



The Biotechnology Education Company®

EDVOTEK®

**287**

EDVO-Kit #

## The Science of *Drosophila* Genetics

### Storage:

Store entire experiment  
at room temperature.

### EXPERIMENT OBJECTIVE

The objective of this experiment is for students to understand the life cycle of the fruit fly, *Drosophila melanogaster*, in a genetics experiment over several generations. Actual results will be compared with predicted results and a chi-square analysis performed. Students will observe the independent assortment and sex-linkage of genes.

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## EDVO-Kit # 287 The Science of *Drosophila* Genetics

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This experiment is designed for 10 lab groups

## Experiment Components

- A Acetocarmine Stain
- B EDVOTEK® BugOut™ Solution
- C NaCl

*Drosophila* Culture Tubes  
Foam Lids  
EDVOTEK® Instant *Drosophila* Growth Media

**Storage:**  
Store entire experiment at room temperature.

### Shipped Separately:

- 1 Vial Wild type *Drosophila*
- 1 Vial Sepia *Drosophila*
- 1 Vial Vestigial wing *Drosophila*
- 1 Vial White eye *Drosophila*

**Flies must be requested from EDVOTEK® 3 weeks prior to early Set-up/Preparation for experiment.**

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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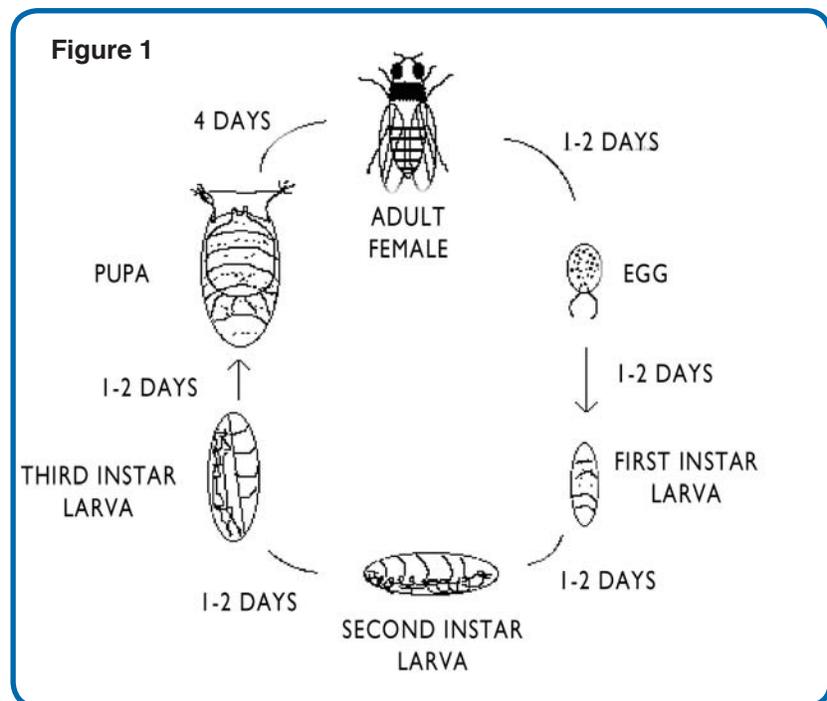
## Requirements

- Dissecting microscopes
- Microscope
- Slides
- Sodium chloride
- Pasteur pipets
- Petri plates
- Filter paper
- Camel-hair brushes
- Mineral or vegetable oil
- Alcohol

## Background Information

*Drosophila melanogaster*, or fruit fly, is the small fly often found living on fruit. It is amazing that this diminutive creature has played a major role in the development of modern genetics during the 20th century. The short 14 day life cycle, as shown in Figure 1, and the relative ease of handling contributed to the selection of *Drosophila* as a model system for eucaryotic genetic analysis by the American biologist, Thomas Hunt Morgan. Important genetic concepts, such as gene linkage, crossing over, and mutational events can trace their roots to early studies on *Drosophila*. The importance continues today as molecular biologists try to understand diverse biological phenomenon, such as transposable elements, heat shock response, oncogenes, and development of human cancer.

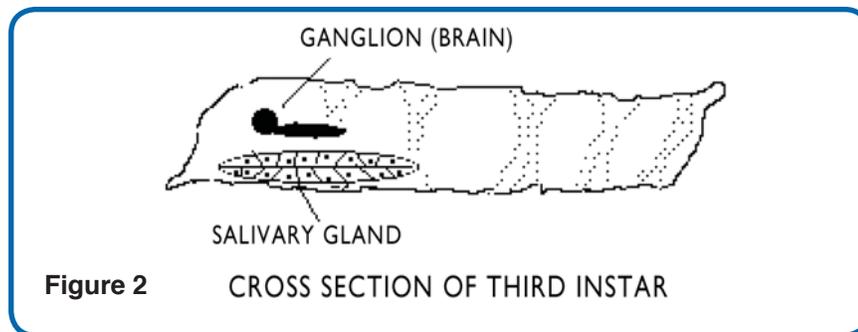
After fertilization, embryonic development occurs within the egg, followed by the larva, pupa and adult stages. The length of this cycle will vary depending upon the temperature. The stages from the egg to the end of the third instar larva is approximately 8 days at 20°C (room temperature) and approximately 5 days at 25°C. The pupa, which is the last stage before the adult fly emerges, is approximately 6-7 days at 20°C (room temperature) and approximately 4-5 days at 25°C. The entire life cycle is, therefore, 15 days at 20°C and 10 days at 25°C



## Background Information

The EGG is only 0.5 mm in length. Filaments extend from the egg and prevent it from sinking into the media upon which it was laid. The egg is covered by a chorion under which lies a rigid chitinous layer. The female stores many sperm during mating. Although many sperm enter the egg, only one functions in fertilization.

The LARVAL stages, which follow the hatching, are periods of intense eating. After hatching, there are 2 additional molts. The larval period consists of three stages called instars. The media will become gutted by the action of these hungry larva. The salivary chromosomes for Part B of this exercise are obtained from the third instar larva.



The PUPA is the stage immediately preceding the adult stage. The larvae will crawl from the media up the side of the vial to a relatively dry area. The larvae will pupate within the skin of the third instar. It will eventually become dark and hard. The adult fly organs and body shape are developing within the pupal case. The fly will emerge from the pupal case by pushing its way out. The newly emerged fly is rather long and the wings appear shortened. After a short time, the body becomes round and the wings assume their full length. Newly emerged flies are lighter in color than older flies.

The ADULT flies may live several weeks. The female mates about 10 hours after emerging from the pupa. It is essential, therefore, to collect virgin females within 10 hours of emergence. The female will usually mate only once and store the sperm for her lifetime.

The experiments performed in this laboratory are simple and introduce key concepts of classical genetics. Inheritance of phenotypes, including eye color and wing structure, will be observed through a series of controlled mating experiments. Concepts of dominant, recessive and sex-linked genes will be demonstrated.

## Background Information

In diploid organisms, chromosomes are arranged as homologous pairs. For a given simple phenotypic trait, there will be 2 copies of a gene, one on each chromosome of the pair. More complex phenotypes require the expression of many genes and are not considered in the following discussion. Pairs of genes are said to be alleles of each other or to form allelic pairs. For each autosomal, non sex-linked gene or trait, there are two alleles. If alleles are identical, the geneticist considers the particular organism **homozygous** for that gene. In the monohybrid cross performed in this experiment, pure bred red eyed flies producing only red eyed progeny are homozygous for red eyes. The same is true of pure bred sepia eyed flies that produce only sepia eyed progeny. They are **homozygous** for sepia eyes. The sperm and eggs of these pure bred flies contain chromosomes with the same allele, either red or sepia.

Consider what happens when a homozygous red eyed fly mates with a homozygous sepia eyed fly. Every F1 (the first Filial generation) offspring has red eyes. The red eye phenotype would be considered the **dominant** trait, and sepia eyes the **recessive** trait. By convention, we will designate the dominant trait with an upper case letter, R, the recessive trait with a lower case letter, r. What is the genetic make up of these F1 flies? The parents are designated either RR or rr, one letter signifying one gene for each chromosome. Since there is independent and random segregation of the homologous chromosome pairs during meiosis, the parental homozygous flies only produce gametes of one type, R, from the red eyed flies and r from the sepia eyed flies. The genotype of the fertilized zygote must be Rr. F1 flies are heterozygous for red eyes, since they have a mixture of two different alleles. One chromosome carries the dominant gene/allele for red eyes, R. The other chromosome carries the recessive

gene/allele for sepia eyes, r. We can represent the genotype of the parental egg and sperm, and the resulting genotype of the fertilized egg which produce the F1 generation, using a **Punnet Square** shown in Figure 3. The possible genotypes for the egg and sperm are indicated outside of the box. The genotypes of the fertilized egg/F1 generation are shown within the box.

	R	R
r	Rr	Rr
r	Rr	Rr

Figure 3

What happens if F1 flies mate to each other? In this case, each F1 parent will produce two different sperm or egg (called gametes). The gametes are either R or r. We can easily predict the genotypic makeup of the F2 generation based on knowledge of reproduction and statistics. A Punnet square is used for this analysis, which can also be determined mathematically.

As shown in Figure 4, there are only 2 different sperm or eggs, R or r, and therefore, 4 different possibilities for the fertilized zygotes, RR, Rr, Rr or rr. The genotypic ratio is 1:2:1. However, the phenotypic ratio is 3:1. Since red, R, is dominant over sepia, r, three offspring will have red eyes.



## Background Information

One is homozygous red, RR, and the other 2 are heterozygous red, Rr. Only one offspring will be sepia, rr, also called the double recessive. Remember that these are statistical ratios observed only in large populations. All simple monohybrid crosses involving a single trait have a 1:2:1 genotypic ratio, and a 3:1 phenotypic ratio.

	R	r
R	RR	Rr
r	Rr	rr

Figure 4

Three genetic crosses are performed in this experiment. The monohybrid cross contrasts expressions of the same trait, red eye versus sepia eye. The dihybrid cross involves expression of two traits, red/sepia eyes and normal/vestigial wings. The third is a sex linked cross for red and white eye color with the trait carried on the X sex chromosome.

To determine whether the experimental or observed data is a good fit or an approximation of the expected or calculated data, statistical analysis will be done using the **Chi-Square test**. In general, the higher the Chi-Square value, the greater the difference between the experimental or observed frequencies versus the expected frequencies. The class will collect and share data. Actual results will be compared with expected results for each cross. The Chi-Square statistic provides an assessment of whether the difference between the observed and expected (calculated) data is too great to have occurred on the basis of chance alone. **If the difference is too great, (probability < 5%), we would reject the null hypothesis.** An alternative hypothesis is accepted if the Chi-square test indicated that the data varies too much from the expected 3:1.

The formula for chi-square is:

$$\chi^2 = \frac{\sum(o-c)^2}{c}$$

o = the observed frequencies

c = the calculated frequencies

$\Sigma$  = the **sum of the values** (or the differences, squared, divided by the number calculated) for each possible genotype.

**Degrees of Freedom = (# of Phenotypic Classes) - 1**

As an example, analyze a hypothetical monohybrid cross using the Chi-square ( $\chi^2$ ) statistic. Analyze a single Mendelian trait which determines eye color. R is for red-eye, which is dominant to the recessive r, for sepia eyes. Cross homozygous pure bred parents RR X rr. The F1 generation will be all Rr. Crossing the F1 generation, Rr X Rr, Punnet square analysis examines the null hypothesis which predicts a 0.75 probability of the red eyed phenotype (RR

## Background Information

and Rr), and a 0.25 probability of the sepia eyed phenotype (rr). Expected frequencies are 0.75 and 0.25. Analysis of 1000 flies yields results of 735 red eyed flies and 265 sepia eyed flies. Are these results valid for the null hypothesis? Table 1 shows the calculations for X<sup>2</sup> analysis of this data. The X<sup>2</sup> value equals 1.2.

Determine the **degrees of freedom (df)** for the experiment, by taking the number of phenotypic class minus 1. In a monohybrid cross there are two phenotypic classes, so the **df = 1**. A probability value, or p-value, is used to evaluate the significance of a Chi-Square. Scientists often set the cutoffs for significance at p-value of 0.05 (5%) or less. (In other words, there is a 95% probability that the differences between experimental versus expected frequencies can be attributed to chance). Statisticians consider anything above the 0.05 value to be significant. In Table 2 (page 9), the bold P = 0.05 column indicates that 95% of experimental trials will have a X<sup>2</sup> value which lies to the left of the 0.05 column value. Only 0.05 or 5 % of all possible trials would have a X<sup>2</sup> value greater than the value in the P= 0.05 column. These data, with one degree of freedom (2 phenotypic classes -1), has a P value which falls between 0.2 and 0.3. Using the hypothesis, we could expect a X<sup>2</sup> value of 1.2 to occur in 20% to 30% of such experiments. Therefore, the observed results could occur simply by chance alone and the null hypothesis is not rejected. If the calculated X<sup>2</sup> value was greater than 3.841 for one degree of freedom, a statistician would consider that result significant and unlikely to occur by chance alone. It would be necessary to reject the null hypothesis. Both the calculated frequency and the hypothesis on which it was based, appear sound. Thus there is a "Goodness of Fit" between the observed results and the calculated expectancy.

TABLE 1

Phenotype	Observed	Calculated	O - c	(O - c) <sup>2</sup>	(O - c) <sup>2</sup> / c
Red Eyes	735	750	- 15	225	0.30
Sepia Eyes	265	250	15	225	0.90
<b>TOTALS</b>	1000	1000			<b>x<sup>2</sup> = 1.20</b>



## Background Information

TABLE 2

	P = 0.99	P = 0.95	P = 0.90	P = 0.8	P = 0.7	P = 0.5	P = 0.3	P = 0.2	<b>P = 0.05</b>	P = 0.01
Degrees of Freedom										
1	0.000157	0.000393	0.0158	0.0642	0.148	0.455	1.074	1.642	<b>3.841</b>	6.635
2	0.020	0.103	0.211	0.446	0.713	1.386	2.408	3.219	<b>5.991</b>	9.210
3	0.115	0.352	0.584	1.005	1.424	2.366	3.665	4.642	<b>7.815</b>	11.345
4	0.297	0.711	1.004	1.649	2.195	3.357	4.878	5.989	<b>9.488</b>	13.277
5	0.554	1.145	1.610	2.343	3.000	4.351	6.064	7.289	<b>11.070</b>	15.086
6	0.872	1.635	2.204	3.070	3.828	5.348	7.231	8.558	<b>12.592</b>	16.812
7	1.239	2.167	2.833	3.822	4.671	6.346	8.383	9.803	<b>14.067</b>	18.475
8	1.65	2.733	3.490	4.594	5.527	7.344	9.524	11.030	<b>15.507</b>	20.090
9	2.09	3.325	4.168	5.380	6.393	8.343	10.656	12.242	<b>16.919</b>	21.666
10	2.56	3.940	4.865	6.179	7.267	9.342	11.781	13.442	<b>18.907</b>	23.209
20	8.26	10.851	12.443	14.578	16.266	19.337	22.775	25.083	<b>31.400</b>	37.566
30	14.95	18.493	20.599	23.364	25.508	29.336	33.530	36.250	<b>43.773</b>	50.892
50	29.71	34.764	37.689	41.449	44.313	49.335	54.723	58.164	<b>67.505</b>	76.154
100	70.07	77.930	82.358	87.945	92.129	99.334	106.906	111.667	<b>124.342</b>	135.806

## Experiment Overview

### EXPERIMENT OBJECTIVE:

The objectives of this experiment are:

1. To understand the life cycle of the fruit fly, *Drosophila melanogaster*, as observed in a genetics experiment over two generations.
2. To investigate Mendel's Law of Independent Assortment of two genes in crosses for two generations and determine whether the genes are autosomal or sex-linked.
3. To compare actual results with predicted results using Chi-square analysis.

### WORKING HYPOTHESIS

If pure breeding parents of two genes are crossed for two generations, then one should be able to determine whether the genes are autosomal or sex-linked, dominant or recessive, and using Chi-square analysis see if actual results follow Mendel's Law of Independent Assortment



### LABORATORY SAFETY

Gloves and safety goggles should be worn routinely as good laboratory practice.

### MATERIALS FOR THE EXPERIMENT

Each Lab Group should have the following materials:

- Vials of pure bred parental flies for one, two, or three crosses (if starting at beginning of mating)
- Vial of experimental mated cross (if starting at collecting of F<sub>1</sub> offspring)
- 4 vials containing media for each cross
- 1 bottle EDVOTEK® BugOut™ Solution
- Camel-hair paintbrush to manipulate flies
- Filter paper
- Foam lids
- Pasteur pipets
- Petri plate
- *Drosophila* culture tubes
- *Drosophila* growth media
- Dissecting microscope
- Microscope
- Vegetable or mineral oil for morgue
- Alcohol for morgue
- Flask with fitted cork (morgue)
- NaCl solution
- Slide and cover
- Acetocarmine Stain
- Glass slide



**PART A: Setting Up the F1 and F2 Crosses**

Obtain a vial of parental flies from your teacher. Anesthetize the flies as instructed by your teacher and practice looking at the flies under the dissecting microscope. You should note the sex differences. Also note the type of mutation written on the vial.

1. Obtain a vial of F1 flies from your teacher.
  - Note the vial number and parental cross marked on the vial in your notebook.
  - Using standard fly handling techniques, collect the F1 flies and observe their phenotypes.
  - Record the results below.
2. Place 5-6 male and female F1 flies into a fresh vial with media.
3. After 10 days, remove the F1 parents and discard into the fly morgue.
4. The F2 generation flies will begin to emerge within several days.
  - Remove the F2 generation flies as they begin to emerge to an empty vial without media and anesthetize as described under the section titled Using EDVOTEK® BugOut™ solution.
  - Observe the flies under the dissecting microscope. Record the F2 results over a 3-4 day period. The more data you accumulate the more significant the results.
5. Analyze the data using the chi-squared statistic as previously described. Obtain the class results for the other crosses in this experiment and analyze the results using chi-squared as well.

## Recording Your Data and Class Data

## Monohybrid Cross

The monohybrid cross (vials numbered 1 to 4) should yield all red eyes in the F1 generation and a 3:1 ratio of red to sepia in the F2. Record red eye and sepia eye results for the F1 and F2 generation in your notes. Determine the  $\chi^2$  values.

F1 Phenotype	Your Data		Class Data	
	Males	Females	Males	Females
Red Eyes				
Sepia Eyes				
F2 Phenotype	Your Data		Class Data	
	Males	Females	Males	Females
Red Eyes				
Sepia Eyes				

## Sex Linked Cross

The gene for eye color is carried on the X chromosome. As with humans, *Drosophila* males are XY, and females are XX. The possible F1 and F2 phenotypes are listed. They depend on the sex of the parents used in your initial crosses. Red-eyed males  $X^{w+}Y$  crossed with white-eyed females  $X^wX^w$ , should produce all  $X^{w+}X^w$  red-eyed females and all  $X^wY$  white-eyed males in the F1 generation. White-eyed males  $X^wY$  crossed with pure bred red-eyed females  $X^{w+}X^{w+}$  will produce red-eyed females,  $X^{w+}X^w$  and red-eyed males  $X^{w+}Y$  in the F1 generation. Determine possibilities for the F2 generation. Record your data and class data. Determine the  $\chi^2$  values.

F1 Phenotype	Your Data		Class Data	
	Males	Females	Males	Females
Red Eyes				
White Eyes				
F2 Phenotype	Your Data		Class Data	
	Males	Females	Males	Females
Red Eyes				
White Eyes				



## Recording Your Data and Class Data

## Dihybrid Cross

The gene for normal wings is dominant (V) to vestigial wings (v). Mate a homozygous red-eyed, vestigial-winged fruit fly with a sepia-eyed, normal-winged fruit fly. The expected F<sub>1</sub> phenotype would be all red-eyed, normal-winged fruit flies. The expected F<sub>2</sub> would be 9 red-normal : 3 red-vestigial : 3 sepia-normal : 1 sepia-vestigial. Record your data and class data. Determine the X<sup>2</sup> values.

F1 Phenotype	Your Data		Class Data	
	Males	Females	Males	Females
Red Eyes/Normal Wings				
Red Eyes/Vestigial Wings				
Sepia Eyes/Normal Wings				
Sepia Eyes/Vestigial Wings				

F2 Phenotype	Your Data		Class Data	
	Males	Females	Males	Females
Red Eyes/Normal Wings				
Red Eyes/Vestigial Wings				
Sepia Eyes/Normal Wings				
Sepia Eyes/Vestigial Wings				

## Part B: Isolation and Staining of *Drosophila* Salivary Gland Chromosome

During the course of this experiment, the salivary gland chromosomes from a larval stage may be isolated and stained. In insects belonging to the Diptera order, the cell nuclei of certain tissues are drastically enlarged. The chromosomes within these nuclei undergo many additional rounds of replication. All replicates of the same chromosome fail to separate and are lined up in parallel arrays, this is called polyteny.

The polytene chromosomes in the salivary gland of *Drosophila* can be stained and about 5000 individual bands can be resolved. The homologous pairs of chromosomes will remain lined up as somatic pairs. Since *Drosophila* have 8 chromosomes, 4 pairs may be observed. Differences between the pairs can be seen in the banding patterns. Puffs would indicate a region of high gene/transcriptional activity.

1. Obtain 0.7% NaCl from your teacher. Place a single larval stage *Drosophila* onto a clean microscope slide. Using a Pasteur pipet, place 2 drops of 0.7% NaCl onto the larva to prevent dehydration. View the larva under a dissecting microscope.
2. In one motion decapitate the larva, thereby teasing out the two *Drosophila* salivary glands which are on either side of the larva midline (refer to Figure 2).
  - Place one dissecting needle directly behind the head, and the other near the middle of the larva's body.
  - Pull the needle holding the head. The salivary glands will be pulled out of the body. The salivary glands appear as pea pods, not grapes.
3. Gently remove any material adhering to the glands and place a drop of acetocarmine stain onto the slide.
4. With a dissecting needle, drag the salivary glands into the stain and incubate at room temperature for 10 minutes.
5. Place a coverslip onto the stained salivary glands and squish the glands under the cover slip by gently pressing on the coverslip with a pencil eraser tip.
6. Examine the chromosome squash for banding. If no chromosomes are visible, the gut may have been selected and stained instead of the salivary glands. Try again.



## Experiment Results and Study Questions

### LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

#### Before starting the experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

#### During the Experiment:

- Record (draw) your observations, or photograph the results.

#### Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

### STUDY QUESTIONS

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. If the genes analyzed by the dihybrid cross were linked on the same chromosome, how would that affect your results?
2. Why is it important that the parental females be virgins?
3. Why are the adult flies removed?
4. Why are *Drosophila* favorite experimental models for geneticists?
5. Why is it necessary to analyze a large number of offspring before making conclusions about genotypic and phenotypic ratios?
6. Why did you collect data for both males and females?
7. If you were a molecular biologist and had obtained two gene clones for eye color one isolated from pure bred wild type red eyed flies, the other from the mutant sepia eyed flies, how would you analyze these genes to identify the type of gene mutation?

## Experiment Results and Study Questions

8. What possible genotypes and phenotypes would you observe if the F<sub>2</sub> flies from the dihybrid cross were allowed to randomly mate?
9. Assume that during a hypothetical monohybrid cross, the double recessive gene were lethal and expressed itself early in the fly development. Would that skew the results in a large population? Would the recessive eventually disappear from the population?
10. Based on the example in the background section, set up a Punnet Square to predict the genotypic and phenotypic ratios obtained from a Dihybrid cross in the F<sub>1</sub> generation and F<sub>2</sub> generation?

HINT: Red eyes + vestigial wings = RRvv. Sepia eyes + normal wings = rrVV. The genotypes for all F<sub>1</sub> flies is RrVv. Therefore each F<sub>1</sub> fly can produce 4 types of gametes. Determine these and set up a 4 X 4 Punnet square.

11. Use the  $\chi^2$  statistic to determine the values for the monohybrid, dihybrid, and Sex Linked crosses for your individual data and for the class data. Are the hypotheses valid?



## Notes to the Instructor

### OVERVIEW OF LABORATORY INVESTIGATIONS

The "hands-on" laboratory experience is a very important component of the science courses. Laboratory experiment activities allow students to identify assumptions, use critical and logical thinking, and consider alternative explanations, as well as help apply themes and concepts to biological processes.

EDVOTEK® experiments have been designed to provide students the opportunity to learn very important concepts and techniques used by scientists in laboratories conducting biotechnology research. Some of the experimental procedures may have been modified or adapted to minimize equipment requirements and to emphasize safety in the classroom, but do not compromise the educational experience for the student. The experiments have been tested repeatedly to maximize a successful transition from the laboratory to the classroom setting. Furthermore, the experiments allow teachers and students the flexibility to further modify and adapt procedures for laboratory extensions or alternative inquiry-based investigations.

### ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK® web site.

**[www.edvotek.com](http://www.edvotek.com)**

In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

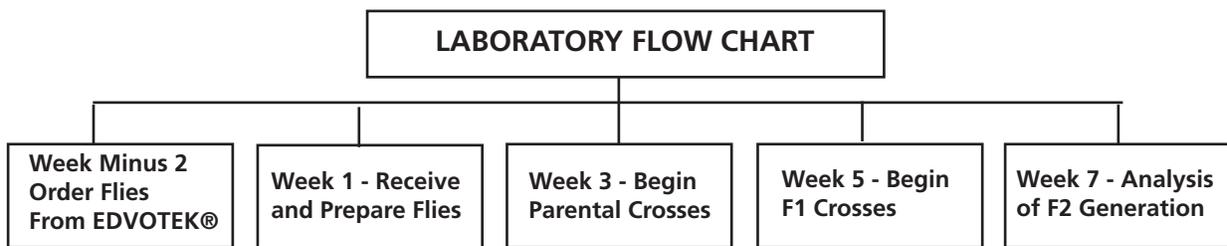
Safety Data Sheets can be found on our website:

**[www.edvotek.com/safety-data-sheets](http://www.edvotek.com/safety-data-sheets)**

## Pre-Lab Preparations

### APPROXIMATE TIME REQUIREMENTS

1. EDVOTEK® requires three weeks notice for shipment of live flies.
2. Allow several hours to set up the initial parental crosses. Students can set up the parental crosses. Each student group would require a class period to set up one parental cross. The lab will require several weeks for completion and is dependent upon the *Drosophila* life cycle. See Laboratory Flow Chart.



This experiment takes 4-6 weeks to complete. Students can set up parental crosses based on available class laboratory time. If the instructor sets up parental crosses, students should begin at Student Experimental Procedures (page 12). Request flies from EDVOTEK® at least three weeks in advance of laboratory to allow time for processing and shipping (refer to Laboratory Flow Chart). Flies should be kept in an incubator at 20-25°C. Some rooms become too cold and slow down fly growth. Conversely, some rooms are hot and shorten the 10 day life cycle, making collection of virgin females difficult.

### GENERAL FLY HANDLING TECHNIQUES

Remember that female fruit flies will use sperm from a single insemination for their entire life span. It is essential you collect virgin female flies for the first Parental (P1) crosses. Females will be capable of mating 10 hours after emerging from the pupae. Therefore, female flies must be less than 10 hours old when collected.

1. Three to five days after arrival of flies, set up and label 4 new vials: Wild type, Sepia, Vestigial, and White eyes.
  - In each of the vials, add 1 scoop of EDVOTEK® Instant *Drosophila* Growth Media and 1 scoop of water. Use either a small coffee type scoop or a 20ml beaker (15ml of media and 15ml of water). There will be about 1 inch of media in the bottom of the vial.
  - Allow the media to set up for 5-10 minutes.
  - Transfer the adult flies from the vials received to the appropriately labeled fresh vial. **This is done without the BugOut™ solution.**



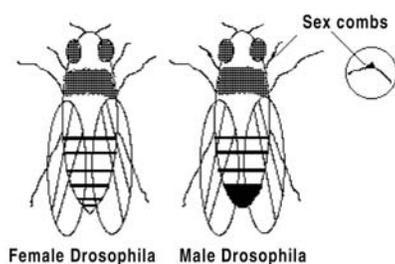
## Pre-Lab Preparations

2. Tap vial with adult flies on lab bench to knock flies off the cap and towards the bottom of vial.
  - Immediately remove the foam plug and quickly invert and overlap a vial which contains media.
  - Tap flies into the empty vial and cap immediately. These Parental flies, which have mated during shipping, can be observed by the students later.
3. Virgin females will emerge from the pupae within vials sent from EDVOTEK®.
  - Collect within 10 hours after emergence.
  - Check vials morning and evening. Males can be collected from an overnight hatching, or at the end of the day.
  - Use 5-7 mating pairs per cross.

### PROCEDURE FOR USING EDVOTEK® BUGOUT™ SOLUTION

1. Do not anesthetize fruit flies in a vial containing media. They will drop into the media and die.
2. Transfer newly emerged flies to a vial without media and quickly cap.
  - Anesthetize the flies by placing two drops of EDVOTEK® BugOut™ solution (component B) onto a small strip of filter paper with a Pasteur pipet.
  - Wedge the filter paper between the foam lid and the wall of the vial. The flies will stop moving in a few minutes.
3. Transfer immobilized flies onto the inverted lid of a petri dish.
  - Place the flies under the dissecting microscope and select the male and female flies as required for your crosses.
  - The flies can be moved by gently pushing with a piece of filter paper. Figure 5 illustrates differences between male and female flies. Male flies have a blunt posterior with a broad black band. They also have sex combs on their front legs. Females have a narrow pointed posterior and lack sex combs. The females also lack the broad black band on their posterior. This is clearly visible under the microscope.

**Figure 5:**  
Male & Female Flies



## Pre-Lab Preparations

4. To transfer the anesthetized flies to a new vial containing media, remove the foam plug from the vial.
  - Pick up sexed flies by gently brushing them onto a piece of filter paper using another piece of filter paper. Do not allow anesthetized flies to fall onto media or they will die.
  - Hold the new vial slightly horizontally. Slide flies into the new vial so that they land on the side wall of the vial.
  - Replace the foam plug and place the vial horizontally onto the bench until the flies awaken.

## THE MORGUE

Set up several fly morgues to collect unwanted flies. Add 50-60ml of mineral or vegetable oil and 50-60ml of alcohol to a 500ml flask. Cap with a cork. Unwanted flies can be tapped into the morgue where they will get stuck in the oil and die.

## PART A. MATING THE PARENTAL GENERATION

### Setting up the Parental P1 Crosses

1. Set up parental crosses approximately 2 weeks before needed by students.
  - Obtain vials of pure bred parental flies including, red eyed (wild type), sepia (brown) eyed flies, white eyed flies, and vestigial wings.
  - The lab is designed for 10 lab groups. Set up 10 cross vials for student groups and 2 extras.
2. For each of the 12 cross vials add 1 scoop of EDVOTEK® Instant *Drosophila* Growth Media and 1 scoop of water. A small coffee type scoop is acceptable, otherwise use a 20ml beaker and use 15ml of media and 15ml of water.
  - There will be about 1 inch of media in the bottom of the vial. Allow the media to set up for 5-10 minutes.
  - Place a foam plug at the top of each vial.



## Pre-Lab Preparations

3. Collect and anesthetize newly hatched flies only. Collect 5-7 mating pairs per cross. Refer to General Fly Handling Techniques and set up the following crosses:
  - 4 vials red eye X sepia eye (monohybrid cross)
  - 4 vials red eye/vestigial wings X sepia eyes/normal wings (dihybrid cross)
  - 4 vials white eyed female X red eyed wild type male (sex linked cross)

If you are setting up the initial crosses, your students should read this section for techniques and then skip to Student Experiment Procedures, Part A.

If students set up initial parental crosses, each group should set up only one cross. The instructions refer to setting up 4 crosses, which is how the instructor should proceed. Refer to General Fly Handling Techniques for basic procedures.

### 4. The Monohybrid Cross

Obtain 4 vials containing media and label 1-4. Include date and label 'monohybrid cross' (red eye X sepia eye). When adding flies to vials, place vials on their sides to keep flies from falling into media.

- Select 5 virgin females red eye and 5 sepia eyed male for vials 1 and 2.
- Select 5 virgin sepia eyed females and 5 red eyed males and place these into vials 3 and 4. (There will not be any difference since these phenotypes are not sex linked).

### 5. The Dihybrid Cross

- Obtain 4 vials with media and label 5 through 8. Include the date and the type of cross. In tubes 5 and 6, set up the following cross: Virgin female red eyed/vestigial wings X male sepia eyed/normal wings.
- In tubes 7 and 8, cross male red eyed/vestigial wings X female sepia eyed/normal wings.

### 6. The Sex Linked Cross

## Pre-Lab Preparations

When setting up the sex linked cross, pay careful attention to the sex of the flies.

- Obtain 4 vials containing media and label 9 through 12. Include the date and the type of cross.
  - In two vials labeled 9 and 10, cross newly emerged virgin female white eyed flies X red eyed males.
  - In two vials labeled 11 and 12, cross newly emerged virgin red eyed females X white eyed males.
7. Let the flies mate. Remove parental flies after about 10 days at 20-25°C before F1 generation begins to emerge.

F1 flies will begin to emerge within 12 days. Students will begin with the F1 progeny. Alternatively, they may have performed the previous crosses.

8. Save some of each parental generation for student observation.

### PART B. ISOLATION AND STAINING OF *DROSOPHILA* SALIVARY GLAND

- Prepare 0.7% NaCl by adding 0.7 gm of NaCl to 100ml distilled water. Mix.



## Experiment Results and Analysis

## SAMPLE RESULTS

## Monohybrid Cross

P<sub>1</sub> RR red eye male X rr sepia eye female

F<sub>1</sub> 156 Rr

F<sub>2</sub> Rr X Rr

Actual	Expected
80 red eye	81 red eye
26 sepia eye	27 sepia eye

## Chi-Square for Monohybrid Cross

$$\begin{aligned}\chi^2 &= \frac{(80-81)^2}{81} + \frac{(26-27)^2}{27} = \\ &= .0123 + .037 = \\ &= .0493\end{aligned}$$

The **Null Hypothesis is not rejected**. There is no statistically significant difference between the observed and the expected data.

With one degree of freedom, the calculated  $\chi^2$  is 0.0493, it can be deduced from the Chi-Square Table, there is an 80 - 90% probability that any deviation of the observed values from the expected values are due to random chance alone.

## Dihybrid Cross

P<sub>1</sub> red eye, vestigial-wing female X sepia eye, normal-wing male  
Rrvv X rrVV

F<sub>1</sub> 178 RrVv (red eye, normal-wing)

F<sub>2</sub> RrVv x RrVv

Actual	Expected
175 red eye, normal wings	180 red eye, normal wings
57 red eye, no wings	60 red eye, no wings
63 sepia eye, normal wings	60 sepia eye, normal wings
18 sepia eyes, no wings	20 sepia eye, no wings

## Experiment Results and Analysis

### Chi-Square for Dihybrid Cross

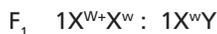
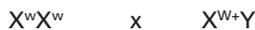
$$\begin{aligned}\chi^2 &= \frac{(175-180)^2}{180} + \frac{(57-60)^2}{60} + \frac{(63-60)^2}{60} + \frac{(18-20)^2}{20} = \\ &= .0139 + .15 + .15 + .20 = \\ &= .639\end{aligned}$$

The **Null Hypothesis is not rejected**. There is no statistically significant difference between the observed and the expected data.

With three degrees of freedom, the calculated  $\chi^2$  is .639. It can be deduced from the Chi-Square Table, that there is an 80 to 90% probability that any deviation of the observed values from the expected values are due to random chance alone.

### Sex-linked Cross

P<sub>1</sub> white eye female x red eye male



F1 Phenotype	Males	Females
Red Eyes		201
White Eyes	175	



F2 Phenotype	Actual		Expected	
	Males	Females	Males	Females
Red Eyes	78	87	77	77
White Eyes	70	73	77	77



## Experiment Results and Analysis

## CHI-SQUARE FOR SEX-LINKED CROSS

$$\begin{aligned}\chi^2 &= \frac{(87-77)^2}{77} + \frac{(78-77)^2}{77} + \frac{(73-77)^2}{77} + \frac{(70-77)^2}{77} = \\ &1.30 + 0.013 + 0.21 + 0.64 = \\ &= 2.16\end{aligned}$$

The Null Hypothesis is not rejected. There is no statistically significant difference between the observed and the expected data.

With three degrees of freedom, the calculated  $\chi^2$  is 2.16. It can be deduced from the Chi-Square Table, that there is an 50 to 70% probability that any deviation of the observed values from the expected values are due to random chance alone.

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