LABORATORY 4. GENE LINKAGE AND CHROMOSOME MAPPING

LEARNING OUTCOMES

When students have completed this laboratory practice, they should be able to:

- Obtain and analyze simulated F_1 data that illustrates trihybrid and test-crosses.
- Describe the difference between independent assortment and gene linkage by analyzing progeny numbers.
- To map three sex-linked genes by analyzing real-case data from a *Drosophila* cross
- To map three genes on a chromosome based on simulated data of a trihybrid test cross.

INTRODUCTION

LINKAGE AND CHROMOSOME MAPPING

When two genes are located on the **same chromosome** at a relatively **small distance**, recombination frequencies do not yield the expected ratios under the assumption of independent assortment. Instead, such genes tend to segregate together and are termed "**genetically linked**". Most of the time, this linkage is not complete due to the occurrence of crossing over between homologous chromosomes (recombination) during meiosis. Then, new combinations of alleles and phenotypes are produced at the end of meiosis (**recombinant gametes**). Since the **frequency of crossovers** depends on the **physical distance** between two genes, their distance can be determined using the **frequency of recombination**.

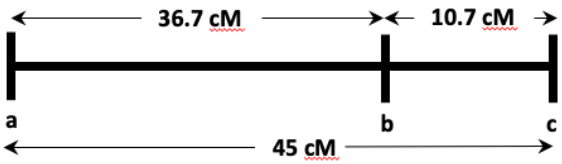
If the relative distance between more than two linked genes is determined, a genetic map can be constructed showing the relative positions of the genes on a chromosome. Such maps can be viewed as a one-dimensional model and can be useful for further genetic analysis.

CHROMOSOME MAPPING

The position of genes on chromosomes is usually determined indirectly by **calculating the frequency of recombination** between linked genes. The following recombination values from three individual **2-point crosses** (hypothetical examples of test-crosses) may be used to illustrate the method for constructing linkage maps.

Cross #	Details of cross	% Recombination
1	**P_1**= AB/AB X **P_2** = ab/ab	
	F_1= AB/ab; then, **test cross** to ab/ab	**36.7**% between **a** and **b** genes
2	**P_1**= BC/BC X **P_2**= bc/bc	
	F_1= BC/bc; then, **test cross** to bc/bc	**10.7**% between **b** and **c** genes
3	**P_1**= AC/AC X **P_2** ac/ac	
	F_1= AC/ac; then, **test cross** to ac/ac	**45.0**% between **a** and **c** genes

Based on the recombination values from the three **test-cross experiments**, we can map the genes a, b, and c as follows:



Thus, the **gene order** (i.e. a-b-c) and the **distance** between the genes can be established on the basis of the **percentage of recombination**. In genetics, this percentage of recombination is equivalent to **centimorgans** (cM) or **map units** (m.u.) which are the units for measuring **genetic linkage** (not true physical distance). However, the previous example of individual two-point crosses for mapping three genes has a **problem**: the distance between outside genes (a-c) does not match the sum of the intermediary distances (a-b) + (b-c):

a-b (36.7 cM) + b-c (10.7 cM) ≠ 45 cM

This discrepancy can be solved by further experiments. A **three-point** (trihybrid) **test-cross** provides more information regarding the recombination events that will allow solving the issue. For example:

P_1= ABC/ABC X **P_2**= abc/abc

Then, a **test-cross**:

F_1= ABC/abc X **P_2**=abc/abc

A test-cross progeny example would be:

Class	Phenotype	Type of gametes	Amount	%
1	ABC	Parental	261	**53.8**
2	abc	Parental	277	
3	Abc	Single crossover	173	**35.5**
4	aBC	Single crossover	182	
5	ABc	Single crossover	44	**9.5**
6	abC	Single crossover	51	
7	AbC	Double crossover	5	**1.2**
8	aBc	Double crossover	7	

IMPORTANT TIP: Note that the **highest** two classes (261 and 277) correspond to the offspring produced by **parental gametes** (the ones on each homolog chromosome of the **F_1 parental,** ABC/abc). The two **smallest** classes (5 and 7) correspond to the offspring produced by **double crossovers** gametes (or double recombinants: AbC and aBc).

When compared to the method based on separate dihybrid test crosses, a **three-point test cross** allows the identification of **double crossovers** between flanking markers (A and C in the example). A dihybrid test-cross, however, does not detect such double crossover individuals. For example, if gene B is not considered, the phenotype of the double crossover progeny (**A**b**C** and **a**B**c**) is identical to the parental classes (**A**B**C** and **a**b**c**) and would go unrecognized as recombinant progeny.

While calculating the percentage of recombination between genes, **all** of the crossovers taking place between any two genes

must be added together before dividing by the total number of progeny (i.e. single + double crossovers). If we apply this rule to the test cross progeny results, then we can determine the correct order of genes and estimate the distances between them.

Distance between a and b = (173+182+5+7)/total= 0.367 or 36.7%

Distance between b and c = (44+51+5+7)/total = 0.107 or 10.7%

Distance between a and c = (173+182+44+51+2(5+7))/total= 0.474 or 47.4%

Thus, a three-point **test cross** experiment offers us:

- The advantage of being able to **detect double crossover** progeny produced by simultaneous crossovers between the a and b, and b and c genes.
- The ability to estimate the level of interaction between those genes by calculating **the coefficient of coincidence**.

Coincidence (C) is calculated by **comparing** the **observed** and **expected** number of **double crossovers**. The expected number of double crossovers is estimated by **multiplying** the **probability** of **single crossovers** between a-b and b-c.

Thus, the **expected frequency** of **double crossovers (DC)** for the three-point test cross is **0.367 X 0.107 = 0.039.** The observed frequency of double crossovers is 5+7/total= **0.012**. By applying the following formula, we can obtain the coefficient of coincidence (C)

C = observed frequency **DC**/expected frequency **DC**

C= 0.012 / 0.039 **= 0.307**.

This value (0.307) indicates that there is a deviation from the expected. vs. observed number of double crossovers (this ratio should be = 1, if no interaction is present). This deviation is known as crossing over **interference (I)**.

To calculate the interference value (I), we can use the following formula:

I = 1 - coefficient of coincidence (C)

I = 1 - 0.307 = **0.692.**

A positive interference value indicates that a crossover in one region (a–b) of the chromosome **decreases** the chance of obtaining a crossover in an adjacent region (b–c), or vice versa. A coefficient of coincidence **C=0** indicates **complete interference (I=1)**, whereas a **C=1** indicates **no interference (I=0)**. A coefficient of coincidence greater than one will result in a **negative** interference value, which implies that a crossover in one region **increases** the chance of getting a crossover in an adjacent region.

Considering this background information, students should be able to analyze the F_2 results from a trihybrid cross performed in the laboratory to determine the **mode of inheritance and map in a chromosome** three sex-linked genes: the **yellow (y), white (w) and miniature (m)** mutations.

MAIN MATERIALS

- A dissection microscope
- A frozen cool-pack and a container with ice

- A couple of paint brushes
- Your vial (**F_1 X F_1 cross**) containing F_2 flies.

For the computer analysis you will need:

- Desk or laptop computer. Small devices as cell phones or tablets **are not recommended**.
- Any web browser. The most common choices are Chrome, Mozilla, or Safari.
- Access to the **Virtual Genetics Lab** platform (www.ampossot.com/virtual_lab)
- Notebook and pen (or pencil)
- Calculator

PROCEDURE FOR LABORATORY WORK

1. Work in **pairs** of students. Collect your vial from the previous lab practice. They are located in the cart at the front of the lab room.

Remember the cross in this vial:

F_1 females (WT) x F_1 males (yellow, white, miniature)

2. Check your tube for the presence of **F_2** flies.

3. Transfer the flies to a clean vial and use ice to anesthetize them. Observe them using the dissecting scope.

4. Using a brush, move the flies around and observe the phenotypes. Identify male and female flies.

5. Score the flies. Remember that in this generation **you are expecting a wide range of phenotypic variation** (see page # 46). Keep this information safe, as you need this data to complete your assignment (question # 1). Discard the flies in the morgue.

6. Return the tube to the rack. You will need the flies for a second round of scoring next week. Remember that these flies are the **second generation or F_2 flies**.

PROCEDURE FOR THE GENETICS VIRTUAL LAB

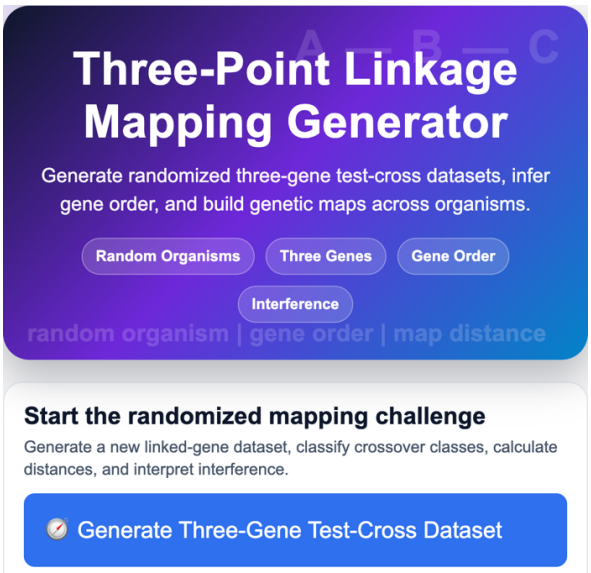
1. Launch the Virtual Genetics Lab. For this lab, you will use the "Three-Point Mapping in *Drosophila* tool" and the "Three-Point Linkage Mapping Generator" tool. The tools are available on Canvas or here:

"Three-Point Mapping in *Drosophila*" tool

www.ampossot.com/3point

"Three-Point Linkage Mapping Generator" tool

www.ampossot.com/mapgen



2. Click on the "Start" button. Read the information presented in the app and start performing the suggested tasks. This part of the lab is individual, and every student will have a different set of data.

3. Complete and submit your assignment by the deadline.

If you need further assistance, please contact your assigned TA or the lab coordinator. Their contact information is available in Canvas.