accepting the null-hypothesis. This is due to our level of significance being above the required minimum five percentile that is necessary to accept. Essentially what this means is that both of our first generation parents were heterozygous and that one allele for each of our chosen traits (Shaven Bristles and Black Body) displays complete dominance. Both parents have recessive alleles, and at the same time are able to display the dominant phenotype. Our original hypothesis ratio of 9:3:3:1 is correct here.

### **Experiment D: Linkage Groups**

#### *Hypothesis*

In Experiment D we will be performing two trials, one with traits that are close in relation to map units on the same chromosomes, and one with traits that are far. We expect to have an outcome of the 9:3:3:1 ratio on both trials (see fig. 4).

	<b>AB</b>	<b>Ab</b>	<b>aB</b>	<b>ab</b>
<b>AB</b>	AABB	AABb	AaBB	AaBb
<b>Ab</b>	AABb	AAbb	AaBb	Aabb
<b>aB</b>	AaBB	AaBb	aaBB	aaBb

<i>ab</i>	AaBb	Aabb	aaBb	aabb
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Figure. 4. – This figure shows the Punnett square used in both trials and proves the expected ratio of 9:3:3:1.

### *Experimental Design*

In this experiment it is necessary to choose traits that are on the same chromosome to test Mendel's law of independent assortment. In order to test this theory, we needed to select traits that were on the same chromosome, and at the same time we wanted to test how often crossover occurred. To do this we did two trials, one with chromosomes that are closer together on the chromosome (this would decrease the chance of crossover substantially) and one trial with chromosomes that are farther apart (this would increase the chance of crossover).

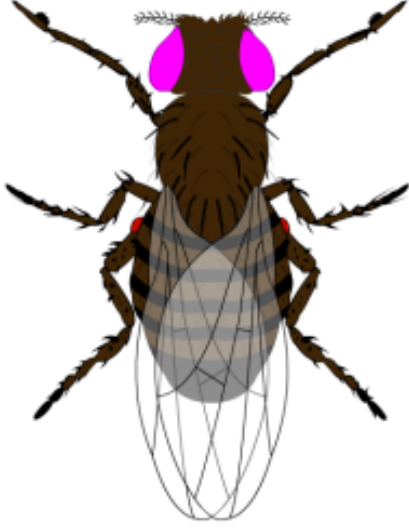
### *Materials and Methods*

For this experiment we conducted two trials. For the first trial using closer traits, we ordered the appropriate fruit flies that were relative to the experiment (table 16). When the flies arrived, we put flies of both phenotypes into a jar and placed it in an incubator for about two weeks. After the two weeks were up we then used a chemical known as ether to knock the flies out of the mating jar into a slide so that we could observe them under the microscope. Upon putting them under the microscope slide, we separated the flies into different groups according to their gender and phenotype and recorded the data (table 17). We then took one fly from each phenotype of the new offspring and used it in a new mating session. Once we picked the flies, we repeated the steps above, and upon separation of the flies we noted the phenotypes and various

traits observed excluding sex (table 18). We then created a table of data in regards to the populations per phenotype (table 19). After this we created a chi-squared analysis table in relation to the collected data (table 20). The entire list of steps was repeated for the second trial regarding map units that were farther apart. (table 21,22,23,24,25).

### *Results and Discussion*

Table. 16. – This table shows the starting parents for the first cross between close map unit traits in Experiment D.

 A detailed illustration of a male fruit fly (Drosophila melanogaster) from a dorsal perspective. The fly has a dark brown, almost black, body. Its eyes are a vibrant purple. The wings are transparent with a network of veins. The legs are dark brown and segmented. The fly is positioned in the center of the left column of the table.	<p style="text-align: center;"><b>Parent for Cross #1</b></p> <p style="text-align: center;">Male; Purple Eyes; Black Body</p>
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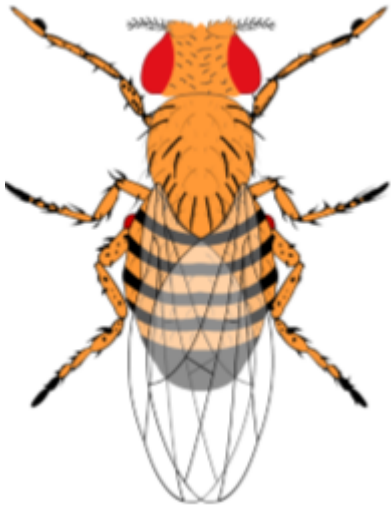
	<p><b>Parent for Cross #1</b></p> <p>Female; Wild Type</p>
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Table. 17. – This table shows the offspring of the first cross of close map unit traits in Experiment D.

	<p><b>Offspring of Cross #1</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 590</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
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**Offspring of Cross #1**

Male; Wild Type



Number with this phenotype: 580


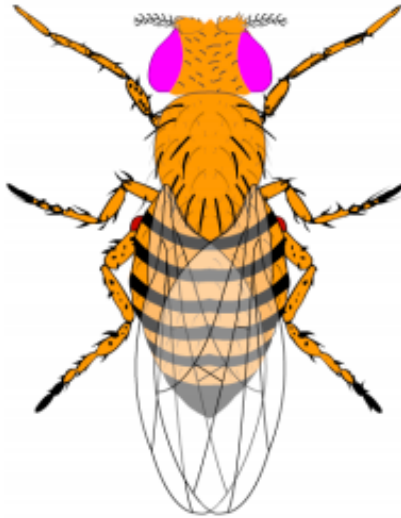
**Parents:**

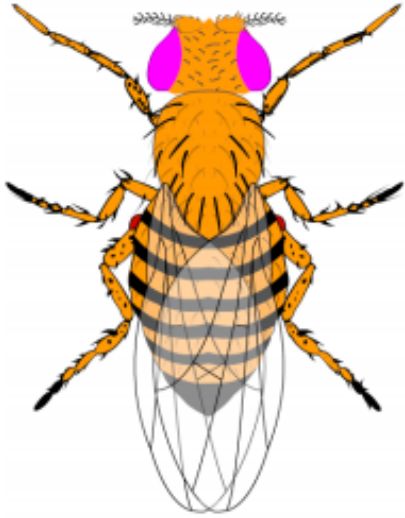
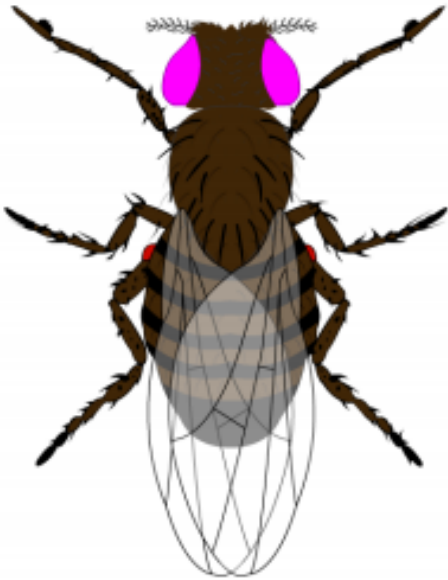
Female; Wild Type

**X**

Table 18. – This table shows the second cross of the close map unit traits in Experiment D.

	<p><b>Offspring of Cross #2</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 454</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Black Body</p> <p>Number with this phenotype: 13</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>

	<p><b>Offspring of Cross #2</b></p> <p>Male; Black Body</p> <p>Number with this phenotype: 9</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Purple Eyes</p> <p>Number with this phenotype: 10</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>

	<p><b>Offspring of Cross #2</b></p> <p>Male; Purple Eyes</p> <p>Number with this phenotype: 9</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Purple Eyes; Black Body;</p> <p>Number with this phenotype: 151</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>



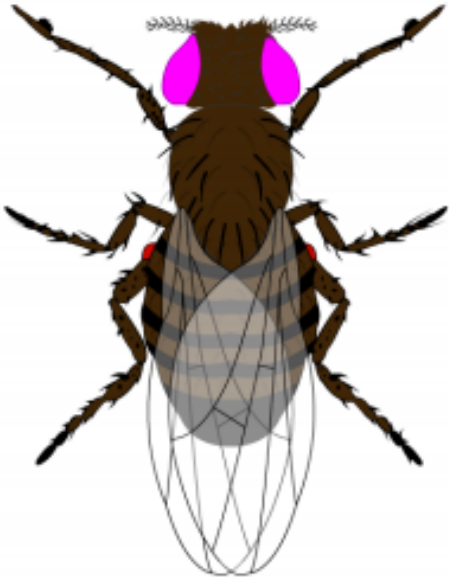

	<p><b>Offspring of Cross #2</b></p> <p>Male; Purple Eyes; Black Body;</p> <p>Number with this phenotype: 145</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p>X</p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 418</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p>X</p>

Table 19. – This table shows the populations per phenotype and their appropriate ratios.

<p><b>Cross #2 – Parents:</b></p> <p>(Wild Type)</p> <p>X</p> <p>(Wild Type)</p>
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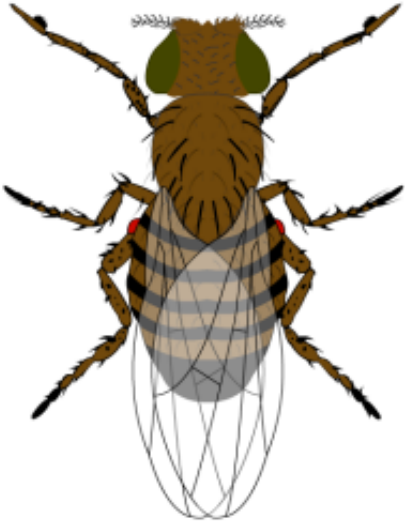
Sex Ignored			
<u>Phenotype</u>		<u>Number</u>	<u>Proportion</u>
Wild Type		872	0.7213
Purple Eyes; Black Body		296	0.2448
Black Body		22	0.0182
Purple Eyes		19	0.0157
	<b>Total</b>	1209	

Table 20 shows us that our values that we originally expected due to the expected 9:3:3:1 ratio are completely off. The ratio is not relevant of our results here and the observed number has a significance level of zero in comparison to our values that we expected. This table helps illustrate that our experiment must either have a mistake or a misinterpretation in regards to the originally expected value. This also lets us know that crossover either did not occur enough or occurred too much. This can be attributed to the fact that the law of independent assortment states that in order for crossover to occur the traits that are used must be found on two different chromosomes. Contrary to that, if the traits used are far enough apart, it acts as if the traits are found on two different chromosomes and crossover happens like normal. It is still possible for chromosomes that are closer than twenty map units to undergo crossover, but due to the randomness of crossover it has less of a chance of occurring exactly between the two traits and carrying on the trait to the next generation. This is the reason our results are skewed and the reason why our chi-squared analysis does not notify us of any significance to our expected values. The reason why we originally expected the 9:3:3:1 ratio is due to the fact that Mendel's dihybrid crosses usually always represent that ratio if they are in accordance to the three laws mentioned previously in the report.

Table 20. – This table shows us the chi-squared analysis between our phenotypes and populations (sex is ignored).

<u>Phenotype</u>		<u>Observed</u>	<u>Hypothesis</u>	<u>Expected</u>	<u>Chi-Square Term</u>
Wild Type		872	680	681.13	53.4887
Purple Eyes; Black Body		296	226	226.37	21.4146
Black Body		22	226	226.37	184.5152
Purple Eyes		19	75	75.12	41.9296
	<b>Total</b>	1209	1207	1209	301.3455
Chi-Squared Test Statistic: 301.3455					
Degrees of Freedom: 3					
Level of Significance: 0					

Table. 21. – This table shows the starting parents for the second cross between far map unit traits in Experiment D.

	<p style="text-align: center;"><b>Parent for Cross #1</b></p> <p style="text-align: center;">Male; Sepia Eyes; Ebony Body</p>
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	<p><b>Parent for Cross #1</b></p> <p>Female; Wild Type</p>
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Table. 22 – This table shows the offspring of the second cross of far map unit traits in Experiment D.

	<p><b>Offspring of Cross #1</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 582</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
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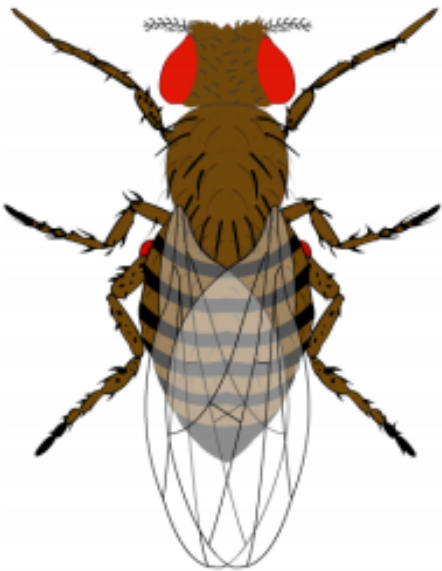
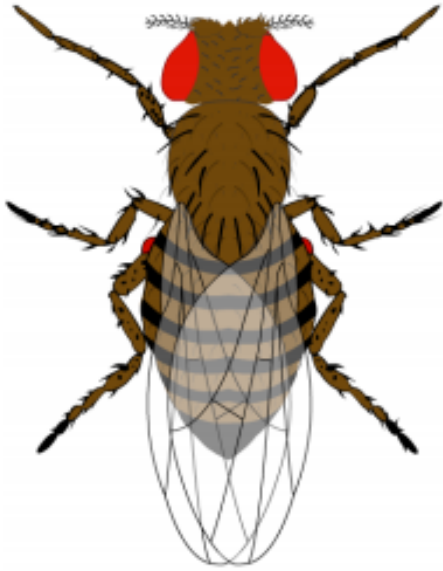
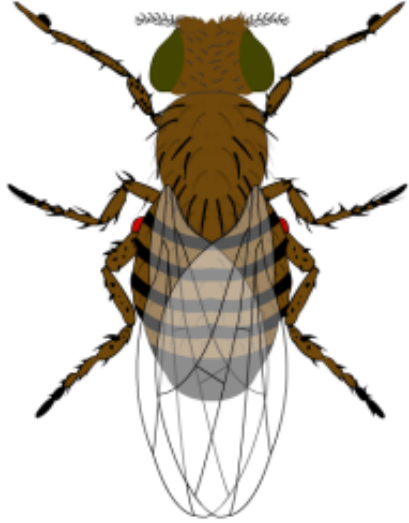
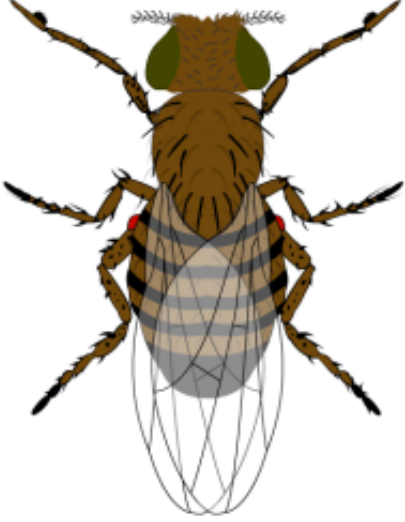


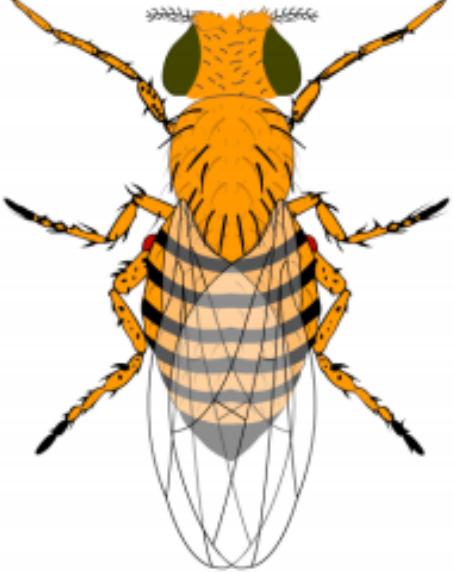
	<p><b>Offspring of Cross #1</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 590</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
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Table 23 – This table shows the offspring of the second cross between far map units in Experiment D.

	<p><b>Offspring of Cross #2</b></p> <p>Female; Ebony Body;</p> <p>Number with this phenotype: 34</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
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	<p><b>Offspring of Cross #2</b></p> <p>Male; Ebony Body</p> <p>Number with this phenotype: 44</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Sepia Eyes; Ebony Body</p> <p>Number with this phenotype: 104</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>

	<p><b>Offspring of Cross #2</b></p> <p>Male; Sepia Eyes; Ebony Body</p> <p>Number with this phenotype: 111</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 406</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>

	<p><b>Offspring of Cross #2</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 408</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
	<p><b>Offspring of Cross #2</b></p> <p>Female; Sepia Eyes</p> <p>Number with this phenotype: 52</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>



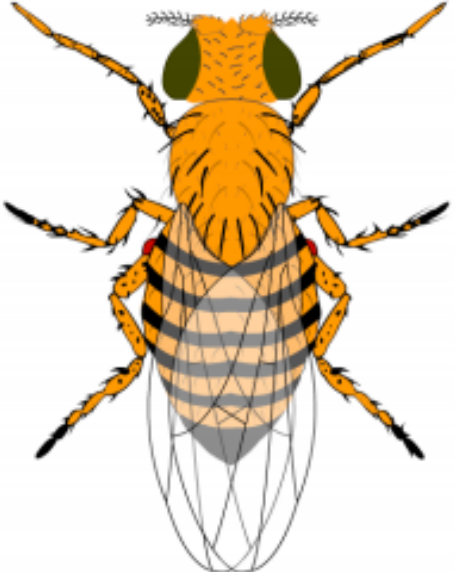
	<p><b>Offspring of Cross #2</b></p> <p>Male; Sepia Eyes</p> <p>Number with this phenotype: 55</p> <p><b>Parents:</b></p> <p>Female; Wild Type</p> <p><b>X</b></p>
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Table 24 – This table shows the populations of the phenotypes as a whole in proportion to the total of far map units in Experiment D.

<p><b>Cross #2 – Parents:</b></p> <p>(Wild Type)</p> <p><b>X</b></p> <p>(Wild Type)</p> <p>Sex Ignored</p>			
<u>Phenotype</u>		<u>Number</u>	<u>Proportion</u>
Wild Type		814	0.6705
Sepia Eyes; Ebony Body		215	0.1771
Sepia Eyes		107	0.0881
Ebony Body		78	0.0643
	<b>Total</b>	1214	

Table 25 shows us the chi-squared analysis of our second trial in Experiment D using traits that are farther apart than twenty map units. For the particular traits chosen, they are about fifty map units apart. Take in mind that the table ignores sex. In the table it can be shown that while our results do not follow a level of significance that proves our results are actually significant to the observed data, the predicted values for a few of the phenotypes are actually a lot more accurate than some of which we had seen in the previous trial. This is due to the fact that due to the law of independent assortment, if two traits are far enough apart on one chromosome they act as if they are on separate chromosomes. The reason for this is that cross over has more surface area and a larger chance of occurring as it must occur somewhere in the middle of the two chromosomes. However, due to the fact that the traits we chose were only fifty map units apart, the percentage wasn't as large as it could have been, this is why the data we have does not accept the null hypothesis. The table shows that the chi-squared value is 89.507 which would mean our level of significance is equal to zero. The point being, the results were closer than that of the last experiment and this all has to do with crossover.

Table 25 – This table shows the chi-squared analysis of the second trial in Experiment D.

<u>Phenotype</u>		<u>Observed</u>	<u>Hypothesis</u>	<u>Expected</u>	<u>Chi-Square Term</u>
Wild Type		814	682	683.69	24.8370
Sepia Eyes; Ebony Body;		215	227	227.56	0.6935
Sepia Eyes		107	227	227.56	63.8738
Ebony Body		78	75	75.19	0.1053
	<b>Total</b>	1214	1211	1214	89.5097
Chi-Squared Test Statistic: 89.5097					
Degrees of Freedom: 3					
Level of Significance: 0					

*Conclusion*

According to both table twenty-five and table twenty, both of our trials are rejecting the null hypothesis as their level of significance (zero) is lower than the minimum required five percentiles. This is very much due to the fact that, like mentioned above, due to Mendel's independent law of assortment, traits must be on separate chromosomes for cross over to occur. However, it is possible for crossover to occur if the traits are far enough apart, as it will behave as if it was on a separate chromosome. The chance of crossover, as shown in our first trial, is extremely low for traits that are less than twenty map units apart, any close trait to be exact, because the amount of space between the traits for crossover to occur creates a low chance that it will in that exact spot. The farther away the traits are, as shown in trial two, the more chance and more crossover you will observe, but your data will still be skewed as crossover is random and there is no direct prediction as how it will behave when conducting an experiment using traits on the same chromosome. We did witness more crossover happen in our second trial than our first trial, our offspring not only show this but the tables as well. In the chi-squared analysis table, looking at each individual phenotype and examining the chi-squared value for that exact phenotype gives you a more accurate number than any of the observed phenotypes in the first trial. There are a few exceptions of course, but this is all due to the randomly occurring crossover process. Our chi-squared value in the second trial (regarding farther traits on the same chromosome) also gives us a much much lower total chi-squared value, meaning that it is closer to being a correct prediction. If perhaps we had chosen traits that were on the very tips of the chromosomes, rather than the ones we had in trial two, then it's very possible we may have been able to acquire accurate predictions as the chance for crossover would have been almost inevitable.

## Experiment E: Lethal Alleles

### *Hypothesis*

In this experiment, due to having two heterozygous flies that are recessive for the lethal alleles, we expect the outcome to have a 3:1 ratio. This can be shown by the Punnett square below.

	A	a
A	AA	Aa
a	Aa	aa

Figure. 5. – This figure shows a Punnett square for a monohybrid cross. As shown it comes out to a 3:1 ratio.

### *Experimental Design*

In this experiment the trait curly wing was selected for both flies. This means that the flies had to have a recessive trait of curly wings because if they were homozygous recessive with a lethal allele they would essentially be dead. We selected the curly wing trait as we knew that this trait was not on a sex linked chromosome and was not a normal trait, but a mutation that would deem it lethal for a fly to possess. Since both of our flies would need to be heterozygous, it is inferred here that the curly wing trait is recessive as we did not order dead flies.

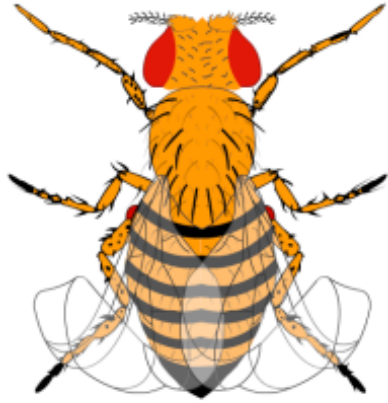
### *Materials and Methods*

First, we ordered fruit flies relative to the experiment shown in table 26, two heterozygous flies recessive for the trait curly wings. After ordering the appropriate flies we emptied the flies into a mating jar where they sat for approximately two weeks. In order to get

the flies out of the jar and onto our microscope slide, we used a chemical called ether. We observed the flies underneath a microscope and proceeded to use a small brush to separate the flies into piles according to their phenotypes (see table 27) and collected the number of each fly with each phenotype. Unlike the other experiments, in this experiment we did not cross the flies twice and create an F2 generation. Instead, we kept with the first cross and created our table showing the proportions and populations per phenotype, ignoring sex (see table 28). After this we continued on with the experiment to do the chi-squared analysis and decide whether or not our results were significant to our original hypothesis (see table 29).

### *Results and Discussion*

Table 26. – This table shows the original parent flies for the cross in Experiment E.

	<p><b>Parent for Cross #1</b></p> <p>Female; Curly Wings</p>
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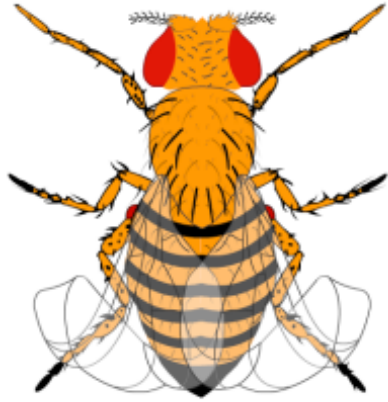
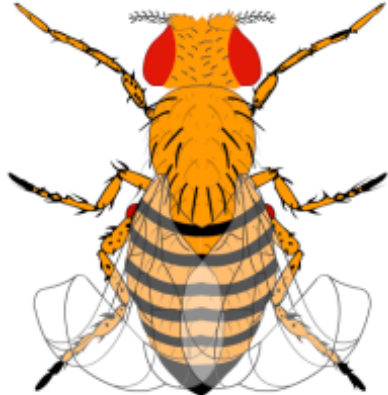

	<p><b>Parent for Cross #1</b></p> <p>Male; Curly Wings</p>
---	--

Table 27. – This table shows the offspring from the cross between heterozygous parents in Experiment E.

	<p><b>Offspring of Cross #1</b></p> <p>Male; Curly Wings</p> <p>Number with this phenotype: 401</p> <p><b>Parents:</b></p> <p>Female; Curly Wings</p> <p><b>X</b></p> <p>Male; Curly Wings</p>
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	<p><b>Offspring of Cross #1</b></p> <p>Female; Curly Wings</p> <p>Number with this phenotype: 387</p> <p><b>Parents:</b></p> <p>Female; Curly Wings</p> <p><b>X</b></p> <p>Male; Curly Wings</p>
	<p><b>Offspring of Cross #1</b></p> <p>Female; Wild Type</p> <p>Number with this phenotype: 212</p> <p><b>Parents:</b></p> <p>Female; Curly Wings</p> <p><b>X</b></p> <p>Male; Curly Wings</p>


	<p><b>Offspring of Cross #1</b></p> <p>Male; Wild Type</p> <p>Number with this phenotype: 194</p> <p><b>Parents:</b></p> <p>Female; Curly Wings</p> <p><b>X</b></p> <p>Male; Curly Wings</p>
---	--

Table 28. – This table shows the results and populations per phenotype (ignore sex) in Experiment E.

<b>Cross #1 – Parents:</b>		
(Wild Type)		
<b>X</b>		
(Wild Type)		
Sex Ignored		
<u>Phenotype</u>	<u>Number</u>	<u>Proportion</u>
Curly Wings	788	0.65
Wild Type	406	0.34
<b>Total</b>	1194	

Table twenty-nine shows us that our original hypothesis of 3:1 is wrong. The results can be interpreted instead as a 2:1 ratio. This is due to the fact that the trait that was used here (recessive curly wings) was a lethal allele. This means that an entire group of the flies that should have been an outcome from the breeding was dead upon birth. It's important to note that a



*Drosophila* fly can not live if it is homozygous recessive for curly wings. If the fly has this genotype, then it will immediately die and this is why we see the 2:1 ratio shown in the table. If we take a look at our values, we will see that the phenotype Wild Type has almost exactly half of our curly wing recessive phenotype and does support this conclusion. The chi-squared test statistic comes out to 9.1917 and tells us for sure that the original hypothesis is incorrect. Our level of significance is below the threshold for accepting the null-hypothesis and due to that this experiment does not accept, but rather rejects, the null-hypothesis.

Insert table here

### *Conclusion*

In conclusion to the results discovered in Experiment E, our data does not accept the null-hypothesis. We originally made an incorrect prediction about the expected outcome as we based our Punnett Square on the fact that the trait was simply a trait, and not a lethal trait that would essentially wipe out an entire group of genotypes. Had we done this differently, and predicted a 2:1 ratio, more than likely the results would have come out in support of the null-hypothesis and shown us that our significance from the expected values to the observed values is correct. Like mentioned above, in order for a fly to live with the curly wing trait, the trait must be recessive and the fly must be heterozygous. If this rule is not followed then like many mutations in other organisms, the fly, if it lives at all, will die almost immediately upon birth.

## **Experiment F: Linkage Map**

### *Materials and Methods*

For this experiment we will need to order one fly possessing three x-linked traits and another fly that is a Wild Type. The traits we are using in this portion are as follows: fork bristles, white eye color and miniature wings. After getting the flies in we have arranged them inside of a mating jar to be incubated for about two weeks. After the two weeks is up they are then evacuated from the mating jar onto a lab slide using a chemical called ether. Once on the lab slide they are put under a dissecting scope and sorted using a brush. Once separated, we will take a female from the cross and put it into the mating jar, and then order a male fruit fly with all of the three traits the original fly parent had. Once again the flies are incubated for two weeks to provoke breeding. After, they are yet again knocked out of the mating jar with ether and placed on lab slides for separation and data collection. After analyzing the results (ignoring sex) a table has been made showing the data collected for each phenotype (see table 30). Using the data collected we are going to create a linkage map. In order to do this, we need to sort the phenotypes into pairs, the no recombinant will be the largest, and the double crossovers are the smallest (see table 31). Once put into pairs the pairs are analyzed to select the gene that is switched in the double crossover class. This gene will be found in the middle of the phenotype string. The gene order is then written down and the remaining classes are analyzed and recorded (see table 32). The distance between the genes is then calculated using the following formula (see fig. 6 and fig. 7).

$$\text{Map Distance} = \left[ \frac{\text{Number of Nonrecombinants}}{\text{Total}} \right] * 100$$

Figure. 6. – This figure shows the equation to find map distance.

*Results and Discussion*

Table 30. – This table shows the total amount of collected data per phenotype.

<b>Phenotype</b>	<i>Total Population</i>
+	5332
<b>F</b>	1104
<b>W</b>	1673
<b>F;W</b>	366
<b>M</b>	351
<b>F;M</b>	1763
<b>W;M</b>	1077
<b>F;W;M</b>	5263
<b>Total Flies:</b>	16929

Table 31 – This table shows the phenotypes arranged into pairs.

<b>Phenotype</b>	<i>Total Population</i>		
+	5332		
<b>F;W;M</b>	5263		
<b>F;W</b>	366		
<b>M</b>	351		
<b>W</b>	1673		
<b>F;M</b>	1763		
<b>W;M</b>	1077		
<b>F</b>	1104		
<b>Total Flies:</b>	16929		



Non Recombinant



Double Crossover



Non Recombinant



Double Crossover

Table 32. – This table shows the gene order after analyzing which gene was switched.

Phenotype	Total Population		
+	5332		
<b>F;W;M</b>	5263		Non Recombinant
<b>F;W</b>	366		
<b>M</b>	351		Double Crossover
<b>W</b>	1673		
<b>F;M</b>	1763		Non Recombinant <i>W-M Singles</i>
<b>W;M</b>	1077		
<b>F</b>	1104		Double Crossover <i>M-F Singles</i>
<b>Total Flies:</b>	16929		

$$W - M \text{ Distance} = \frac{1673 + 1763 + 366 + 351}{16929} * 100 = 24.5319 \text{ Map Units}$$

$$M - F \text{ Distance} = \frac{1077 + 1104 + 366 + 251}{16929} * 100 = 16.5279 \text{ Map Units}$$

Figure 7. – This figure shows the math equations used to calculate map unit distance between each pair of genes.

As shown in the above figure and in the previous figure that explains the math formula used to calculate map distance, we added up all of our population of one gene pair and divided by the total population. This will give us a nasty looking decimal number so in order to get away

from that and into a reasonable range, we multiple it by one-hundred. This gives us the final number which is in terms of map units. This number explains exactly how many map units one gene is from another on the chromosome.

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