

# Chapter 15

## **The Genetics of Eye Color in *Drosophila melanogaster***

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### Note to Instructors:

This exercise has been set up as requiring six weeks. It can be done completely by students in which case short periods of time are required each week for six weeks. Alternatively, the crosses could be set up for the students and they could begin the exercise further along e.g. at Week 3. The two-week chromatography exercise can be put in anywhere between Week 1 and Week 5. It is assumed that students doing this exercise will have had some experience with basic Mendelian genetics. If not, then the exercise can be modified to include basic principles of meiosis, Mendelian genetics and gene/enzyme relationships.

### Schedule:

- Week 1: set up parental crosses
- Week 2: remove parents
- Week 3: chromatography of eye pigments  
F<sub>1</sub> self cross
- Week 4: remove F<sub>1</sub> parents  
analyze chromatograms
- Week 6: analyze F<sub>2</sub> data
- Week 7: report due



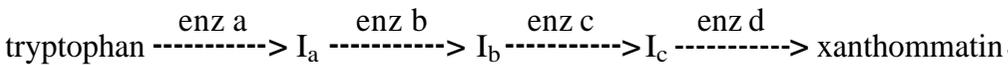
## INTRODUCTION

The purpose of this exercise is to investigate the inheritance of various eye colors in mutant strains of *Drosophila*. We will also use these mutants to demonstrate the relationship between the information carried by the DNA molecule (genotype) and the characteristics of that organism (phenotype) as well as to demonstrate the way that gene products interact to produce phenotypic characteristics.

### PIGMENT PRODUCING PATHWAYS

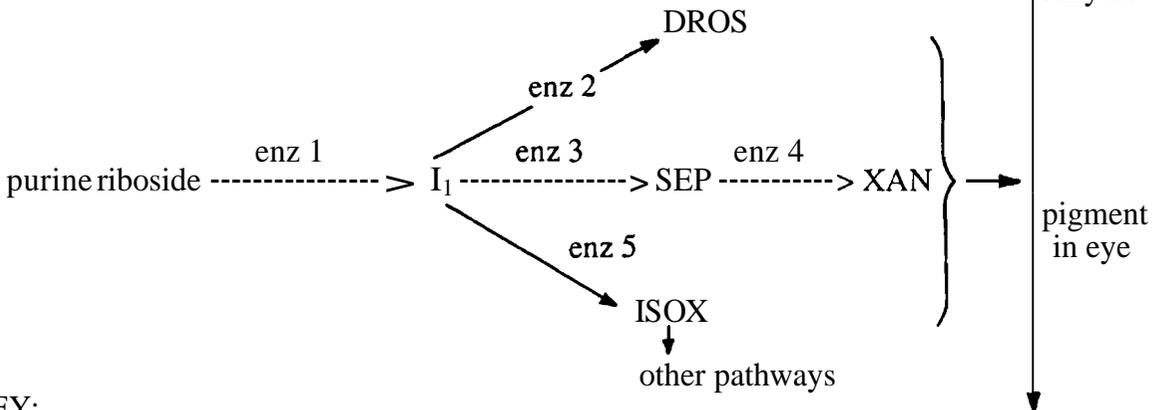
There are two separate biochemical pathways leading to the production of eye pigments in *Drosophila*. One produces the brown pigments, ommochromes, and the other produces red pigments, pterins. See Figure 1. In addition to pigment production, the pigment must also be bound to a granule in the pigment cell of the eye. Failure of this binding process, for example by a mutation, results in the lack of pigment in the eye regardless of the pigments produced.

#### A. Ommochrome pathway (brown pigments):



where enz a, enz b, etc. are the enzymes and I<sub>a</sub>, I<sub>b</sub>, etc. are the intermediates of the pathway.

#### B. Pterin pathway (red pigments):



#### KEY:

DROS = drosopterin  
 SEP = sepiapterin  
 XAN = xanthopterin  
 ISOX = isoxanthopterin

where enz 1, enz 2, etc. are the enzymes and I<sub>1</sub> is an intermediate in the pathway.

Figure 1. Pigment production pathways in *Drosophila melanogaster*. (simplified)

## WEEK 1

### Drosophila crosses

Each pair of students will be given a specific cross to follow through three generations: the parental generation (P), the first generation of offspring (F<sub>1</sub>) and the second generation of offspring (F<sub>2</sub>). For our crosses *Drosophila* are grown on a cornmeal medium to which dextrose, agar, yeast extract and a mold inhibitor have been added.

### Phenotypes

Examine the flies on demonstration and be sure you can accurately identify each phenotype. Use the space below for any notes you wish to make on these phenotypes. Each strain differs from the wild-type in eye color only. Note: there is a "wild" type for all traits; it is the phenotype that was first observed in wild populations of the organism.

Stocks to be used in crosses:

wild type	(+)
scarlet	(st)
brown	(bw)
white	(w)
sepia	(se)

### Distinguishing the Sex of Adult Flies

In order to set up crosses it is necessary to mate males and females. Therefore it is very important to be able to distinguish the sexes. Also, some genes are sex-linked and show different patterns of inheritance in males and females. Therefore, in determining the mode of inheritance of a genetic trait, it is important to distinguish the sex of the flies and to record the number of flies of each sex in each phenotype.

Adult males and females can easily be distinguished in a number of ways using the dissecting microscope (see Figure 2).

1. The abdomen of the female is longer (seven segments) and pointed at the end with several dark transverse stripes. The male abdomen has only five segments with two narrow dark stripes and a heavily pigmented tip.
2. The males have a sex comb of about 10 black bristles on the forelegs; females do not have a sex comb.
3. Males have obvious external genitalia on the ventral surface; females do not.

### Crosses

The following crosses are to be set up:

Cross 1A: brown-eyed female x wild-type male

Cross 1B: wild-type female x brown-eyed male

Cross 2A: scarlet-eyed female x wild-type male

Cross 2B: wild-type female x scarlet-eyed male

Cross 3A: brown-eyed female x scarlet-eyed male  
Cross 3B: scarlet-eyed female x brown-eyed male

Cross 4A: white-eyed female x wild-type male  
Cross 4B: wild-type female x white-eyed male

Cross 5A: sepia-eyed female x wild-type male  
Cross 5B: wild-type female x sepia-eyed male

Cross 6A: white-eyed female x sepia-eyed male  
Cross 6B: sepia-eyed female x white-eyed male

Note: crosses A and B are reciprocal crosses, i.e. the strains involved in the crosses are the same but the phenotype of the female and male parents is reversed.

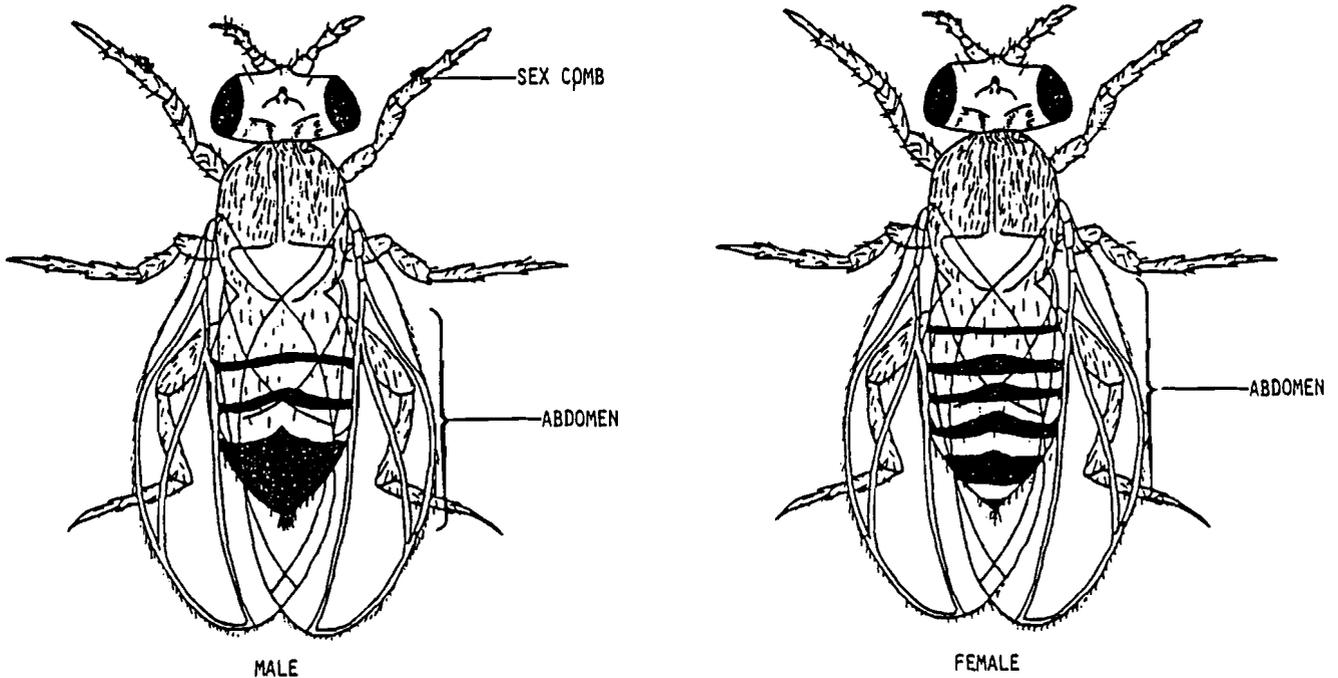


Figure 2. Distinguishing characteristics of male and female *Drosophila*.

### Procedure

1. Etherize your parental flies for 2-3 min. CAUTION: Ether is flammable. Keep away from all open flames.
2. Place 5 females and 5 males for each cross in a fresh vial.
3. Indicate your names and cross number on each vial.
4. Leave the vials on their side until the flies revive.
5. Your vials will be incubated at 25°C and returned to you next week.

## WEEK 2

### *Drosophila* crosses - Removal of Parents

In your culture vials the adults are the original parents. Note the presence of the other stages of the life cycle (see Figure 3).

#### Procedure

1. Etherize your flies - do not worry about over-etherizing.
2. Discard your parental flies into the morgue (a soap solution which will kill the flies).
3. Your vials will be incubated at 25°C and returned to you next week.

## WEEK 3

### Chromatography of Pterin Pigments

We will use paper chromatography to separate the pterin pigments which will then be identified next week on the basis of their fluorescence in ultraviolet light. The ommochrome pigments will not appear on the chromatograms.

#### Materials

filter paper, pencil, ruler, glass rod, forceps, beaker with solvent (2:1 n-propanol: 1% NH<sub>4</sub>OH)

strains of *Drosophila*:

wild-type (+)	
sepia	(se)
white	(w)
brown	(bw)
scarlet	(st)

Procedure: Work in pairs.

1. Obtain a piece of filter paper 15 cm x 23 cm and, with a pencil, lightly draw a line 1.5 cm from the longer edge. This will be the lower edge. Be careful not to get fingerprints on the filter paper.
2. With a pencil, initial the upper right hand corner.
3. With a forceps, place one white (w) fly on the pencil line 3 cm from the left edge of the paper (see Figure 4). Holding the fly in place, use a glass rod to crush the head onto the pencil line. Discard the body of the fly. Label this spot in pencil below the line - see Figure 4. Repeat with four more white flies. Make sure all the heads are crushed in exactly the same spot.
4. Wash the glass rod and dry it with a paper towel. Place one brown fly 3 cm from the white flies. Crush the head. Label this spot in pencil below the line - see Figure 4. Repeat with four more brown flies. Make sure all the heads are crushed in exactly the same spot.

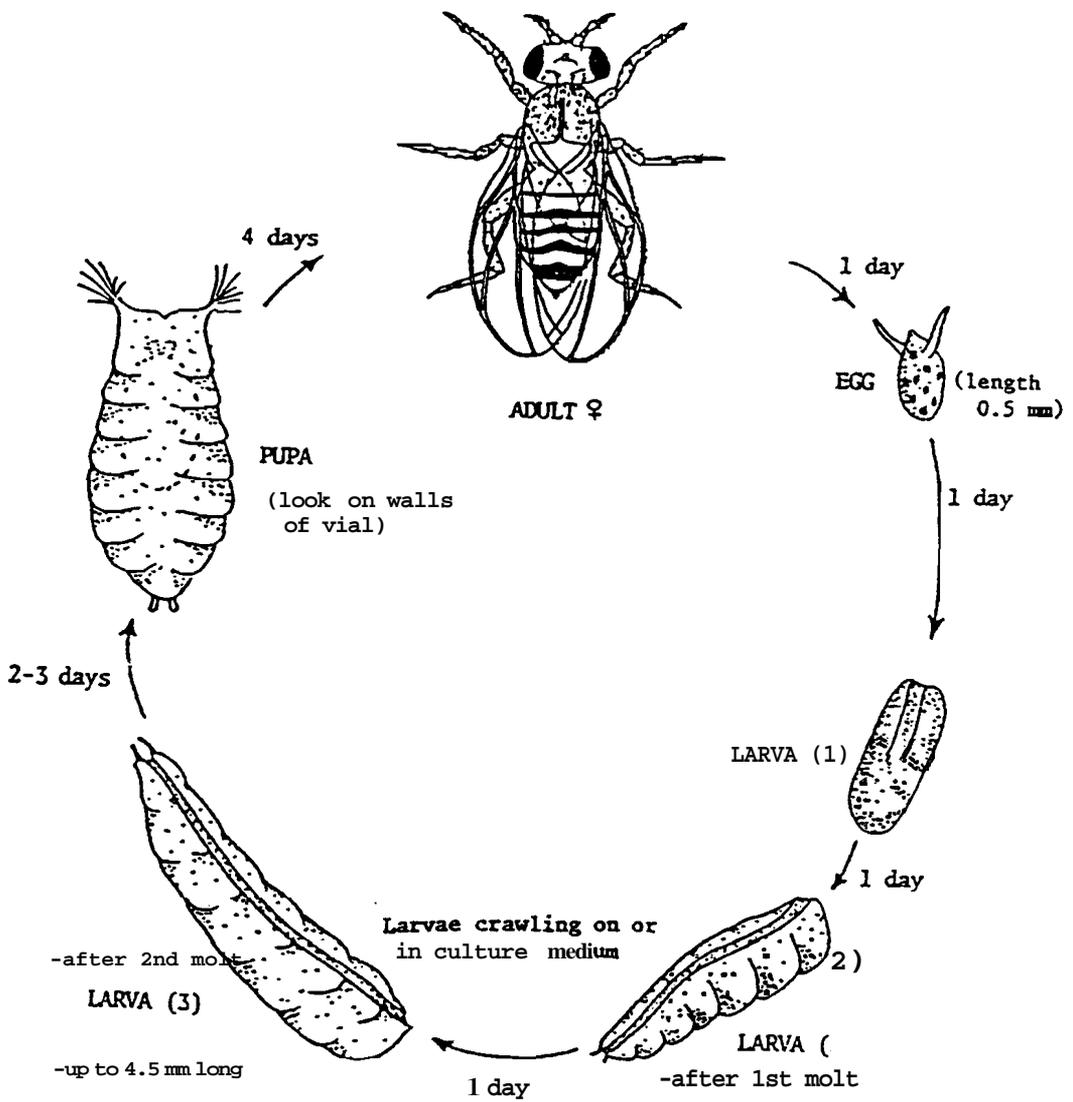


Figure 3. Life cycle of *Drosophila melanogaster*.

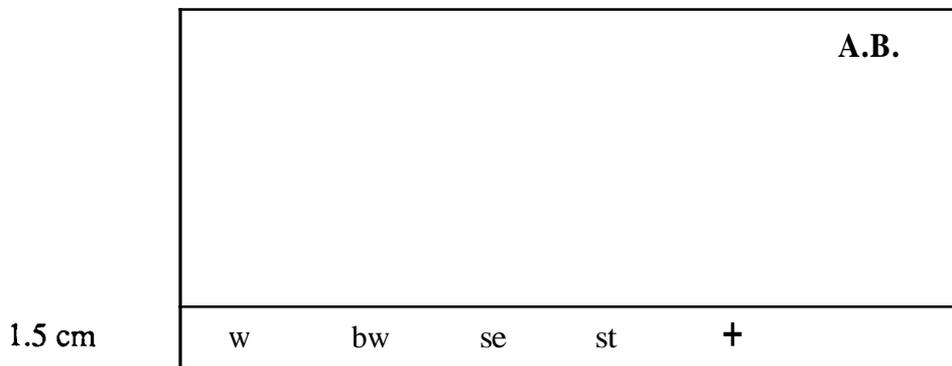


Figure 4. A chromatogram spotted and labelled.

5. Repeat step 4 with sepia (se), scarlet (st) and wild-type (+) flies.
6. Allow the spots to dry.
7. Roll your filter paper into a cylinder so that the spots are on the inside. Staple the cylinder so that the edges are neither touching nor overlapping. (Figure 5).

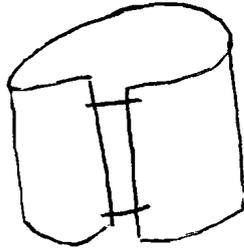


Figure 5: A chromatogram folded and stapled, ready for running in solvent.

8. Place this cylinder in a beaker which has approximately 1 cm of solvent in it (the solvent must not come higher than the pencil line). Cover the beaker with foil and let sit for 2 hours 20 minutes or until the solvent front is 1 cm from the top of the paper.
9. Remove the cylinder from the beaker and place your chromatogram where indicated by your T.A. until next week.

### *Drosophila* crosses - F<sub>1</sub> self Cross

1. Etherize your flies for 2-3 minutes.
2. Separate into males and females.
3. Note the phenotype of each sex and record in the space below.

F<sub>1</sub> females:

F<sub>1</sub> males:

### **Do not discard your flies!**

4. Check your results with your T.A.
5. Select five males and five females and place in a fresh vial.
6. Label the vial with your name and the number of your parental cross.

7. Leave the vial on its side until the flies revive.
8. Your vials will be incubated at 25°C and returned to you next week.
9. Discard any leftover F<sub>1</sub> flies into the morgue.

## WEEK 4

### Drosophila crosses - Removal of F<sub>1</sub> Parents

1. Etherize your flies in your F<sub>1</sub> self vial.
2. Remove all flies from this vial.
3. Discard flies into morgue.
4. Your flies will be returned to you in two weeks.

### Analysis of Chromatograms

#### Introduction

The pathway for the production of the red pigments, the pterins, in *Drosophila* is outlined in Figure 1B. Pigments such as drosopterin (DROS), sepiapterin (SEP), xanthopterin (XAN) and isoxanthopterin, (ISOX) can be identified on the basis of their fluorescence in ultraviolet light. When ultraviolet light is absorbed by organic compounds, such as pigments, some of the absorbed energy can be emitted as heat or as light of a longer wavelength, i.e., in the visible range. When the separated pigments are observed in ultraviolet light, each pigment fluoresces a characteristic color. This color, as well as its relative position on the chromatogram, can be used to identify the pigment. None of the brown pigments (ommochromes) will appear on the chromatogram.

#### Results

1. Examine your chromatogram in ultraviolet light. CAUTION - Ultraviolet light can be dangerous. Do not look directly at the source.
2. Compare your results with the sample chromatogram of wild-type pigments on the next page (Figure 6) and complete Table 1. The pigments may not appear as separate spots but they may blend into each other. Use the relative distance each pigment has moved from the original position of the sample to help in your identification.

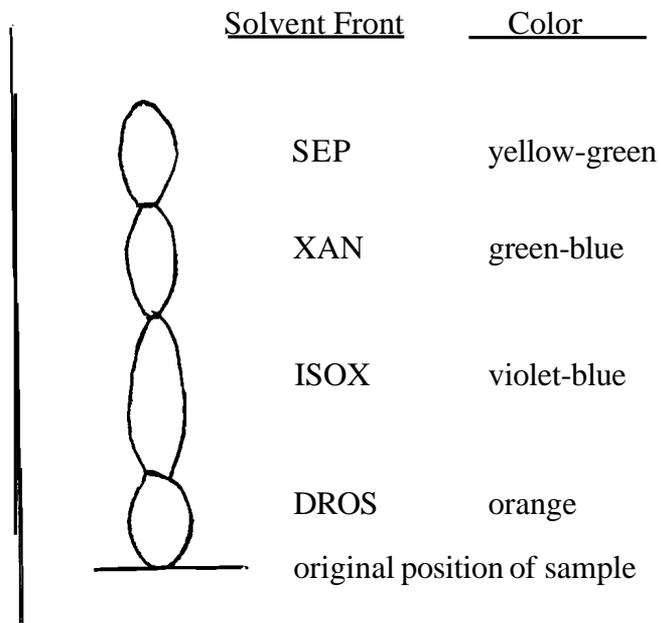


Figure 6. Sample chromatogram of wild-type *Drosophila* eye pigments

Table 1. Results of chromatographic separation of *Drosophila* eye pigments.

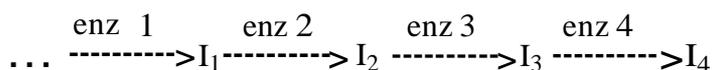
KEY:

- = absent
- + = small amount
- ++ = moderate amount
- +++ = large amount

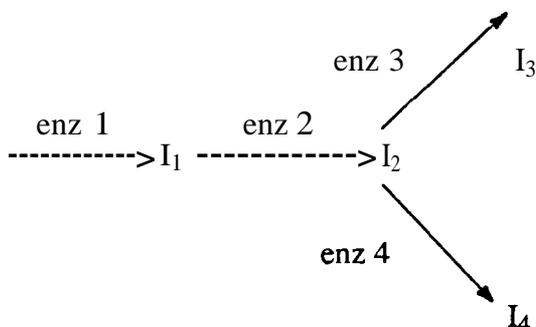
Strain	DROS	ISOX	XAN	SEP
+				
se				
w				
bw				
st				

## Analysis of results

If a mutation in the DNA occurs such that one enzyme in the biochemical pathway is not functional, then the intermediate acted on by that enzyme could accumulate. For example, in the pathway



if enz 3 is not functional, then  $I_2$  could accumulate. If there is a branch in the pathway e.g.



and the non-functional enzyme occurs after the branch, e.g. enz 3, then  $I_2$  could accumulate and/or extra  $I_4$  could be produced (the extra  $I_4$  doesn't necessarily accumulate - why?).

Compare the pigments present in each of the mutant strains with the pigments found in the wild type and by the presence or absence or amount of each and try to determine which enzyme in the pigment pathways (see Figure 1) could be non-functional. Remember the brown (ommochrome) pigments do not appear on the chromatogram, therefore you must also consider the phenotype i.e. the actual eye color of each strain. Xanthommatin is the only ommochrome bound to granules, therefore if a mutant has no brown pigments assume enz d (see Figure 1A) is non-functional.

Remember if one enzyme in the pterin pathway is blocked (excluding the first one), you should see an increase in at least one of the intermediates or products of the pathway. Also keep in mind that the pigments must be bound to the granules in the eye for any color to be observed.

Complete the following table:

Strain	Defective enzyme	Reasons
w		
bw		
se		
st		

## WEEK 6

### Drosophila crosses - Analysis of F<sub>2</sub>

#### Results

1. Etherize your flies.
2. Separate into males and females.
3. Determine the phenotypes of each sex and the number of each phenotype. Record your results here and on the blackboard.

	PHENOTYPE(S)	NUMBER
females		
males		

4. Using the data tables provided, record the results of all crosses.

#### Analysis of Results

For each cross 1 - 6 you must use the results provided to:

1. Decide on the mode of inheritance of each phenotype i.e. how many genes are involved in each cross, how many alleles of each gene and which alleles are dominant, recessive, linked, etc. You must provide the reasons for your decision. Use Analysis Table 1 for this part of your assignment.
2. Do a Chi-Square test:  $\chi^2 = \frac{\Sigma(\text{obs} - \text{exp})^2}{\text{exp}}$

to confirm your suggested mode of inheritance. Show all your work, including the calculation of expected values. If sex-linkage is involved you must do separate Chi-Square tests for males and for females in each of the reciprocal crosses.

3. Relate the results of each cross to the enzyme defective in each of the parents, the F<sub>1</sub> and all the F<sub>2</sub> phenotypes observed. If sex-linkage is not involved, you may do either cross A or B. Use Analysis Table 2 for this part of your assignment.

Remember: Explain all your reasoning and show all your work. Be sure to explain any abbreviations or symbols used. Your assignment is due in one week.

(6 copies of this table are needed)

Data Table:

Cross:

	Cross A Phenotype	Cross B Phenotype
a. <u>Parental cross</u> P female  P male		
b. <u>F<sub>1</sub> Progeny</u> F <sub>1</sub> females  F <sub>1</sub> males		
c. <u>F<sub>2</sub> Progeny</u> (from F <sub>1</sub> self) include numbers of each phenotype F <sub>2</sub> females  F <sub>2</sub> males		

- d. Examine the F<sub>1</sub> and F<sub>2</sub> data. If sex does not appear to be a factor i.e. results for the F<sub>1</sub> and F<sub>2</sub> of the reciprocal crosses are similar, then add all the numbers for each F<sub>2</sub> phenotype i.e. F<sub>2</sub> females cross A + F<sub>2</sub> males cross A + F<sub>2</sub> females cross B and F<sub>2</sub> males cross B. Calculate the F<sub>2</sub> phenotypic ratio on the basis of the combined data.

phenotypes:

number observed:

ratio observed:

If sex does appear to be a factor i.e. the results for the F<sub>1</sub> and F<sub>2</sub> of the reciprocal crosses are not similar, then do not combine your data. Calculate the phenotypic ratio separately for F<sub>2</sub> males and F<sub>2</sub> females and for crosses A and B.

Analysis Table      This table has been condensed)

Cross	Mode of inheritance	Reasons
1		
2		
3		
4		
5		
6		

Analysis Table 2. (6 copies of this table are needed. Also note that this table has been condensed.)

Cross:

	Defective enzyme	Reason(s)
P female		
P male		
F <sub>1</sub>		
F <sub>2</sub>		

## REFERENCES

- Hadorn, E. 1962. Fractionating the Fruit Fly. *Sci. Am.* Vol. 206, pp 101-110.
- Phillips, J. P. and Forrest, H. S. 1980. Ommochromes and Pteridines. *The Genetics and Biology of Drosophila*. M. Ashburner and T.R.F. Wright, Ed., Academic Press, N.Y. Vol. 2d, pp 541-623.