The Genetics of *Drosophila melanogaster*

Thomas Hunt Morgan, a geneticist who worked in the early part of the twentieth century, pioneered the use of the common fruit fly as a model organism for genetic studies. The reason it is so widely used is because it is easily cultured in the lab, has a short generation time, has a diploid number of 8 (4 homologous pairs of chromosome), are inexpensive, have easily seen traits, and can produce many offspring.

The life cycle (from egg to adult) takes about 10 days at room temperature. Eggs are laid and hatch into first instar larvae. These larvae feed voraciously on the culture medium provided. You can observe this by looking at a culture bottle - you should see many tunnels in the medium made by small white larvae (or maggots). These first instar larvae go through several instar stages and eventually the third instar larvae crawl up the sides of the bottle away from the culture medium. There they stop and their larval cuticle hardens forming a dark brown pupa. Metamorphosis takes place during the pupal stage. Larvae tissues degenerate and reorganize forming an adult fly inside the pupal case. When metamorphosis is complete, the adult fly emerges from the pupal case. After the fly emerges, the wings expand and dry, the abdomen becomes more rotund, and the color of the body darkens.

**Sexing flies:** Male and female fruit flies can be distinguished from each other in three ways:

1) Only males have a sex comb, a fringe of black bristles on the forelegs.
2) The tip of the abdomen is elongate and somewhat pointed in females and more rounded in males.
3) The abdomen of the female has seven segments, whereas that of the male has only five segments.
**Genetic notation:** In fruit fly genetics, the normal fly is called a "wild type" and any fly exhibiting a phenotypic mutation is called a "mutant". Mutant flies are given names that generally denote the type of mutation the fly exhibits. For example, the mutant "ebony" has a much darker body than the wild type fly. Each mutation is also given a letter code. Thus, in the case of ebony, the code is a lower case e. The wild type fly is denoted by a +. A white eyed mutation is given the code w. A sepia eye mutation is given the code se. The wild type is still denoted by a +.

**Types of Inheritance in a Cross:** Assume you identify 100 flies and record the following data for the offspring of an unknown cross involving a single trait.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>35</td>
<td>41</td>
<td>76</td>
</tr>
<tr>
<td>Sepia eye</td>
<td>11</td>
<td>13</td>
<td>24</td>
</tr>
</tbody>
</table>

What are the possible crosses that could have produced this pattern of offspring?

1. se se X + + (homozygous sepia crossed with homozygous wild type)
2. + se X + se (heterozygous wild type crossed with heterozygous wild type)
3. + se X se se (heterozygous wild type crossed with homozygous sepia)
4. + se X + + (heterozygous wild type crossed with homozygous wild type)

There appears to be a ration of 3:1 wild type to recessive. Cross 2 would be the likely parents. To test your hypothesis that the observed ratio of 76:24 is the same as the expected ratio of 3:1, we can use a statistic called the chi square statistic.
**Chi square statistic:**

A chi square ($X^2$) statistic is used to investigate whether observed and expected ratios differ due to chance. In statistical testing we always use a null hypothesis that any difference is due to chance. In our case, we can use the actual observed number of flies of each type as our observed values. We can find the expected number of flies of each type for a 3:1 ratio by using the same number of flies (100 flies) and dividing by 4 to give us expected values of 75:25 for a 3:1 ratio of 100 flies.

Calculate the chi square statistic ($x^2$) by completing the following steps:

1. For each observed number in the table subtract the corresponding expected number ($O - E$).
2. Square the difference [$ (O - E)^2 $].
3. Divide the squares obtained for each cell in the table by the expected number for that cell [$ (O - E)^2 / E $].
4. Sum all the values for $(O - E)^2 / E$. This is the chi square statistic.

The calculation would be:

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>Expected</th>
<th>$(O - E)$</th>
<th>$(O - E)^2$</th>
<th>$(O - E)^2 / E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>76</td>
<td>75</td>
<td>1</td>
<td>1</td>
<td>0.013</td>
</tr>
<tr>
<td>Mutant type</td>
<td>24</td>
<td>25</td>
<td>-1</td>
<td>1</td>
<td>0.042</td>
</tr>
<tr>
<td>totals</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$x^2 = 0.055$

For our data there are two categories, wild type and sepia, so degrees of freedom is 2-1=1. Determine the probability of your chi-square value on the distribution table below. Is the probability 5% or greater ($p = or > 0.05$)?

If so, you can accept the null hypothesis that a ratio of 76:24 is the same as a 75:25 ratio. It means that we do have a 3:1 ratio of wild type to mutant offspring and that these offspring could have come from a cross between two heterozygotes.
Go to the web site: http://www.sciencecourseware.org/vcise/drosophila/
Click on enter as a guest.

Of course, fruit flies also have sex chromosomes and they contain a subset of genes as well. If the gene is located on a sex chromosome, we use a slightly different notation. Under normal diploid conditions a female fruit fly has two X chromosomes, a male has an X and a Y chromosome. Sex-linked genes are located on one of the sex chromosomes (usually the X chromosome). Thus, the genotypic notation for a mutant gene for white eye color on the X chromosome would look like:

- \( X^+ X^+ \) = wild type female
- \( X^w X^w \) = white-eyed female
- \( X^+X^w \) = wild type heterozygote female
- \( X^w Y \) = white-eyed male
- \( X^+ Y \) = wild type male

You will perform the following cross.

\[ \text{P: } X^+ X^+ \text{ crossed with } X^w Y \]

\[ \text{F}_1 \text{ Hypothesis: } (\text{you may wish to work out a Punnett square}) \]

\[ \text{F}_2 \text{ Hypothesis: } (\text{you may wish to work out a Punnett square}) \]
To perform the cross:

1. Click on computer to order flies.
2. Click on “order flies”.
3. A female wild type fly appears on the screen. Click on the green cart symbol to add her to your shopping cart.
4. A male wild type fly appears on the screen. Click on eye color to the right of the fly. Select white. Then click on the green cart symbol to add him to your shopping cart.
5. Now click on your shopping cart and select ‘check out’. Click on yes when asked if you are sure.
6. When flies arrive, click on box. Then click on the mating jar to mate the P generation flies.
7. Click on the mating jar, then the incubator door to select the flies in the mating jar. The flies are knocked out and placed under a dissection microscope.
8. Click on sort flies.
9. Click on the pile on the left side of the screen and examine.
   Record gender_____ Number ______ Phenotype _____ Genotype______
   Then click on ‘use in new mating jar’, followed by ‘ok’ and ‘zoom out’.
10. Click on the pile on the right side of the screen and examine.
   Record gender_____ Number ______ Phenotype _____ Genotype______
   Then click on ‘use in new mating jar’, followed by ‘ok’ and ‘zoom out’.
   Finally, click on ‘return to lab’.
11. Click on mating jar to mate the F₁ flies.
12. Click on the mating jar, then incubator and select the mating jar for cross #2. Once knocked out, click on ‘sort flies’. There are three piles: Record the number and gender of each in the space below.
   Pile 1: gender ____________ number ____________
   Pile 2: gender__________ number ____________
   Pile 3: gender__________ number ____________
13. Click on send data to computer, followed by clicking on ‘yes’.
14. Click on analyze data.
15. Click on chi-square analysis.
16. Calculate expected values based on your F₂ hypothesis and insert them into the table.
17. Click on ‘test this hypothesis’.
18. Print out your chi-square analysis
19. On the chi-square analysis, explain if you accept the hypothesis. Why or why not?
Now lets examine genetic linkage. Linked genes are located on the same chromosome. We are going to run THREE different dihybrid crosses and use the data to determine the order of genes on chromosome two of *Drosophila melanogaster*.

1. Click on computer to order flies.
2. Click on “order flies”.
3. A female wild type fly appears on the screen. Click on the green cart symbol to add her to your shopping cart.
4. A male wild type fly appears on the screen. Click on **eye color** to the right of the fly. Select brown. Then click on **wing size** and select vestigial. Now click on the green cart symbol to add him to your shopping cart.
5. Now click on your shopping cart and select ‘check out’. Click on yes when asked if you are sure.
6. When flies arrive, click on box. Then click on the mating jar to mate the P generation flies.
7. Click on the mating jar, then the incubator door to select the flies in the mating jar. The flies are knocked out and placed under a dissection microscope.
8. Click on sort flies.
9. Click on the pile of female flies.

What is their genotype? _______________ their phenotype? _______________

Then click on ‘use in new mating jar’, followed by ‘ok’ and ‘zoom out’.

Click on ‘return to lab’.

10. Click on order flies.
11. Click on male. Then click **eye color** and select brown. Click on **wing size** and select vestigial. Add to card and check out.
12. Click on box and mating jar to mate these males with your females from cross #1.
13. Click on incubator and select the mating jar for cross #2. Once knocked out, click on ‘sort flies’. (There should be eight piles)
14. Click on send data to computer, followed by clicking on ‘yes’.
15. Click on analyze data.
16. Click on ‘ignore gender’ since there is not a significant difference between male and females results.
17. Click on chi-square analysis.
18. Calculate expected values based on your F2 hypothesis and insert them into the table.
19. Click on ‘test this hypothesis’.
20. Print out your chi-square analysis.
21. On the chi-square analysis, explain why your results are significant and calculate the crossing over frequency of the **eye color** and **wing size** genes.
Repeat the same procedures but for **body color and wing size**.

1. Click on computer to order flies.
2. Click on “order flies”.
3. A female wild type fly appears on the screen. Click on the green cart symbol to add her to your shopping cart.
4. A male wild type fly appears on the screen. Click on **body color** to the right of the fly. Select black. Then click on **wing size** and select vestigial. Now click on the green cart symbol to add him to your shopping cart.
5. Now click on your shopping cart and select ‘check out’. Click on yes when asked if you are sure.
6. When flies arrive, click on box. Then click on the mating jar to mate the P generation flies.
7. Click on the mating jar, then the incubator door to select the flies in the mating jar. The flies are knocked out and placed under a dissection microscope.
8. Click on sort flies.
9. Click on the pile of female flies
   
   What is their genotype? _______________ their phenotype? _______________

Then click on ‘use in new mating jar’, followed by ‘ok’ and ‘zoom out’.
Click on ‘return to lab’.
10. Click on order flies.
11. Click on male. Then click **body color** and select black. Click on **wing size** and select vestigial. Add to card and check out.
12. Click on box and mating jar to mate these males with your females from cross #1.
13. Click on incubator and select the mating jar for cross #2. Once knocked out, click on ‘sort flies’. (There should be eight piles)
14. Click on send data to computer, followed by clicking on ‘yes’.
15. Click on analyze data.
16. Click on ‘ignore gender’ since there is not a significant difference between male and females results.
17. Click on chi-square analysis.
18. Calculate expected values based on your F<sub>2</sub> hypothesis and insert them into the table.
19. Click on ‘test this hypothesis’.
20. Print out your chi-square analysis
21. On the chi-square analysis calculate the crossing over frequency of the **body color** and **wing size** genes.
Repeat the same procedures but for **body color and eye color**.

1. Click on computer to order flies.
2. Click on “order flies”.
3. A female wild type fly appears on the screen. Click on the green cart symbol to add her to your shopping cart.
4. A male wild type fly appears on the screen. Click on **body color** to the right of the fly. Select black. Then click on **eye color** and select brown. Now click on the green cart symbol to add him to your shopping cart.
5. Now click on your shopping cart and select ‘check out’. Click on yes when asked if you are sure.
6. When flies arrive, click on box. Then click on the mating jar to mate the P generation flies.
7. Click on the mating jar, then the incubator door to select the flies in the mating jar. The flies are knocked out and placed under a dissection microscope.
8. Click on sort flies.
9. Click on the pile of female flies
   
   What is their genotype? ___________________ their phenotype? ___________________

Then click on ‘use in new mating jar’, followed by ‘ok’ and ‘zoom out’.

Click on ‘return to lab’.
10. Click on order flies.
11. Click on male. Then click **body color** and select black. Click on **eye color** and select brown. Add to card and check out.
12. Click on box and mating jar to mate these males with your females from cross #1.
13. Click on incubator and select the mating jar for cross #2. Once knocked out, click on ‘sort flies’. (There should be eight piles)
14. Click on send data to computer, followed by clicking on ‘yes’.
15. Click on analyze data.
16. Click on ‘ignore gender’ since there is not a significant difference between male and females results.
17. Click on chi-square analysis.
18. Calculate expected values based on your $F_2$ hypothesis and insert them into the table.
19. Click on ‘test this hypothesis’.
20. Print out your chi-square analysis
21. On the chi-square analysis calculate the crossing over frequency of the **body color** and **eye color** genes.
22. **Draw a map of the chromosome showing the body color, eye color, and wing size genes.**