LABORATORY 3. *Drosophila* BREEDING EXPERIMENT (SEX-LINKED TRAITS)

LEARNING OUTCOMES

At the end of this lab practice, students should be able to:

- Obtained and analyze simulated F_1 data that illustrate sex-linkage.
- Propose a genetic model of inheritance for two traits based on the analysis of simulated *Drosophila* crosses.
- Analyze hypothetical crosses that illustrate a gene-interaction case (i.e., recessive epistasis).

INTRODUCTION

Interaction of Genes and Epistasis

Many **dihybrid** crosses deviate from the classical **9:3:3:1** ratio. Alleles of independently segregating genes interact with one another to produce novel phenotypes in further generations (**F_1** and **F_2**). In some cases, alleles of one gene prevent the expression of alleles of other genes, yielding different ratios such as **9:7, 9:3:4, 15:1**. The term **epistasis** is used to describe when the action of allele(s) of one gene prevent the expression of the allele(s) of another gene. Crosses involving an epistatic mode of inheritance have been used to determine the particular position of genes in a metabolic pathway or network. A detailed description of the different epistatic models is available in the "Gene Interaction" chapter on the recommended textbook.

Sex Linkage

The term **sex-linkage** refers to the **genes** that are tied to the sex chromosomes, rather than non-sex chromosomes (autosomes). In humans and many other organisms, these genes are termed **X-linked** or **Y-linked**. In *Drosophila*, Thomas Hunt Morgan provided the first experimental proof to demonstrate that alleles of a gene were sex-linked. His investigation of the inheritance of the **white-eyed** trait resulted in assigning the **gene for white eyes to the X chromosome.** In his studies, he also demonstrated that the white mutation (recessive form, w) is **epistatic** over the sepia (se) mutation. Hence, when the **w** mutation is present, the expression of the **se** allele is **repressed**. Please consider this information when solving some of the genetics problems in the lab assignment.

If you would like to explore more information about sex-linked genes in *Drosophila*, please refer to the book **"Sex-linked Inheritance in *Drosophila*"** by Thomas H. Morgan and Calvin B. Bridges freely available at the following link:

https://www.gutenberg.org/files/34368/34368-h/34368-h.htm

The "forked-line" method

Punnet squares are straightforward when dealing with one or two genes. However, when using three or more genes, the method becomes more complicated. For example, if considering three unlinked genes with two alleles, each parent can produce 8 different gametes (2^n), which will generate 64 possible genotypic combinations in the Punnet square (8 x 8). In this case, it is more effective to use the forked-line method to determine the possible outcomes of the cross (phenotypic and/or genotypic probabilities).

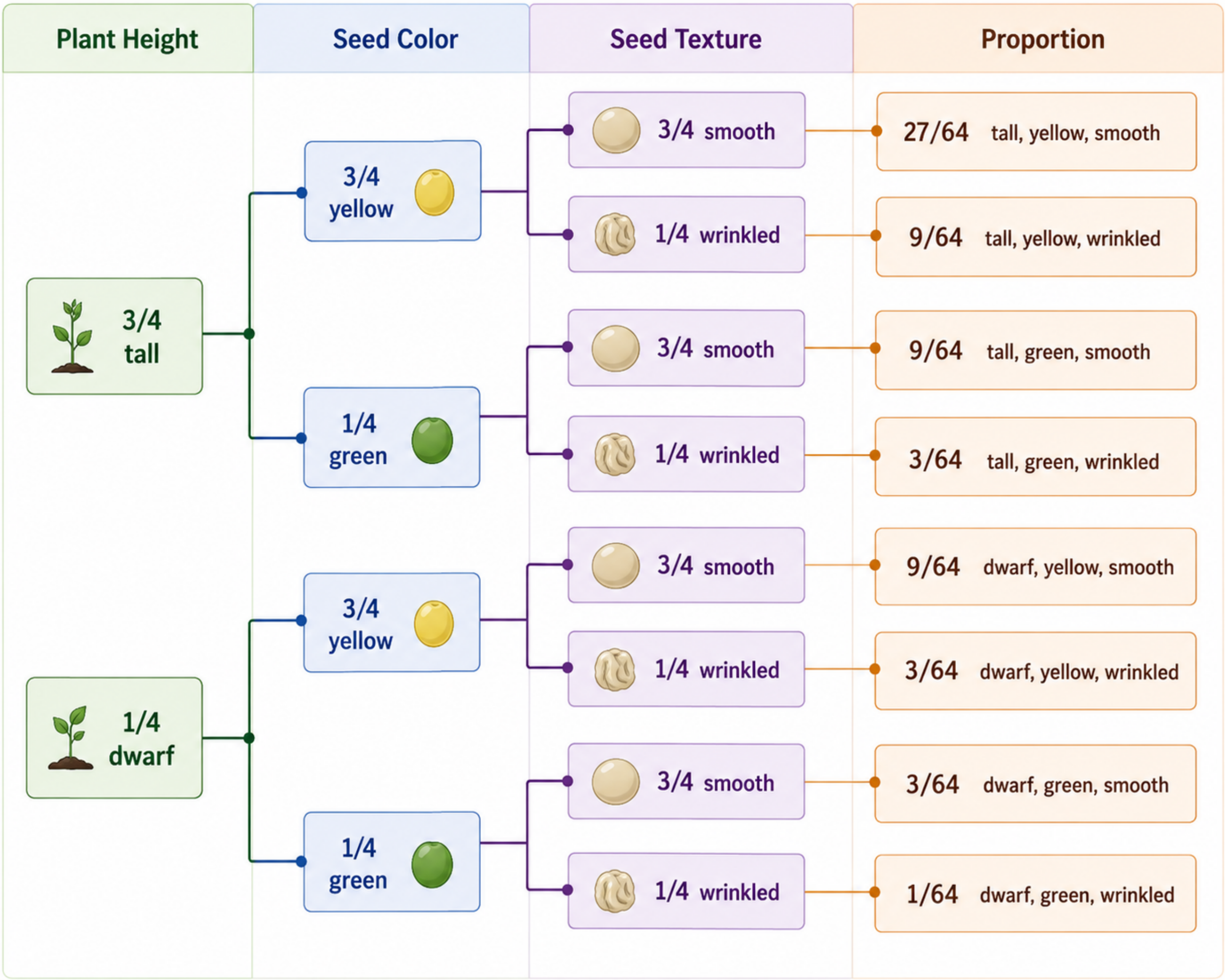
In the forked-line method, each branching point represents the expected segregation of phenotypes or genotypes for **only one** particular trait based on the parental genotypes. Then, multiplying the expected fractions across the diagram will provide the expected frequency (or probability) of any phenotypic or genotypic combination. Let's consider the following example to estimate the phenotypic frequencies of a tri-hybrid cross.

Traits:

Plant height:	**T:** tall;	**t:** dwarf
Seed color:	**Y:** yellow;	**y:** green
Seed texture:	**R:** smooth;	**r:**wrinkled

P_1: Tt Yy Rr **X** **P_2:** Tt Yy Rr

Tip: If you consider only **one trait**, for example **plant height**, the cross **Tt x Tt** will produce ¾ T_ (tall) and ¼ tt (dwarf) plants. Keep branching the fork with the remaining traits (genes).



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_1 parental flies.

For the computer simulations 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_1** parental flies.

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

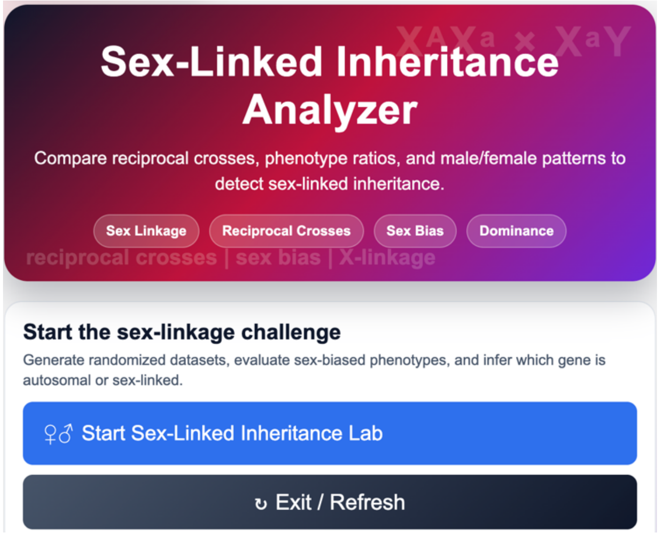
4. Using a brush, move the flies around and observe the phenotypes. Identify male and female flies.

5. Score the flies and put them in the **morgue**. Keep this information safe, as you need this data to complete your assignment.

6. Return the tube to the rack. **It is extremely important that you remove the F_1 x F_1 parentals from the tube.** Then, you will analyze the flies that will start emerging next week. These will be the **second generation or F_2 flies**.

PROCEDURE FOR THE VIRTUAL GENETICS LAB

1. Launch the Virtual Genetics Lab. For this lab, you will use the "Sex-linked Inheritance Analyzer" tool. It is available on Canvas (lab # 2 tab) or here:



Sex-Linked Inheritance Analyzer

Compare reciprocal crosses, phenotype ratios, and male/female patterns to detect sex-linked inheritance.

Start the sex-linkage challenge

Generate randomized datasets, evaluate sex-biased phenotypes, and infer which gene is autosomal or sex-linked.

www.ampossot.com/sex

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

3. Perform the number of crosses described in your assignment. Record the number of offspring phenotypes and **ratios** in the provided templates. An easy way to obtain phenotypic ratios is dividing the **higher-class number** by the **lowest class number**. Example: If you get 60 wild-type flies and 19 vestigial flies, the ratio will be 60/19 wild-type: 19/19 vestigial flies. That is 3.15 wild-type: 1 vestigial flies. **If only one phenotypic class is present, the ratio would be 1:0**.

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