

Inheritance of Aldehyde Oxidase in *Drosophila melanogaster*



(adapted from Morgan, J. G. and V. Finnerty. 1991. Inheritance of aldehyde oxidase in *Drosophila melanogaster*. Pages 33-47, in *Tested studies for laboratory teaching*. Volume 12. (C. A. Goldman, Editor). Proceedings of the 12th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 218 pages.)

A. Objectives

Become familiar with

1. The model organism *Drosophila melanogaster*
2. Use of the dissecting (stereo) microscope
3. Handling and sex typing of *D. melanogaster*
4. Certain biochemical aspects of genetic research
5. Inheritance patterns in *Drosophila melanogaster*.
6. Gene mapping and linkage analysis

B. Before coming to lab

Read the background section of this lab and review chapters 14 (Mendel) and 15 (Chromosomal Basis of Inheritance) in Campbell's *Biology*. Fill in the blanks in the Background section.

C. During lab

Work in groups of two students.

D. After lab week 2

Turn in this laboratory exercise to your instructor for her or him to check your answers to the embedded questions (including review questions). The points you get for this lab will count towards your lab notebook grade.

E. Background

The experiments in this exercise have been chosen because they exemplify some of the classic areas of genetic research. The study of genetics focuses on an understanding of the principles which govern the inheritance of specific traits. Initial experiments were concerned with the transmission of hereditary factors from generation to generation and led to the discovery of specific laws which define the pattern of inheritance of individual genes. Later experiments identified chromosomes as the physical structures where the units of heredity reside and provided firm cytological evidence for the theorems of transmission genetics. More recent investigations are concerned with the biochemical and molecular basis of gene expression. *Drosophila melanogaster*.

One of the classic tools of genetic research is the fruit fly, *Drosophila melanogaster*. This organism has been employed in genetic studies for nearly 80

years and has played an important role in the development of our knowledge of heredity. *Drosophila melanogaster* has been a particularly important eukaryotic genetic system for such studies because it has a very low chromosome number. The haploid (N) number of chromosomes is 4 and the chromosomes are designated X(1), 2, 3, and 4. The 2, 3, and 4 chromosomes are the same in both sexes and are referred to as autosomes to distinguish them from the X and Y sex chromosomes. Female *Drosophila* are characterized by having two X chromosomes while male *Drosophila* have an X and a Y chromosome. The Y chromosome only has genes necessary for male fertility, and does not carry any other genes. The 4 chromosome is so small, constituting only about 5% of the total genome, that, for all practical purposes, it can be ignored. Thus, almost the entire genetic content of the *Drosophila* genome resides on only three chromosomes: X, 2, and 3. Another characteristic of *Drosophila* that makes it an excellent genetic research tool is its short generation time. At 25°C, a *Drosophila* culture will produce a new generation in 10 days: 1 day in the egg (embryo) stage, 5 days in the larval stage, and 4 days in the pupal stage. Each experiment in this exercise will make use of the fruit fly, *Drosophila melanogaster* (see Figure 1).

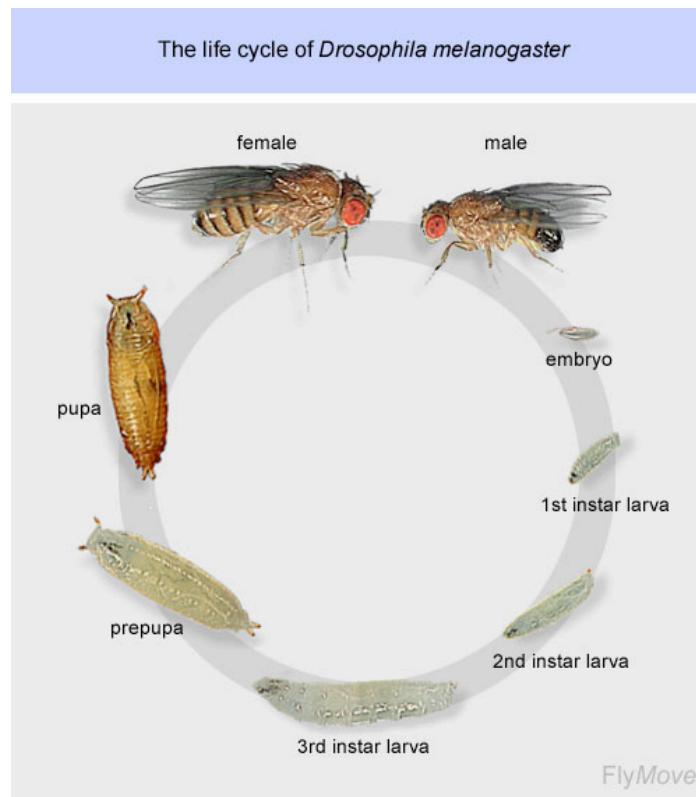


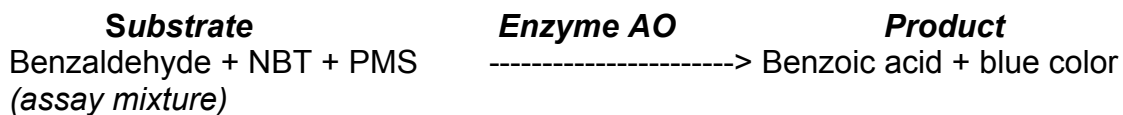
Figure 1: *Drosophila melanogaster* life cycle (Source: http://www.anatomy.unimelb.edu.au/researchlabs/whittington/img/life_cycle.jpg)

Enzyme Spot Assay

The trait that will be studied in each experiment is the presence or absence of an enzyme, aldehyde oxidase (AO), which catalyzes the oxidation of a number of

aldehydes, including acetaldehyde and benzaldehyde. AO activity is controlled by one gene, the *aldox* gene. Although *Drosophila* possess AO activity, its physiological importance to the organism is not well understood. Mutant strains which exhibit no AO activity are available and their viability and fertility are normal. This latter observation indicates that AO activity is not a vital enzyme activity for the fly when reared in a laboratory setting.

The technique used to test for AO activity is called an **enzyme spot assay**. This test operates on the following principle: In the presence of AO, the substrate—benzaldehyde, mixed with a color indicator, nitroblue-tetrazolium (NBT), and phenazine methylsulfate (PMS)—will oxidize to form benzoic acid and a blue color. Without AO, the reaction will not proceed and no blue color will be produced. This reaction can be diagrammed as follows:



You will be given two vials. Vial 1 contains flies that will serve as a **positive control**, vial 2 contains flies that will serve as a **negative control**.

1. Which of the two vials contains flies that can produce AO?

Vial 1 _____

Vial 2 _____

2. If you homogenize the flies in assay mixture, what will happen?

Flies from vial 1 _____

Flies from vial 2 _____

3. What will the positive control tell you?

F. Procedures Week 1

Experiment 1 and 2: Establishing the Enzyme Reaction Controls

1) Anesthetize the flies in Vials 1 and 2 as follows:

- From your ether drop bottle, place 2 or 3 drops of ether on the cotton plug of your vial. Be sure to re-cap the ether bottle tightly. Keep in mind that ether fumes are explosive.
- Invert your vial so that the adult flies will fall asleep on the cotton plug rather than on the culture medium.
- When flies have become immobilized on the cotton plug, they can be removed for examination.
- Flies can be examined on a white card and moved with a toothpick using

the stereoscopic microscope.

- e) A fly “re-etherizer” is provided in case the adults begin to awaken before phenotype classification is concluded.
- 2) In Figure 2 below, label the characteristics for female and male flies as outlined in a) through e) below. From each vial, identify 2 or 3 females and 2 or 3 males and return the remainder to their appropriate vial. (Vial 1 will be used again in later experiments.) The following criteria may be used to distinguish adult males and females:
- a) *Size*: The female is generally larger than the male.
 - b) *Shape of abdomen*: The female abdomen is larger and the tip is more pointed than the male abdomen.
 - c) *Abdominal pigmentation*: Alternating dark and light dorsal bands can be seen on the entire rear portion of the female abdomen; the last few segments of the male are uniformly pigmented.
 - d) *External genitalia*: On the ventral portion of the abdomen, the female has anal plates and *lightly pigmented* ovipositor plates. The male has anal plates and a *darkly pigmented* genital arch and penis.
 - e) *Sex comb*: On males, there is a tiny brush-like tuft of hairs on the basal tarsal segment of the first pair of forelegs. This is the most accurate method for sexing males.



Figure 2: Female and male *Drosophila melanogaster* (Source: <http://bioweb.wku.edu/courses/Biol114/Online/Genetics/Drosophila.htm#fly>)

- 3) Keeping track of the sexes, place the flies from Vial 1 in one row of a spot assay plate, one fly per well.
- 4) Place the flies from Vial 2 in different row. Note: The next two steps need to be done quickly since the assay mixture is light sensitive. Cover the spot plate with an index card if there is any delay between steps.
- 5) Add 1 drop of assay mixture to each well.
- 6) Homogenize the flies (why?) with a “fly masher.” Wipe off the masher after each fly.
- 7) Place the spot plate in a desk drawer.
- 8) After 5 minutes, check the reactions and record your results in Table.
- 9) Rinse and shake off the excess water from your spot plate.

Table 1: AO Activiy in Male and Female Flies from Vials 1 and 2

Vial #	Fly #	Sex (F, M)	AO activity (+/-)
1	1		
	2		
	3		
	4		
	5		
	6		
2	1		
	2		
	3		
	4		
	5		
	6		

Experiment 3: Determining Pattern of Inheritance of the Aldox Gene

The third experiment initiates our study of the pattern of inheritance of the *aldox* gene. Vial 3 contains the F₁ progeny from a mating between a female from a stock homozygous for the *aldox* allele and a male from a stock homozygous for the *aldox*⁺ allele.

Hypothesize

What is the genotype of the female (maternal parent – lacks the enzyme) in our cross?

What is the genotype of the male (paternal parent – has the enzyme in our cross if the gene is not sex-linked)?

What would be the genotype of the male if the gene is sex-linked?

What would be the genotype of the the F₁ progeny if the gene is sex-linked?

What would be the genotype of the the F₁ progeny if the gene is not sex-linked?

Procedure

- 1) Anesthetize the flies in Vial 3.
- 2) Count out 10 males and 10 females.
- 3) Perform the spot assay as described in Experiment 1 with the following addition: from Vial 1 select a single fly and assay it with this and all following experiments. This will provide you with a “positive control” (PC) to compare with your unknown assays. Record your results when you see the “positive control” has turned blue—indicating the assay is working.

- 4) Record the results in Table 2.2.
- 5) Rinse out the spot plate.

Table 2: Data sheet for inheritance investigation

Fly #	Sex (F, M)	AO activity (+/-)	Fly #	Sex (F, M)	AO activity (+/-)
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		
Positive Control					

G. Procedures Week 2

Experiment 4: Determining Parental Genotypes Using Evidence from Progeny

Vial 4 contains F₁ progeny from a mating between unknown parents. Through this exercise, we want to find out the genotype of the parents of the flies in vial 4.

- 1) Anesthetize the flies in Vial 4.
- 2) Count out 25 flies and place them in individual spot plate wells.
- 3) Perform the spot assay including a “positive control” from Vial 1.
- 4) Record the results on two data sheets: Table 3 and the class data sheet.

Table 3: Data sheet for parental genotype determination

Fly #	Sex (F, M)	AO activity (+/-)	Fly #	Sex (F, M)	AO activity (+/-)
1			14		
2			15		
3			16		
4			17		
5			18		
6			19		
7			20		
8			21		
9			22		
10			23		
11			24		
12			25		
13			-		

Positive Control

Using your data, hypothesize what the genotypes of the parent flies must be by reviewing the following questions, some answered by previous experiments:

- 1) Is the trait sex-linked?
- 2) Is one allele dominant?
- 3) What is the ratio of the two traits? (Round-off the ratio.)
- 4) What parental cross can account for the above ratio, taking into consideration the answers to questions 1 and 2?
- 5) What are the expected values for each trait, given the rounded ratio between the traits and the total number of observations?

Enter your hypothesis below:

Total the class data and, using this data, test your hypothesis concerning the genotypes of the parents using a chi-square test as outlined in Table 4.

Table 4: Chi-Square Calculations (observed values represent total class data)

	AO Activity (+)	AO Activity (-)
Observed value (<i>o</i>)		
Expected value (<i>e</i>)		
Deviation (<i>o-e</i>) or <i>d</i>		
Deviation ² (<i>d</i> ²)		
<i>d</i> ² / <i>e</i>		
Chi-square $\sum d^2/e$		
Degrees of freedom (df)		
Probability (<i>p</i>)		

- 6) Do the data support your hypothesis?

- 7) Does this experiment support or contradict your conclusions concerning the pattern of inheritance derived from the previous experiment?

Experiment 5: Cross Involving Two Genes

In this experiment, we want to investigate a **dihybrid cross** and find out whether two genes, the *aldox* gene and a gene that influences eyecolor, *sepia*, are **linked** or not. Vial 5 contains the F₁ progeny from a mating between parents having the following genotypes:

Parent 1		Parent 2
<u>sepia⁺ aldox⁺</u>	x	<u>sepia aldox</u>
sepia aldox		sepia aldox

Where *sepia*⁺ represents the dominant, wild-type eye color allele that produces red eye color and *sepia* represents the mutant, recessive allele that produces a dark brown eye color.

Hypothesize:

- 1) Are the genes linked or not? What do you base your hypothesis on?
- 2) What would be the ratio of phenotype classes of offspring if the genes were linked?
- 3) What would be the ratio of phenotype classes of offspring if the genes were not linked?
- 4) Use a Punnett square to illustrate your prediction.

Procedure

1. Anesthetize the flies in Vial 5.

2. Count out 50 flies and classify them on the basis of eye color (red or brown).
3. Keeping the eye colors separate, perform the spot assay including a “positive control” from Vial 1.
4. Record the results in Table 5.
5. Rinse out the spot plate.

Questions

From the mating outlined, what are the *expected* phenotypic classes and respective ratios?

Record your results of the eye color classification and the spot test for each fly in Table 9. Total your results below. Add your results to the total class data sheet provided by the instructor. Total the class data and record below:

	Your Totals	Class Totals
Red eyes, AO-present	_____	_____
Red eyes, AO-absent	_____	_____
Sepia eyes, AO-present	_____	_____
Sepia eyes, AO-absent	_____	_____

What phenotypic classes were observed, and in what approximate ratio?

In your laboratory notebook, perform the chi-square test on class totals to determine if the results support or falsify your hypothesis. Report your p value and conclusion here:

The map distance between two genes is related to the frequency of recombinants produced during meiosis. The specific relationship between map units and recombinants is the following:

$$\text{Map units} = 100 \times \text{Number of Recombinants} / \text{Total}$$

How frequent are the recombinant classes?

How far is the aldox locus from the sepia locus?

What is the exact map position of the *aldox* locus if the *sepia* locus is 26.0? Are you sure?

Table 5: Data sheet for gene location experiment

Fly #	Eye color	AO activity (+/-)	Fly #	Eye Color	AO activity (+/-)
1			26		
2			27		
3			28		
4			29		
5			30		
6			31		
7			32		
8			33		
9			34		
10			35		
11			36		
12			37		
13			38		
14			39		
15			40		
16			41		
17			42		
18			43		
19			44		
20			45		
21			46		
22			47		
23			48		
24			49		
25			50		
Positive Control					

Experiment 6: Developmental Profile

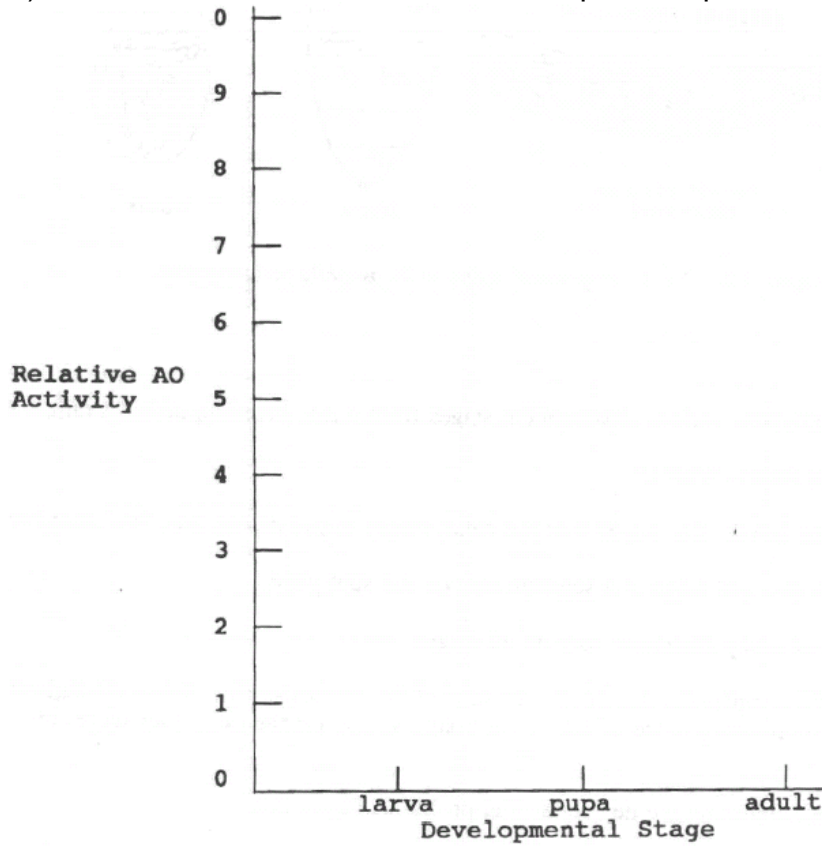
The following experiment is designed to demonstrate the expression of AO activity during the development cycle of the fly. There are four distinct stages in the life cycle of the fruit fly as shown in Figure 1.

Procedure

Use Vial 1 which contains various development stages from a true-breeding *aldox+* strain.

- 1) Anesthetize the flies in Vial 1.
- 2) Select five large larvae, five pupae (not the clear, empty pupae cases), and five adult flies.
- 3) Put each developmental stage in a separate row on the spot plate.
- 4) Perform the spot assay simultaneously on all stages.

- 5) After 5 minutes, compare the degree of coloration of the various assays. Select the darkest stage and assign it an arbitrary value of 10. Then assign values for the other two stages relative to the 10.
- 6) Plot the above number on the developmental profile in Figure 2.



H. Review questions

1. Is AO activity present at each developmental stage?

2. Are some stages more active than others?

3. If flies which are AO-negative are normally viable and fertile, why does the enzyme exist at all?

4. Why would it vary in developmental stages?

5. What makes *Drosophila melanogaster* such a good model organism?

6. How can you tell female and male *Drosophila* apart?

7. Did your results of the controls match your predictions?

8. Does the sex of flies seem to correlate with AO activity?

9. Can you tell which allele is dominant? If so, which?

10. What evidence of your results in Table 2 support your answer?
Can you tell if the aldox gene is on the X chromosome or on an autosome?

11. What evidence of your results in Table 2 support your answer?

12. What does it mean that two genes are linked?