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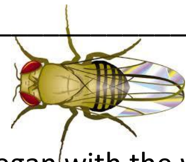


***Drosophila melanogaster* Genetics**

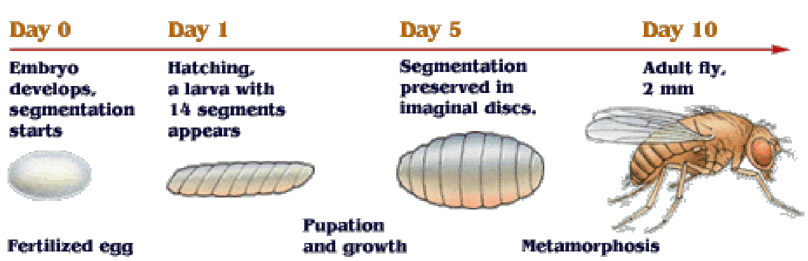
Modern genetics began with the work of Gregor Mendel. Through Mendel's studies of pea plants, he developed the laws of independent assortment and segregation. These two laws state that 1) when organisms are crossed and offspring are produced, the offspring receive one gene from each parent for a particular trait and 2) all traits are inherited independently of one another. Modern genetics has found that in some cases genes are linked and the inheritance of every trait isn't so simple. Mendel saw these laws at work in his pea plants as he observed patterns in the number of each phenotype (physical appearance) in different offspring. These laws are utilized today to explain heredity (what traits are passed from one generation to the next) and variation (the differences between parents and their offspring).

Through his experiments, Mendel determined that there can be multiple forms of the same gene. These alternate forms of genes, called alleles, code for slightly different expressions of a genetic trait. He saw that some alleles exhibited dominance and others exhibited recessive tendencies. When a dominant allele was combined with a recessive allele, only the dominant allele was expressed in the phenotype. In modern genetics, as in Mendel's experiments, a dominant allele is represented with a capital letter, and a recessive allele is represented with a lowercase letter.

Another mode of inheritance, sex-linked inheritance, is determined by a gene located on one of the sex chromosomes. In humans and fruit flies alike, males have one X chromosome and one Y chromosome. Females have two X chromosomes. Some genes are carried on the X chromosome that are not present on the Y. These genes are, therefore, sex-linked.

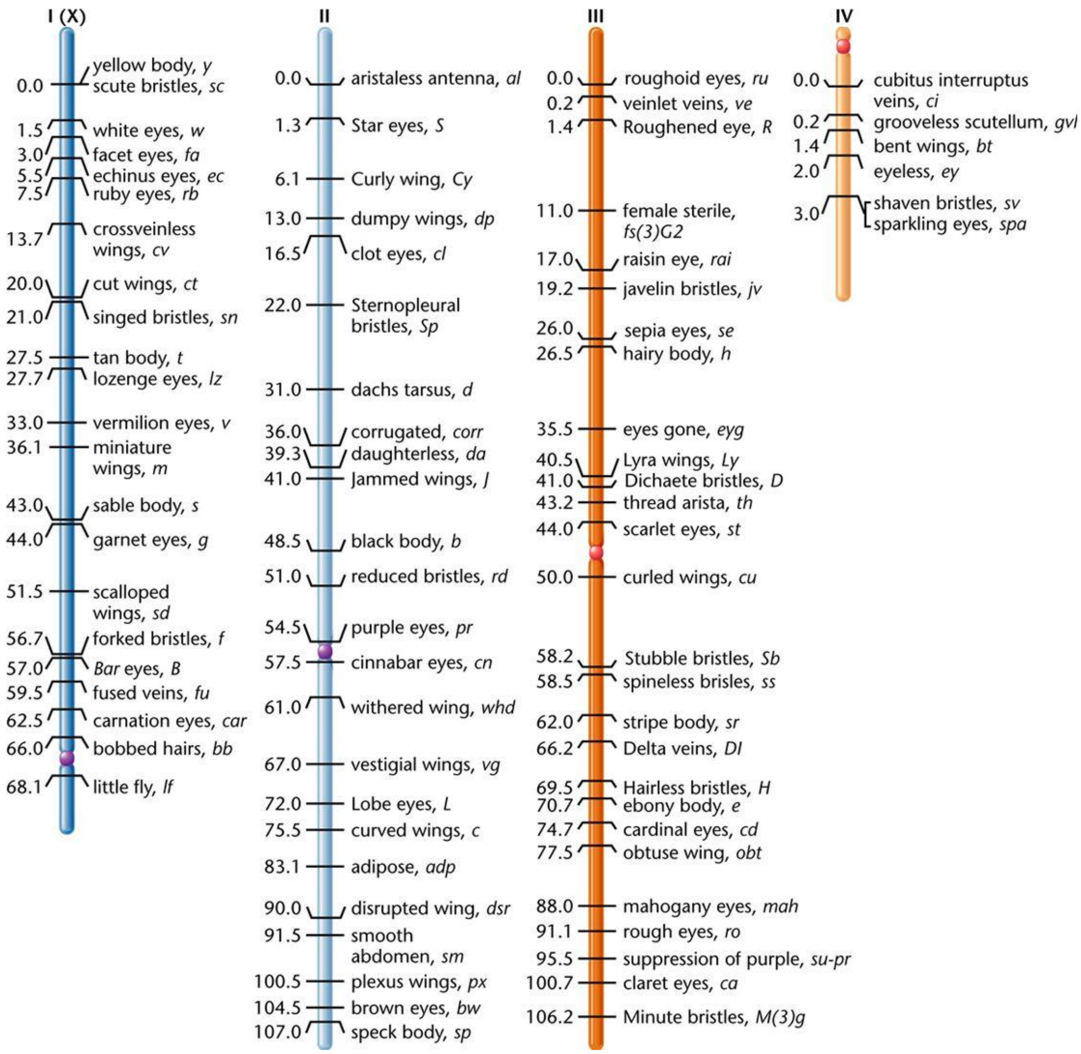
Drosophila has become a model organism for many reasons, including ease of use. Fruit flies are inexpensive to support, and millions fit in a single room. *Drosophila* has simple food and storage requirements, completes its life cycle in approximately 12 days, and has a genome packaged in only four chromosomes (see diagram). Furthermore, *Drosophila* is safe to work with, large enough to be examined with the naked eye, and easily anesthetized for observation and sorting. There is also a great deal of genetic homology (genetic similarities) between *Drosophila* and human beings, making the fruit fly an invaluable tool for disease research. *Drosophila* exhibits complete metamorphosis, going through egg, larval, pupal, and adult stages of life (see life cycle diagram).

In the early 20th century, the geneticist Thomas Hunt Morgan used *Drosophila* to provide the first evidence supporting the chromosome theory of inheritance, which claims that chromosomes carry genes and that all genes on a chromosome tend to be inherited together. Since Morgan's time, *Drosophila* has been used in thousands of experiments, and hundreds of mutations have been characterized.



Sexing Flies:

The most dependable and conspicuous sex-identifying traits of *Drosophila* are the shape and color of the posterior abdomen. Mature males have darker, blunter posteriors than females. Another difference is that males have sex combs on the uppermost joint of their forelegs, and females do not. These are used to mount the female while mating. Male flies typically are smaller than females of the same line, but this is not a reliable indicator of gender.



Crossing/Mating Fruit Flies:

Using *Drosophila*, you will investigate the autosomal monohybrid inheritance and sex-linked inheritance. During your observations, you will keep accurate records of the numbers of flies with mutant traits; this is called "scoring" the flies. You will observe the way in which a mutant trait is passed from one generation to the next, and then construct a hypothesis describing the mode of inheritance for the mutation.

When setting up crosses, the first two lines of flies that are crossed are called the Parental Generation (P_1 generation). The offspring of the P_1 generation are called the First Filial generation (F_1 generation). The offspring of the F_1 generation are the Second Filial generation (F_2 generation), and so on. When labeling crosses, the female description always comes first (ladies first ☺). For example, if we were crossing wild-type females with sepia eyed males, we would label the culture vial "wild-type X sepia). This standardized notation eliminates the need to distinguish between males and females when labeling crosses.

***Drosophila* Genetics Pre-Lab**- Read and annotate ENTIRE lab before answering these questions!

Please read the fruit fly genetics lab carefully and answer the following questions:

1. Gregor Mendel (the father of Genetics) developed two laws important to the field of genetics. What are these two laws (describe them) and how are they used today?

2. What is another name for alternate forms of a gene?

3. Look this up online or in your text:
   What is a genotype?

   What is a phenotype?

4. What happens when a dominant allele is combined with a recessive allele? Which allele is expressed and why (called the Law of Dominance)?

5. Where are sex-linked genes located in fruit flies?

6. List three good reasons we are using *Drosophila* fruit flies to study genetics.

7. How will you figure out if you are working with a male fly or a female fly? Be as specific as possible so you know what to look for!

8. You will be using *Drosophila* to study autosomal monohybrid inheritance and sex-linked inheritance. You can figure out what sex linked inheritance is, but what does autosomal monohybrid inheritance mean…

   a. What are autosomes?

   b. What does mono mean?

   c. What does hybrid mean?

d. What is autosomal monohybrid inheritance in fruit flies?

9. When crossing our fruit flies, the initial first two lines crossed are called the _______________

What are the offspring of this generation called?___________________________________

What are the offspring of that generation called?______________________________________

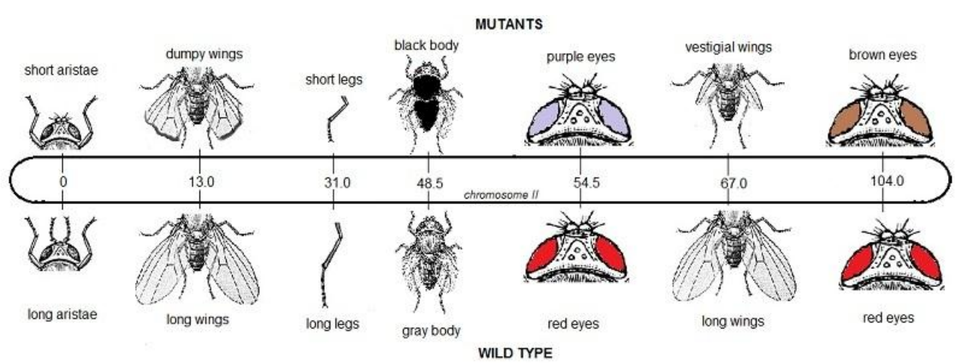
10. How long should we wait after mating the F_1 generation to look at the F_2 generation?

11. Which traits are we looking at specifically in this lab?

12. Circle which would represent an autosomal genotype: X^RX^r or Rr

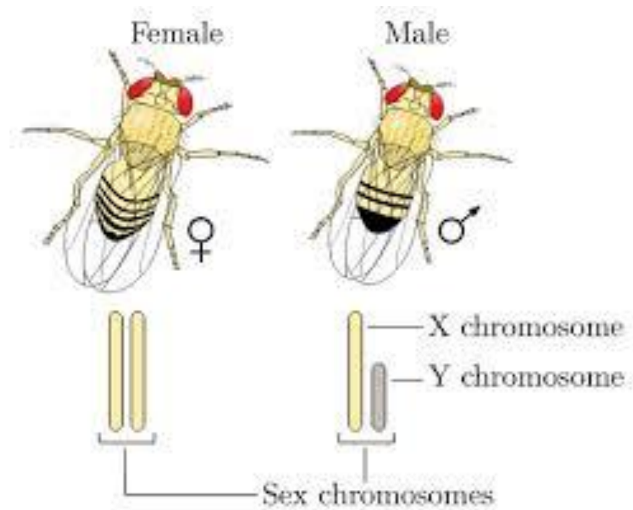
13. Circle which would represent a sex-linked genotype: X^RX^r or Rr

14. Wild-type is a term used to describe the most common alleles in a population. Others are called mutants. Sketch out and color a wild-type fruit fly with three different traits according to the diagram below. Sketch out and color a mutant fruit fly with the same three traits.



Day 1: Sexing Fruit Flies Date:________________________

1. You will receive a vial of anesthetized (sleeping) fruit flies. Please empty these fruit flies on to an index card.
2. Using a stereoscope and a sorting brush begin sexing the fruit flies. Pay attention to eye color, wing shape, and bristles on the legs. Males have sex combs. Sex combs are located on the forelegs of male flies and look like thick black lines right before the joint. They use these to mount the female when mating (see below).



3. Count how many males, females, red-eyed, and white-eyed flies you have in your sample. **Wild-type (normal) flies have red eyes and Mutant (abnormal) flies have white eyes.**

Males w/red eyes (wild type males)	Males w/white eyes (mutant males)
Females w/red eyes (wild type females)	Females w/white eyes (mutant females)

4. These flies came from the parent generation (P_1). Carolina (the lab that sent these flies to us) crossed these parents to produce an F_1 generation. Tomorrow we will look at the F_1 flies to help us determine if eye color is an autosomal or sex-linked trait. An autosomal trait is located on any chromosome other than a sex chromosome. A sex-linked trait can only be found on a sex chromosome.

5. Complete the following punnett squares to predict which ratios of offspring will have white or red eyes. We're going to use R and r as our alleles. R represents the dominant trait and r represents the recessive trait. From Mendel's law of simple dominance, we know that a dominant trait masks the effects of the recessive trait.

<u>Parent Generation (P_1):</u> These are the possible genotypes of the parents. We are only capable of determining the phenotype. More than one genotype can express the same trait as you can see below.

- <u>Autosomal dominant:</u> Autosomal genotypes are expressed simply with capital and lower case letters representing the alleles. If a trait is autosomal dominant, then the offspring will express the trait if they inherit only one dominant allele.

P_1= White female (RR or Rr) X Wild-type male (rr)

Possible genotypes = (RR X rr) or

(Rr X rr)

- <u>Autosomal recessive:</u> If a trait is autosomal recessive, then the offspring must inherit two recessive alleles (one from each parent) to express the recessive trait.

P_1= White female (rr) X Wild-type male (RR or Rr)

Possible genotypes = (rr X RR) or

(rr X Rr)

- <u>Sex-Linked dominant:</u> Sex-linked genotypes are represented with the capital or lowercase letter superscript on the X or Y chromosome, depending if the allele is on the X or Y chromosome. There are usually going to be more traits on the X chromosome than the Y. If a female is expressing a sex-linked dominant trait then her genotype is X^RX^R but it can also be $X^RX^{r.}$ That dominant allele on the X chromosome would cause her to express that trait. If a male has a sex-linked dominant trait then X^RY could be his only possible genotype.

P_1 = White female (X^RX^R or $X^r Y$) X Wild-type male ($X^r Y$)

Possible genotypes = ($X^R X^R$ x $X^r Y$) or

($X^R X^r$ x $X^r Y$)

- Sex-Linked recessive: If a trait is sex-linked recessive, a female must receive two X chromosomes with that gene. Her genotype must be X^rX^r only. If she inherits a dominant and a recessive allele on her X chromosome, then she will show the dominant trait. Males will express the sex-linked recessive trait if the only X chromosome they inherit is recessive. His genotype could only be X^rY.

P_1 = White female (X^rX^r) X Wild-type male (X^RY)

***Po*ssible genotypes = (X^rX^r x X^RY)**

Expected Results for the F_1 Generation: use % ratios (no fractions).

1. Using your punnett squares above, predict the genotypic and phenotypic ratios if this trait is autosomal dominant: *The first is done for you:*

(RR X rr)

Genotypic Ratio ____________100% Rr______________________________

Phenotypic Ratio____________100% white-eyed______________________

	R	R
r	Rr	Rr
r	Rr	Rr

(Rr X rr)

Genotypic Ratio ____

Phenotypic Ratio____

2. Using your punnett squares, predict the genotypic and phenotypic ratios if this trait is autosomal recessive:

(rr X RR)

Genotypic Ratio ____

Phenotypic Ratio____

(rr X Rr)

Genotypic Ratio ____

Phenotypic Ratio____

3. Using your punnett squares, predict the genotypic and phenotypic ratios if this trait is sex-linked dominant:

($X^R X^R$ x $X^r Y$)

Genotypic Ratio ____

Phenotypic Ratio____

($X^R X^r$ x $X^r Y$)

Genotypic Ratio ____

Phenotypic Ratio____

4. Using your punnett squares, predict the genotypic and phenotypic ratios if this trait is sex-linked recessive:

($X^r X^r$ x $X^R Y$)

Genotypic Ratio ____

Phenotypic Ratio____

Expected Phenotypic Ratios (use %, no fractions) for F_1 Offspring - Summary

Autosomal dominant: Red-eyed (wild-type) male: ____________ Red-eyed (wild-type female:____________ White-eyed (mutant) male:_____________ White-eyed (mutant) female:____________	***Sex-Linked dominant:*** Red-eyed (wild-type) male: ____________ Red-eyed (wild-type female:____________ White-eyed (mutant) male:_____________ White-eyed (mutant) female:____________
Autosomal recessive: Red-eyed (wild-type) male: ____________ Red-eyed (wild-type female:____________ White-eyed (mutant) male:_____________ White-eyed (mutant) female:____________	***Sex-Linked recessive:*** Red-eyed (wild-type) male: ____________ Red-eyed (wild-type female:____________ White-eyed (mutant) male:_____________ White-eyed (mutant) female:____________

Day 2: Scoring the F_1 Generation and Setting up the F_2 Vials: Date:___________________

1. Today you will score (count and record) F_1 flies. Take one vial of F_1 flies, nap them (your teacher will do this), pour them out on to an index card and record whether each fly is wild-type (red-eyed) male or female and mutant (white-eyed) male or female. Yesterday you made predictions for each type of inheritance…these were your expected results. Today you will count the actual phenotypes that resulted from the cross of the parent generation (P_1 generation)…these are the observed results.

Tally your observed phenotypes below:

Males w/red eyes (wild type males)	Males w/white eyes (mutant males)
Females w/red eyes (wild type females)	Females w/white eyes (mutant females)

Observed Phenotypic %Ratio:

Total Number of flies: ____________________

% Red-eyed male _____________

% Red-eyed female _____________

% White-eyed male _____________

% White-eyed female _____________

2. Now look at your observed phenotypic ratio and compare it to the predicted phenotypic ratio summary chart. Does your observed match up or are they close to any of your predicted phenotypic ratios? If so, which type(s) of inheritance could be possible for this eye color trait?

You may not be able to tell just yet…that's why we are going to cross the F_1 flies…

Setting up the F_2 Vials: Date:_________________________

Crossing the F_1 generation to produce an F_2 (second filial) generation will allow you to see (for certain) how a certain trait is inherited. If you were not able to determine the mode of inheritance from the P_1 to F_1 generations, you should be able to make a determination after examining the F_2 generation. You will need to put the F_1 flies to sleep as you did yesterday to the parents to manipulate them.

1. Add one level measure of *Drosophila* medium to two empty vials. Your group will have two vials for the F_2 generation.
2. Label each vial with period number, table number, F_2, vial 1 or vial 2.
3. Once the medium is in your tube and settled, sprinkle 4-6 grains of yeast on to the surface. Do not add too much yeast as an excessive amount can damage the culture.
4. Empty the vial of sleeping flies to observe the sample of F_1 generation. You will have both male and females.
5. Put five male/female pairs into each of your F_2 vials. Record how many of each type you are putting into your vial.

Males w/red eyes (wild type males)	Males w/white eyes (mutant males)
Females w/red eyes (wild type females)	Females w/white eyes (mutant females)

6. Complete punnett squares below to obtain the expected genotypic and phenotypic ratios for the F_2 offspring below.

Expected Results for the F_2 Generation:

1. Fill in the punnett squares and predict the genotypic and phenotypic %ratios if this trait is autosomal dominant:

(RR X rr)

Genotypic Ratio ____

Phenotypic Ratio____

(Rr X rr)

Genotypic Ratio ____

Phenotypic Ratio____

2. Fill in the punnett squares and predict the genotypic and phenotypic ratios if this trait is autosomal recessive:

(rr X RR)

Genotypic Ratio ____

Phenotypic Ratio____

(rr X Rr)

Genotypic Ratio ____

Phenotypic Ratio____

3. Fill in the punnett squares and predict the genotypic and phenotypic ratios if this trait is sex-linked dominant:

($X^R X^R$ x $X^r Y$)

Genotypic Ratio ____

Phenotypic Ratio____

($X^R X^r$ x $X^r Y$)

Genotypic Ratio ____

Phenotypic Ratio____

4. Fill in the punnett square and predict the genotypic and phenotypic ratios if this trait is sex-linked recessive:

($X^r X^r$ x $X^R Y$)

Genotypic Ratio ____

Phenotypic Ratio____

Expected Phenotypic %Ratios for F_2 Offspring- Summary:

Autosomal dominant: Red-eyed (wild-type) male: _____________ Red-eyed (wild-type female:_____________ White-eyed (mutant) male:______________ White-eyed (mutant) female:_____________	***Sex-Linked dominant:*** Red-eyed (wild-type) male: _____________ Red-eyed (wild-type female:_____________ White-eyed (mutant) male:______________ White-eyed (mutant) female:_____________
Autosomal recessive: Red-eyed (wild-type) male: _____________ Red-eyed (wild-type female:_____________ White-eyed (mutant) male:______________ White-eyed (mutant) female:_____________	***Sex-Linked recessive:*** Red-eyed (wild-type) male: _____________ Red-eyed (wild-type female:_____________ White-eyed (mutant) male:______________ White-eyed (mutant) female:_____________

NOTE: In time, the F_2 generation of flies will be in the form of eggs and larvae in the vials. You should be able to see trails in the medium left by the larvae. You may also be able to see the black mouthparts of the larvae as they move through the medium.

7-10 Days after setting up the F_2 Vials : Scoring the F_2 Generation Date:_____________

1. Today you will score (count and record) F_2 flies. Take one vial of F_2 flies, nap them (your teacher will do this), pour them out on to an index card and record whether each fly is wild-type (red-eyed) male or female and mutant (white-eyed) male or female.

Tally your observed phenotypes below:

Males w/red eyes (wild type males)	Males w/white eyes (mutant males)
Females w/red eyes (wild type females)	Females w/white eyes (mutant females)

Observed %Phenotypic Ratio:

Total Number of flies: ___________________

% Red-eyed male ______________

% Red-eyed female ______________

% White-eyed male ______________

% White-eyed female ______________

2. Now look at your observed phenotypic ratio and compare it to the predicted phenotypic ratio summary chart. Does your observed match up or are they close to any of your predicted phenotypic ratios? Determine the mode of inheritance for the eye color trait in fruit flies.

Determined Mode of Inheritance: ____