LAB SEVEN GENETICS OF ORGANISMS

OVERVIEW

In this lab you will use living organisms to do genetic crosses. You will learn how to collect and manipulate the organisms, collect data from F_1 and F_2 generations, and analyze the results from a monohybrid, dihybrid, or sex-linked cross. The procedures that follow apply to fruit flies; your teacher may substitute other procedures using other organisms.

OBJECTIVES

Before doing this lab you should understand:

- chi-square analysis of data, and
- the life cycle of diploid organisms useful in genetics studies.

After doing this lab you should be able to:

- investigate the independent assortment of two genes and determine whether the two genes are autosomal or sex-linked using a multigeneration experiment, and
- analyze the data from your genetic crosses using chi-square analysis techniques.

INTRODUCTION

Drosophila melanogaster, the fruit fly, is an excellent organism for genetics studies because it has simple food requirements, occupies little space, is hardy, completes its life cycle in about 12 days at room temperature, produces large numbers of offspring, can be immobilized readily for examination and sorting, and has many types of hereditary variations that can be observed with low-power magnification. *Drosophila* has a small number of chromosomes (four pairs). These chromosomes are easily located in the large salivary gland cells. *Drosophila* exists in stock cultures that can be readily obtained from several sources. Much research about the genetics of *Drosophila* during the last 50 years has resulted in a wealth of reference literature and a knowledge about hundreds of its genes.

The Life Cycle of *Drosophila*

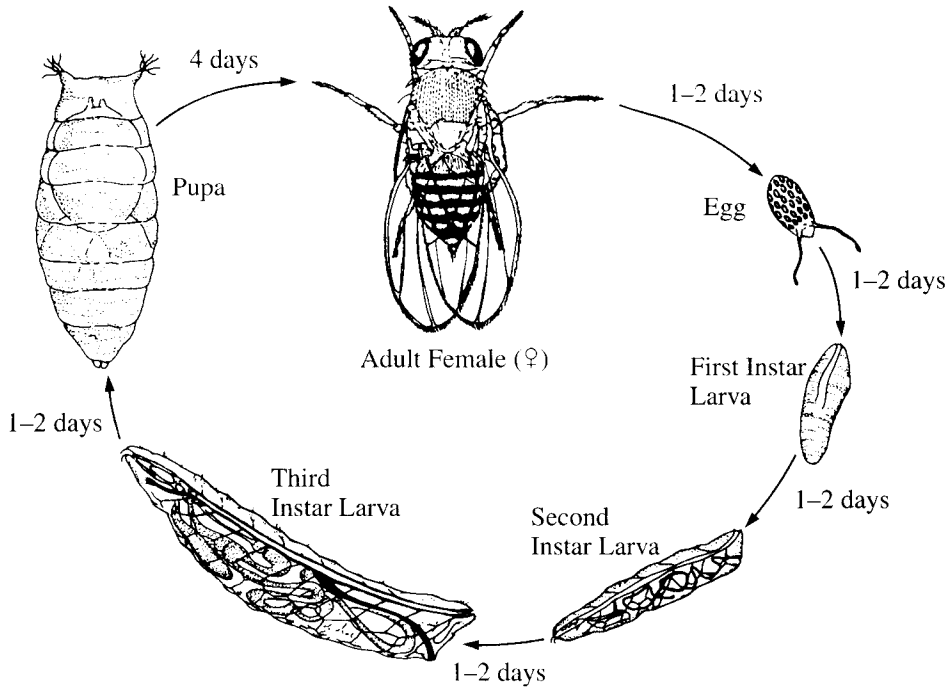
The Eggs. The eggs are small, oval shaped, and have two filaments at one end. They are usually laid on the surface of the culture medium and, with practice, can be seen with the naked eye. The eggs hatch into larvae after about a day.

The Larval Stage. The wormlike larva eats almost continuously, and its black mouth parts can easily be seen moving back and forth even when the larva itself is less distinct. Larvae tunnel through the culture medium while eating; thus, channels are a good indication of the successful growth of a culture. The larva sheds its skin twice as it increases in size. In the last of the three larval stages, the cells of the salivary glands contain giant chromosomes, which may be seen readily under low-power magnification after proper staining.

The Pupal Stage. When a mature larva in a lab culture is about to become a pupa, it usually climbs up the side of the culture bottle or on to the strip provided in the culture bottle. The last larval covering then becomes harder and darker, forming the pupal case. Through this case the later stages of metamorphosis to an adult fly can be observed. In particular, the eyes, the wings, and the legs become readily visible.

The Adult Stage. When metamorphosis is complete, the adult flies emerge from the pupal case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of an adult fly. They live a month or more and then die. A female does not mate for about ten to twelve hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. To ensure a controlled mating, it is necessary to use females that have not mated before (virgins).

Figure 7.1: The Life Cycle of *Drosophila melanogaster*



It is important to realize that a number of factors determine the length of time of each stage in the life cycle. Of these factors, temperature is the most important. At room temperature (about 25°C), the complete cycle takes ten to twelve days.

Design of the Exercise

This genetics experiment will be carried on for several weeks. *Drosophila* with well-defined mutant traits will be assigned to you by your teacher. You are responsible for making observations and keeping records concerning what happens as mutant traits are passed from one generation to the next.

You will be assigned to study a certain mode of inheritance using particular genetic crosses of flies having one or two mutations. The modes of inheritance most commonly used are:

1. **Monohybrid.** In these experiments the mode of inheritance is determined when a single contrasting pair of characteristics is involved.

2. **Dihybrid.** In these experiments the mode of inheritance is determined when two pairs of contrasting characteristics are considered simultaneously.

3. **Sex-linked.** In these experiments the mode of inheritance is determined when the mutant characteristic is associated with the X-chromosome.

To make these experiments interesting and challenging, you will not be told the mode of inheritance, nor the name for the particular mutation(s) you are studying. Study the wild type flies (both male and female) until their phenotypic characteristics are familiar. Flies having one or two mutations can then be identified by making comparisons with the wild type flies. The most commonly studied mutations are in eye color or shape, bristle number or shape, wing size or shape, or antenna size or shape. You should make up your own name for the particular mutation(s) that you identify in your flies.

Procedure

1. Obtain a vial of wild type flies. Practice immobilizing and sexing (determining the gender of) these flies. Examine these flies and note the characteristics of their eyes, wings, bristles, and antennae.

2. To make handling easier, immobilize the flies by chilling them. Since the activity level of the flies is dependent on environmental temperature, the following steps immobilize the flies. (Your teacher may assign a different method.)

   a. Hold the vial containing the flies at an angle and twirl it in ice for several minutes.

   b. When the flies are immobilized, dump them into a small, plastic petri dish containing a #1 Whatman filter paper.

   c. Place the petri dish on top of the ice in order to maintain the cool temperature necessary to keep the flies immobilized.

   d. Use the dissecting microscope to view the flies. The top of the petri dish can be on or off when viewing.

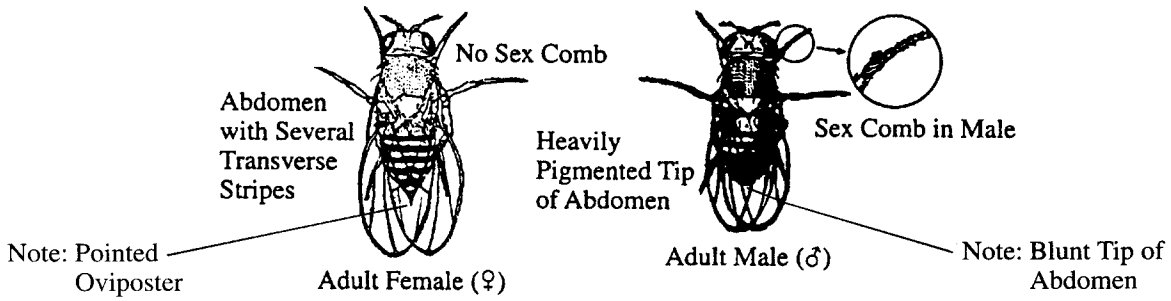
3. Distinguish male flies from female flies by looking for the following characteristics (illustrated in Figure 7.2):

   a. Males are usually smaller than females.

   b. Males have dark, blunt abdomens, and females have lighter, pointed abdomens.

   c. Only the males have sex combs, which are groups of black bristles on the uppermost joint of the forelegs.

Figure 7.2: Female and Male *Drosophila*



4. Obtain a vial containing pairs of experimental flies. Record the cross number of the vial. This number will serve as a record as to which cross you have obtained. These flies are the parental generation (P) and have already mated. The females should have already laid eggs on the surface of the culture medium. The eggs (or maybe larvae now) represent the first filial, F_1, generation and will be emerging from their pupal cases in about a week.

5. **First Week (Today).** Immobilize and remove the adult flies. Observe them carefully under the dissecting microscope. Separate the males from the females and look for the mutation(s). Note whether the mutation(s) is/are associated with the males or the females. Identify the mutation(s) and give it/them a made-up name and symbol. Record the phenotype and symbol in Table 7.1. The findings should be confirmed by your teacher.

6. Place the parents in the morgue (a jar containing alcohol or baby oil). Label the vial containing the eggs or larvae with symbols for the mating. For example, if a sepia-eyed female is crossed with a wild-type male, the label could be "sepia ♀ X wild ♂". Also be sure to label the vial with your name and the date. Place the vial in a warm location.

7. **Second Week.** Begin by observing the F_1 flies. Immobilize and examine all the flies. Record their sex and the presence or absence of the mutation(s) (as observed in the parental flies) in Table 7.1. Consider the conclusions that can be drawn from these data. Place 5 or 6 pairs of F_1 flies in a fresh culture bottle and the rest of the flies in the morgue. For this cross the females need not be virgins. Label the new vial "F_1 X F_1". Also, label the vial with symbols denoting the cross, the date, and your name.

8. **Third Week.** Remove the F_1 flies from the vials and place them in the morgue. The F_2 generation are the eggs and/or larvae in the vial. Place the vial back in the warm location.

9. **Fourth Week.** Begin removing the F_2 flies. Record their sex and the presence or absence of the mutant phenotypes (as observed in the parental flies in Table 7.2). The more F_2 flies collected, the more reliable the data will be. You may have to collect flies over a 3- or 4-day period. Try to collect at least 200 flies.

10. To analyze your data, you will need to learn how to use the chi-square test. Go to the Statistical Analysis Section (page 85) to review this technique.

Table 7.1: F_1 Generation Data **Date** _____

Phenotype and Symbol	Females	Males

Table 7.2: F_2 Generation Data **Date** _____

Phenotype and Symbol	Females	Males

Analysis of Results

1. Describe and name the observed mutation(s).

_____

_____

_____

_____

_____

2. Write a hypothesis that describes the mode of inheritance of the trait(s) you studied. This is your null hypothesis (as described in the Statistical Analysis Section.)

3. Refer to a textbook and review Punnett squares. In the space below construct two Punnett squares to predict the expected results of both the parental and F_1 crosses from your null hypothesis.

Parental Cross	F_1 Cross

4. Refer to the Punnet Squares above. In the box below record the *expected* ratios for the genotypes and phenotypes of the F_1 and F_2 crosses in the experiment.

	Expected Genotypic Ratio	Expected Phenotypic Ratio
F_1		
F_2		

5. Do the actual results deviate from what was expected? If so, explain how.

6. From the results, describe your cross.

   Is the mutation sex-linked or autosomal? _____

   Is the mutation a dominant or recessive? _____

   Is the cross monohybrid or dihybrid? _____

7. Are the deviations for the phenotypic ratio of the F_2 generation within the limits expected by chance? To answer this question, statistically analyze the data using the chi-square analysis. Calculate the chi-square statistic for the F_2 generation in the chart below. Refer to the critical values of the chi-square (χ^2) distribution table (Table 7.5 on page 86) to determine the p (probability) value that is associated with your χ^2 statistic.

Phenotype	# Observed (o)	# Expected (e)	(o−e)	$(o-e)^2$	$\frac{(o-e)^2}{e}$
				$\chi^2 =$	

a. Calculate the chi-square value for these data.

   1. How many degrees of freedom are there? _____
   2. chi-square (χ^2) = _____
   3. Referring to the critical values chart, what is the probability value for this data?

      _____

b. According to the probability value, can you accept or reject your null hypothesis? Explain why.

_____

_____

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Topics for Discussion

1. Why was it necessary for the females of the parental generation to be virgins?

2. Why was it not necessary to isolate virgin females for the F_1 cross?

3. Why were the adult flies removed from the vials at weeks 2 and 4?

LAB SEVEN STATISTICAL ANALYSIS SECTION

Using the Chi-Square Test for Statistical Analysis of Experimental Data

Example 1

Statistics can be used to determine if differences among groups are significant, or simply the result of predictable error. The statistical test most frequently used to determine whether data obtained experimentally provide a good fit or approximation to the expected or theoretical data is the chi-square test. This test can be used to determine if deviations from the expected values are due to chance alone, or to some other circumstance. For example, consider corn seedlings resulting from an F_1 cross between parents that are heterozygous for color.

A Punnett square of the F_1 cross **Gg X Gg** would predict that the expected proportion of green:albino seedlings would be 3:1. Use this information to fill in the Expected (e) column and the (o−e) column in Table 7.3.

Table 7.3

Phenotype	Genotype	# Observed (o)	# Expected (e)	(o−e)
Green	GG or Gg	72		
Albino	gg	12		
	Total:	84		

There is a small difference between the observed and expected results, but are these data close enough that the difference can be explained by random chance or variation in the sample?

To determine if the observed data fall within acceptable limits, a chi-square analysis is performed to test the validity of a **null hypothesis** (that there is no statistically significant difference between the observed and expected data). If the chi-square analysis indicates that the data vary too much from the expected 3:1, an **alternative hypothesis** is accepted.

The formula for chi-square is:

$$\chi_2 = \Sigma \frac{(o-e)^2}{e}$$

where **o** = **observed** number of individuals

e = **expected** number of individuals

Σ = the **sum of the values** (in this case, the differences, squared, divided by the number expected)

1. This statistical test will examine the null hypothesis, which predicts that the data from the experimental cross above will be expected to fit the 3:1 ratio.
2. Use the data from Table 7.3 to complete Table 7.4.

Table 7.4

Phenotype	# Observed (o)	# Expected (e)	(o−e)	$(o-e)^2$	$\frac{(o-e)^2}{e}$
Green	72				
Albino	12				
				$\chi^2 = \Sigma\frac{(o-e)^2}{e} =$	

3. Your calculations should give you a value for $\chi^2 = 5.14$. This value is then compared to Table 7.5.

Table 7.5: Critical Values of the Chi-Square Distribution

	Degrees of Freedom (df)				
Probability (p)	1	2	3	4	5
0.05	3.84	5.99	7.82	9.49	11.1
0.01	6.64	9.21	11.3	13.2	15.1
0.001	10.8	13.8	16.3	18.5	20.5

How to Use the Critical Values Table

1. Determine the **degrees of freedom (df)** for your experiment. It is the number of phenotypic classes minus 1. Since there are two possible genotypes, for this experiment df = 1 (2 samples –1). If the experiment had gathered data for a dihybrid cross, there would be four possible phenotypes and therefore 3 degrees of freedom.

2. Find the p value. Under the 1 df column, find the critical value in the probability (p) = 0.05 row: it is 3.84. What does this mean? **If the calculated chi-square value is greater than or equal to the critical value from the table, then the null hypothesis is rejected.** Since for our example $\chi^2 = 5.14$ and $5.14 > 3.84$, we reject our null hypothesis that there is no statistically significant difference between the observed and expected data. In other words, chance alone cannot explain the deviations we observed and there is, therefore, reason to doubt our original hypothesis (or to question our data collection accuracy.) The minimum probability for rejecting a null hypothesis in the sciences is generally 0.05, so this is the row to use in the chi-square table.

3. These results are said to be significant at a probability of p = 0.05. This means that only 5% of the time would you expect to see similar data if the null hypothesis was correct; thus, you are 95% sure that the data do not fit a 3:1 ratio.

4. Since these data do not fit the expected 3:1 ratio, you must consider reasons for this variation. Additional experimentation would be necessary. Perhaps the sample size is too small, or errors were made in data collection. In this example, perhaps the albino seedlings are underrepresented because they died before the counting was performed.

Example 2

In a study of incomplete dominance in tobacco seedlings, the counts in Table 7.6 were made from a cross between two heterozygous (Gg) plants:

Table 7.6

Phenotype	Genotype	# Observed (O)
Green	GG	22
Yellow Green	Gg	50
Albino	gg	12
	Total:	84

A Punnett square for this cross indicates that the expected counts should be in a 1 green:2 yellow green:1 albino ratio (Table 7.7). The expected values for a total count of 84 organisms are therefore:

$$1 \text{ green} = 1/4 \times 84 = 21$$

$$2 \text{ yellow green} = 1/2 \times 84 = 42$$

$$1 \text{ yellow} = 1/4 \times 84 = \frac{21}{84}$$

Table 7.7

Phenotype	# Observed (o)	# Expected (e)	(o−e)	$(o-e)^2$	$\frac{(o-e)^2}{e}$
Green	22	21	1	1	0.05
Yellow Green	50	42	8	64	1.52
Albino	12	21	9	81	3.86
				$\chi^2 = \Sigma\frac{(o-e)^2}{e} =$	5.43

Go to the chi-square table, this time for two degrees of freedom (there are three phenotypes: 3 – 1 = 2 df). If the χ^2 value were greater than or equal to the critical value of 5.99 we would reject our hypothesis. Since 5.43 is less than the critical value at p = .05, we accept the null hypothesis (this second data set does fit the expected 1:2:1 ratio).

Practice Problem

An investigator observes that when pure-breeding, long-wing *Drosophila* are mated with pure-breeding, short-wing flies, the F_1 offspring have an intermediate wing length.

When several intermediate-wing-length flies are allowed to interbreed the following results are obtained:

Observed

230 long wings
510 intermediate-length wings
260 short wings

a. What is the genotype of the F_1 intermediate-wing-length flies? _____

b. Write a hypothesis describing the mode of inheritance of wing length in *Drosophila* (this is your null hypothesis).

_____

_____

_____

c. Complete Table 7.8.

Table 7.8

Phenotype	# Observed (o)	# Expected (e)	(o − e)	$(o-e)^2$	$\frac{(o-e)^2}{e}$
				$\chi^2 = \Sigma\frac{(o-e)^2}{e} =$	

(i) Calculate the chi-square value for these data.

1. How many degrees of freedom (df) are there? _____
2. χ^2 (chi-square) = _____
3. Referring to the critical values chart, what is the probability value for these data?

   _____

(ii) According to the critical value of χ^2, can you accept or reject the null hypothesis? Explain why.

_____

_____

_____

_____

_____