EXERCISE 6

# Mendelian Genetics: Lessons from the Fruit Fly

Even in prehisoric times, humans recognized that certain physical characteristics of plants, livestock, and people were passed on from one generation to the next. This rudimentary knowledge of genetics was important for improving the cultivation of corn and wheat, as well as the domestication of cattle, horses, and dogs. However, it wasn't until **Gregor Mendel**, an austrian monk who worked on the heritable traits of pea plants in the 1860's, that the way these traits were passed on to offspring was carefully quantified and several fundamental principles of genetics were discovered. Mendel did not know the role of the cell nucleus, chromosomes, or DNA—they were discovered much later—but he did attribute the passing of parental characteristics to their offspring to heritable 'factors'. These 'factors' are now known as genes.

**Thomas Hunt Morgan**, in the early 1900's, used the heritable traits of a common species of fruit fly, *Drosophila melanogaster*, to expand our understanding of genetics. Morgan was the first to show through experiments that genes were located on chromosomes. He also found that certain fruit fly traits (e.g., white vs. red eye color) are found on the same chromosomes that also determine their sex. Fruit flies have several characteristics that make them excellent subjects for genetic studies, as they: 1) are easily reared in the laboratory, 2) are prolific, 3) have a relatively short life cycle (approximately two weeks), 4) have relatively prominent characteristics that can be used in sex determination, and 5) have fairly simple chromosome organization—3 pair of autosomal chromosomes and 1 pair of sex chromosomes.

In this exercise, you'll use normal (wild type) fruit flies and those having one or more mutant forms of body color, eye color, eye shape, wing shape, etc. to investigate some of the basic principles of genetics. Breeding fruit flies is fairly easy, but it requires that you learn a number of skills in sex recognition, making up media, anesthetizing, data collection, and simple statistics. One thing is guaranteed: you'll never view those little pesky flies hovering above a bowl of overripe fruit in the same light.

## *Objectives:*

Upon competing this lab exercise you should be able to:

1. Define or describe the terms in this handout that are in **bold** type.
2. Compare and contrast the following sets of terms: autosome vs. sex chromosomes, genotype vs. phenotype, homozygous vs. heterozygous, dominant vs. recessive alleles, reduction vs. equatorial divisions, segregation vs. independent assortment, and haploid vs. diploid.
3. Summarize chromosome action during meiosis.
4. Show how Mendel's laws of inheritance are related to meiosis.
5. Describe the *Drosophila* life cycle and identify each stage.
6. Identify the characteristics that are used to distinguish between the sexes of adult fruit flies.
7. Prepare media, make crosses, and culture fruit flies for genetics studies.

8. Use *Drosophila* notation, Punnett squares, and the Product Rule to predict outcomes of monohybrid, dihybrid, sex-linked, and linked autosomal trait crosses.
9. Describe how crossing over results in genetic recombination and infer the relative position of a set of linked alleles based on recombination frequency.
10. State the appropriate hypothesis as to the predicted outcome of selected crosses, test the hypothesis against observed data obtained from the cross using the chi-square statistical analysis, and interpret the results.

## *Definitions of Basic Genetics Terms:*

The basic template of an organism's genetic information is coded in the double-stranded DNA of its cells. In most prokaryotes the double-stranded DNA is circular and together with associated proteins make up a rather jumbled mass of a single **chromosome** found in the cell's nucleoid region (remember: prokaryotes lack a nuclear membrane). In eukaryotes the DNA and associated protein exist in discrete chromosomes that are enclosed within the cell's nucleus. Often the chromosomes are paired—that is, each has a **homologue**, having identical size, shape, and genes coding for the same kinds of traits. Eukaryotic cells can be haploid (n), diploid (2n), triploid (3n), or polyploid (>3n) depending on the number of homologues for each chromosome. Diploid cells have paired chromosomes—one homologue of the pair was obtained from the male parent and the other from the female parent—whereas, they are unpaired in haploid cells. For triploid cells, each chromosome type has two other homologues, and polyploid cells have multiple homologues for each chromosome.

The total number of homologous chromosome pairs in a nucleus is usually species specific and varies widely across the plant and animal kingdoms. However, very often one pair, the **sex chromosomes**, is involved in sex determination and the remaining pairs, the **autosomes**, carry genes for traits other than sex determination. The table below contains some examples of the variation observed among species.

**TABLE 25. Number of autosomes and sex chromosomes for a variety of diploid organisms.**

| Species | Autosome Pairs | Sex Chromosome Pairs | Total Number |
|---|---|---|---|
| *Penecillium* | - | - | 2 |
| Garden Pea | - | - | 14 |
| Fruit Fly | 3 | 1 | 8 |
| Chickens | 38 | 1 | 78 |
| Dog | 38 | 1 | 78 |
| Humans | 22 | 1 | 46 |

A **gene** is a discrete unit of DNA that codes for a particular trait (e.g., eye color). Alternate forms of a gene (e.g., brown, blue, or white eyes) are called **alleles** and the allele's physical location on a chromosome is its **locus** (*pl*... loci). The following are loci of various fruit fly traits:

**TABLE 26. Loci of some fruit fly traits. Traits beginning with a capital letter denote the allele is dominant.**

| Trait | Chromosome | Position (Map Units) |
|---|---|---|
| *yellow body* | 1 | 0.0 |
| *Curly wings* | 2 | 6.1 |
| *purple eyes* | 2 | 54.5 |
| *Wrinkled wings* | 3 | 46.0 |
| *eyeless* | 4 | 0.2 |

Diploid organisms possessing the same allele at the same locus on each homologue are **homozygous** for that trait; whereas, those having different alleles at a particular locus are **heterozygous**. For genetics studies, it is important to know which alleles are present on each homologous chromosome—that is, the **genotype**. For example, a fruit fly with the purple-eye allele on both chromosome-3 homologues is homozygous for purple eyes and will physically show purple eyes (i.e., it will have the purple-eye **phenotype**). If, however, the fruit fly has the purple-eye allele on one chromosome and the normal, red-eye allele on its homologue, then it is heterozygous for eye color. The allele appearing in the phenotype of heterozygous individuals is the **dominant** allele and the one hidden in the heterozygous condition is the **recessive** allele. Red eyes are dominant over purple eyes; therefore, flies in the example above would have the red-eye phenotype, but have a heterozygous purple-eye genotype. Recessive traits are only expressed in homozygous genotypes.

Traits are **linked** if their genes are found on the same chromosome (we will see how they are linked, later) and **sex-linked** if they occur on the sex chromosomes.

## *Meiosis in Review:*

Understanding the genetics of most diploid organisms requires that you first have a good foundation in the way chromosomes move and interact during the production of gametes. For simplicity, the following hypothetical example (Fig. 28) has two pair of homologous chromosomes, a pair of autosomes and a pair of sex chromosomes, and crossovers between homologous pairs are not considered. By carefully following these chromosomes from the parents' somatic cells, through meiosis to form gametes, then fertilization to the resulting offspring; one can see the connection between chromosome movement and several of the laws Mendel observed. Note the sex chromosomes in the male and female nuclei represented in the example. One of the male sex chromosomes is 'crooked'. This is similar to the X-Y sex determination system seen in a wide variety of organisms, including fruit flies, horses, and humans. In the male of this example, one homologue of the sex chromosome pair is 'crooked' (the Y) and the other is 'straight' (the X). The female has two 'straight' (X) homologues. Each sperm cell has either one 'straight' sex chromosome or one 'crooked' sex chromosome in addition to one autosome, while all eggs have one 'straight' sex chromosome plus one autosome. All the gametes are haploid (n). Union of eggs and sperm produce male or female diploid (2n) offspring, depending on which sex chromosome the sperm cell was carrying.

The fundamentals of the meiotic divisions can be summarized as follows: 1) during interphase I of meiosis, the chromosomes are duplicated so that, 2) the duplicated chromosomes, duplicates attached at the centromere, can be seen during prophase I, 3) the homologous chromosomes, with their attached duplicates, line up at the cell's equatorial plane, during metaphase I, 4) followed by a **reduction division** during anaphase I through telophase I, where the homologues separate

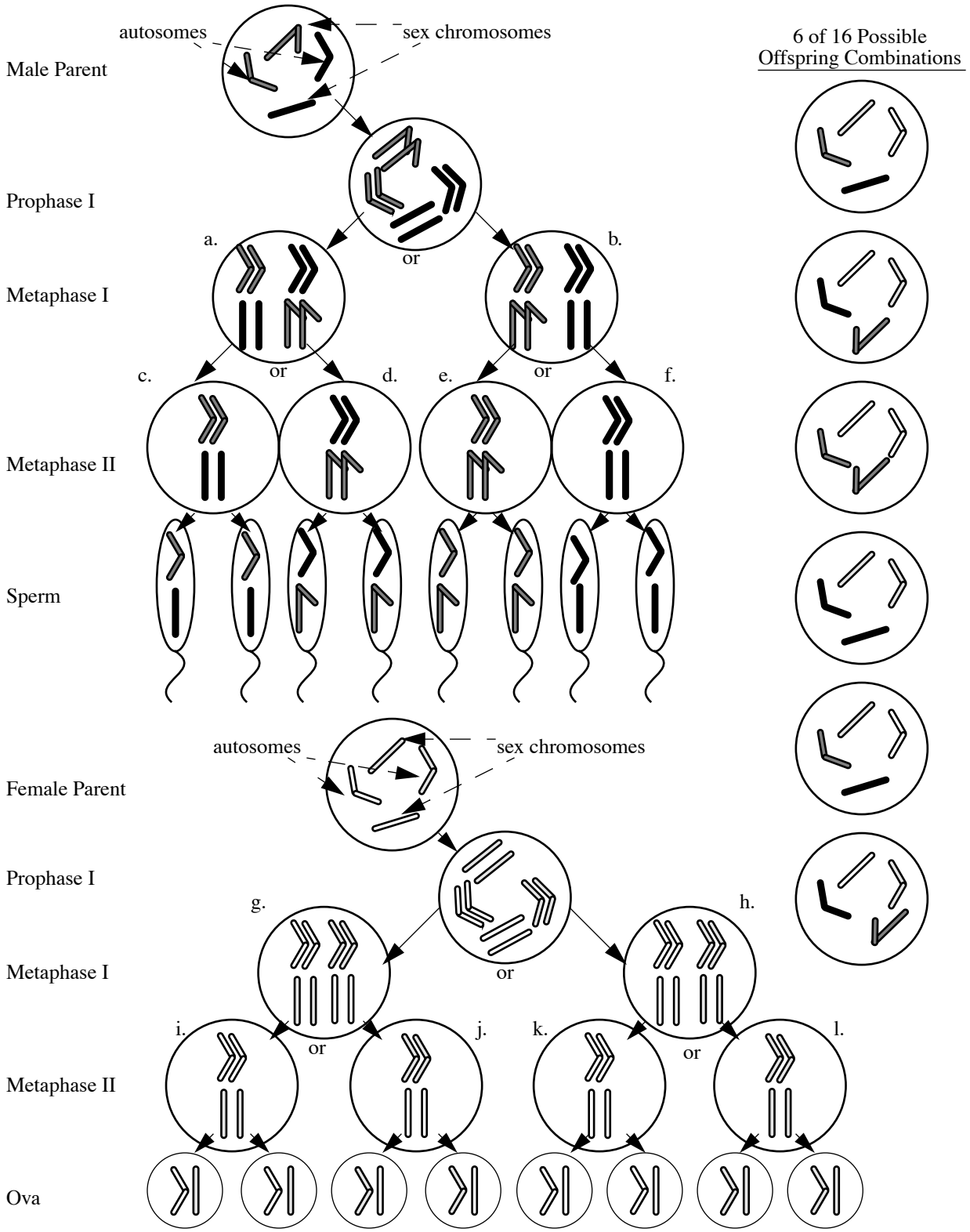
into different cells, 5) the second part of meiosis proceeds with the now unpaired chromosomes lining along the cell's equatorial plane by their centromeres during metaphase II, then 6) the duplicates separate during anaphase II through telophase II—an **equatorial division**—to form the gametes.

Mendel proposed two laws, based on his work with garden peas, that he believed governed the inheritance of traits. The first was the **law of segregation**, which states that if different forms of a trait (**alleles**) are present in the parent, they separate during gamete formation; and the second, the **law of independent assortment**, states that if more than one pair of alleles are present in the parent, they will separately independently during gamete formation. These laws hold true only for special cases, where none of the traits examined are linked, but they do explain the movement of chromosomes. Mendel was fortunate none of the traits he was studying in garden peas occurred on the same chromosome as another; otherwise, he would have had extremely confusing results.

The chromosomes in the example are differentially shaded to make it easy to follow their movement through various meiotic phases. The black chromosomes originally can from the male parent's father and the dark-shaded chromosomes were from the male's mother. Similarly, the white chromosomes originally came from the female parent's father, and the light-shaded chromosomes were from the female's mother. Mendel's law of independent assortment is evident at metaphase I of the example, where the duplicated homologues are lined up on the cell's equatorial plane. At this point, there is an equal probability that the male's dark-shaded autosome and black sex chromosome will end up on the left side of the equatorial plane (Fig. 28a) as there is of the dark-shaded auto some and sex chromosomes ending up on the left side (Fig. 28b). The same pattern is seen for the female's white and light-shaded chromosomes during metaphase I (Fig. 28g and 28h). Because organisms may have hundreds of cells undergoing meiosis during gametogenesis, nearly equal numbers of both possible chromosome combinations would be expected.

Mendel's law of segregation is also the result of chromosome movement during meiosis. Comparing the parent's chromosomes with those of its gametes shows the homologues are segregated though meiosis so that exactly half of the resulting gametes end up each homologue. Segregation can be illustrated by following the male parent's sex chromosomes (the black 'straight' and dark-shaded 'crooked' chromosomes) through the meiotic stages. These chromosomes are lined up on opposite sides of the cell's equatorial plane during metaphase I, then separate into different cells during telophase I. The subsequent equatorial division of meiosis II results in one-half the sperm cells obtaining the black 'straight' chromosome and the other half having the dark-shaded 'crooked' chromosome.

**FIGURE 31. Nuclear status of parental somatic cells, gamete formation, fertilization, and resulting offspring cells of a hypothetical diploid organism. Note: This example does not show crossovers.**



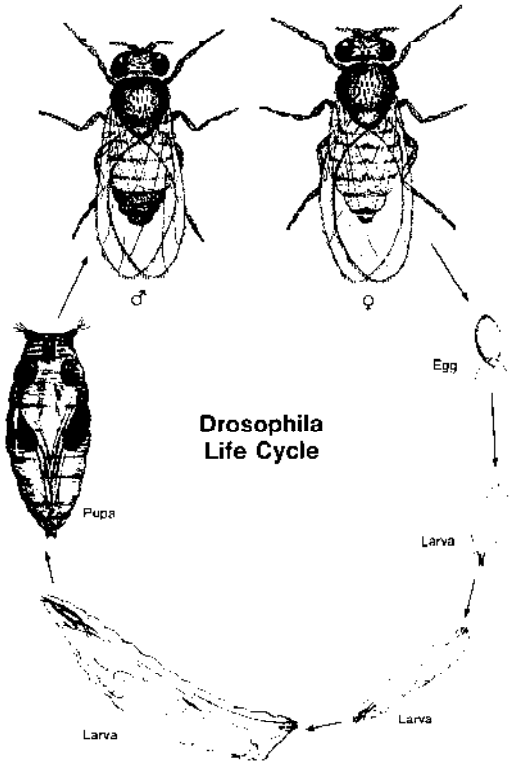
## *Fruit Fly Biology & Culture:*

Fruit flies are fairly easy to culture in the laboratory; only a few specialized materials and some basic skills are required to use them successfully in genetics studies. Here is a short guide to the biology and culture of Drosophila melanogaster.

### Fruit Fly Life Cycle:

Drosophila melanogaster has complete metamorphosis life cycle with an egg, 3 larval instars, pupa, and adult. If cultures are maintained at 21 ˚C, it takes an average of two weeks between when the flies are mated and when their offspring emerge as new adults. Development time is a couple of days shorter if the temperature is 23 ˚C.

**FIGURE 32. Life cycle of *Drosophila melanogaster.* From the Carolina Drosophila manual, Carolina Biological Supply.**




Adults are ready to mate within 8 to 12 hours after they emerge and can live under laboratory conditions for more than a month. Females begin laying eggs within a two days after mating and first-instar larvae are active within three to four days. These soft-bodied larvae grow rapidly as they crawl through rotting fruit eating fungi and bacteria. In the lab, we will culture them on an instant medium developed by the Carolina Biological Supply Company. Larval flies can almost be considered aquatic, because they require a moist environment in which to survive. They continue to grow and molt through two additional larval instars before seeking a drier place to pupate, usually on the side of the culture tube. The pupal stage lasts for approximately six days. Shortly before emergence, the pupae darken and the wingpads become visible as two small black spots. Adult flies emerge through small openings on the anterior end of the pupal cases. Newly emerged adults are pale, skinny, and their wings shriveled. It takes an hour or so before their abdomens become round and their wings expand.

## Culturing

We will use clear plastic vials (1.25" diameter x 4" high) with plastic foam plugs for culturing. The special culture medium developed by Carolina Biological Supply (Formula 4-24® Blue) is in flake form and you simply add equal volumes of Instant Drosophila Medium and tap water to the vials, then allow the mixture to sit for about a minute. The medium is supplied with small plastic cups for measuring media and water. After the medium has been prepared, add about 6–10 grains of dry yeast to each vial. Growing larvae will feed on the yeast, but too much yeast will kill the culture. The vials are now ready for adding flies.

## Virgin Females

Genetics experiments require that the parents of the offspring are known. For example, if you want to determine the outcome of mating a yellow-bodied female with a wild-type male, then you must be certain that the female has not mated with any other males besides wild-type males. Because females can store sperm for weeks after a single mating, it is important to isolate females before they have had the opportunity to mate. After mature pupae are present in the culture containing the females of interest, clear all adult flies from the vial by tapping them into a clean, dry vial with a foam plug. **Be sure all living adults have been removed from the vial.** Vials of adults can be placed in a freezer for 15 minutes, then discarded into a special container labelled as the Fly Morgue. Any flies emerging for about 10 hours after the cultures have been cleared are virgins. You only need this method to obtain virgin females; males used in matings do not need to be virgins.

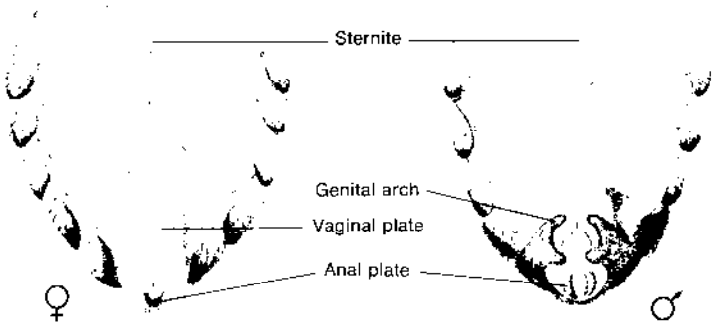
## Anesthetizing

Flies need to be sexed and sorted both for making the matings and for counting their subsequent offspring. To immobilize flies for sorting and counting, we will use FlyNap®, a safe anesthetizing agent that will knock the flies out for an hour or so. To use FlyNap, first tap the flies out into a clean, dry vial with a foam plug, then wet a felt wand with FlyNap and insert the felt end beneath the foam plug so that the wand handle extends above the vial rim. **Make sure the cap is always tightly screwed down on the FlyNap bottle.** The flies should be sufficiently anesthetized in about two minutes. Immediately remove the wand and tap the immobilized flies unto a sorting card. Flies can be sorted under 15X magnification with a dissecting microscope. Use a fine-bristled, artist's paint brush to gently move the flies, and make sure the flies are not left exposed to the illuminator heat for more than a few minutes as this can kill them. Unused flies can be discarded in the Fly Morgue.

## Sexing

Careful sex determination is essential in preparing matings for genetics studies. Sexes can be distinguished soon after emergence and, with practice, even in the pupal case. There are only two features that are reliable for determining sex in all fruit flies: 1) the genitalia, and 2) the presence or absence of sex combs. Place anesthetized flies ventral side up on a card and view them with a dissecting microscope at 30X or higher magnification. The posterior-most abdominal segments of male flies have a darker, scleritized genital arch, penes, and divided anal plate; whereas females lack these features. Instead, females have a light-colored, subterminal vaginal plate with a small, medial slit running lengthwise that is barely visible at 30X.

**FIGURE 33. Ventral view of the abdomens of male and female fruit flies. From the Carolina Drosophila manual, Carolina Biological Supply.**




Males also have a set of dark, closely-spaced bristles—called **sex combs**—on the inside edge of each of their front legs. Females lack sex combs. To make sure you've made the correct sex determination, check to see if each fly you sex possesses both the necessary genitalia and foreleg characteristics.

## Mating

After you've selected about six or eight virgin females of one genotype and the same number of males of the second genotype, you are ready to gently transfer the flies to a vial that has been previously prepared with media. Do not place the flies directly on the media. Rather with the vial lying on its side, place the flies along one dry plastic side of the vial and insert the foam plug. Let the vial remain on its side until the flies are awake and active. Be sure to label the vial with the date, parental genotypes, your name, and lab section.

## Phenotypes

Fruit flies have been widely used in genetics studies for several decades and geneticists along with commercial breeders have isolated hundreds of phenotypes that are now available to make selected matings. Below is list of some of the more common phenotypes available, along with a short description and abbreviation used for each:

**TABLE 27. Abbreviations and descriptions of various common phenotypes.**

| Phenotype | Abbr. | Description |
|---|---|---|
| *Eyeshape:* | | |
| *Normal* | + | wild type |
| *Eyeless* | | eyes reduced |
| *Lobed* | L | small eyes on pedicle |
| *Bar* | B | long, narrow eyes |
| *Eye color:* | | |
| *Red* | + | wild type |
| *White* | w | white eyes |
| *Sepia* | se | eyes brownish to black with age |
| *Antennae:* | | |

**TABLE 27. Abbreviations and descriptions of various common phenotypes.**

| Phenotype | Abbr. | Description |
|---|---|---|
| *Normal* | + | wild type |
| *Spineless-aristapedia* | ss[a] | antennae enlarged and leg-like |
| *Wings:* | | |
| *Normal* | + | wild type |
| *Vestigial* | vg | wings reduced |
| *Apterous* | ap | wingless |
| *Dumpy* | dp | wings truncated |
| *Bristles:* | | |
| *Normal* | + | wild type; long |
| *Singed* | sn | short and curled |
| *spineless* | ss | short and fewer |
| *Body color:* | | |
| *Normal* | + | tan |
| *Yellow* | y | yellow |
| *Ebony* | e | dark brown |

Photos of many of these phenotypes will be available in the lab for you to compare.

### Rearing flies

Fly cultures are most successful if they are kept near 21 ˚C. Larval development is faster at 25 ˚C but harmful fungi, bacteria, and mites often kill cultures at these temperatures. It's a good idea to check the cultures every few days; you may have to start them over if the population becomes infected. After about 6 days the parents should be removed.

### Counts—The Observed Results

Within 14–16 days after the cultures have been started, the offspring will begin to emerge. It's best to anesthetize, sort, and count the newly emerging adults every-other-day, or so, over the 10-day emergence period. Careful records should be kept of the counts; data sheets are provided at the end of this lab exercise for this use.

## *Fundamental Genetics—The Predicted Results*

This section provides an overview of some basic concepts in genetics with an emphasis on the inheritance of traits in fruit flies. Additional information will be given in the lab lecture, the lecture course, and is available in Campbell (1996; chapters 12–14).

## Drosophila Notation

Workers using fruit flies for genetics studies have devised a somewhat standard shorthand method for designating genotypes and phenotypes. You were already introduced to some of this notation in the Phenotype section above, but here it will be treated more extensively. First, normal wild-type flies or a single wild-type allele is designated with a "+", and other alleles are given an abbreviation (a list of common phenotypes and their abbreviations are given in a table above). Abbreviations beginning with a lower case letter indicate the trait is recessive (e.g., "w" for white eyes and "y" for yellow body), whereas those beginning with an upper case letter (e.g., "B" for bar eyes) are dominant alleles.

A fly's genetic condition can be further designated as to whether or not a particular set of traits are linked. Those that are linked are joined by an underlined, and those not linked have separate underlining. For example, the notation "y v f" denotes these three alleles occur on the same chromosome; however, "y v e" shows that "y" and "v" are on one chromosome and "e" is on another chromosome. The notation can also be used to show homozygous and heterozygous conditions. For example, $\frac{\text{y v f}}{+\ +\ +}$ shows all three traits are linked and heterozygous; this individual's phenotype would be wild type because all the mutant alleles are recessive.

## Monohybrid crosses—one pair of alleles

The simplest type of cross is the monohybrid because it involves just one pair of alleles. A typical monohybrid cross with fruit flies might pair homozygous wild-type ($\frac{+}{+}$) males with homozygous recessive, vestigial-winged ($\frac{\text{vg}}{\text{vg}}$) females. The parents in this cross are called the parental, or **$P_1$,** generation. $P_1$ males in this case would produce haploid sperm that carry the "+" allele and eggs of the female parents would carry the "vg" allele. Offspring from this mating are the **$F_1$**, or first filial generation, and in this case, would all be heterozygous for wing shape ($\frac{+}{\text{vg}}$) yet have normal wings. $F_1$ females of this cross would mature to produce eggs, about half of which would contain the "+" allele and the other half with the "vg" allele. In the same way, half of the sperm produced by $F_1$ males would contain the "+" allele and the other half would contain the "vg" allele.

The second filial, **$F_2$**, generation is produced by mating $F_1$ females with $F_1$ males. A simple tool for showing the proportion of offspring with each genotype possible resulting from such a cross is the **Punnett square**. To use a Punnett square, you simply put the possible sperm genotypes along the column headings and the egg genotypes in the row headings of a table. The expected offspring genotypes and their proportions are found by filling in the table with all the possible sperm and egg combinations. A Punnett square is used to determine expected genotypes of the $F_2$ offspring in the above example.

**TABLE 28. Punnett square showing genotypes of $F_1$ sperm and eggs as well as the expected genotypes of the resulting $F_2$ generation**

| Eggs | Sperm | |
|---|---|---|
| | + | vg |
| + | $\frac{+}{+}$ | $\frac{+}{\text{vg}}$ |
| vg | $\frac{+}{\text{vg}}$ | $\frac{\text{vg}}{\text{vg}}$ |

As shown by the above table, $F_2$ offspring are expected to have a genotypic ratio of 1 homozygous wild type: 2 heterozygous vestigial: 1 homozygous vestigial and a phenotypic ratio of 3 wild type: 1 vestigial-winged.

## Dihybrid crosses—two non-linked pair of traits

Dihybrid crosses involve alleles for two separate traits that are found on different chromosome. For example, vestigial wings and rosy eyes are mutant recessive alleles found on Chromosome 2 and Chromosome 3, respectively. A typical dihybrid cross could be made by pairing males that were homozygous for vestigial wings and rosy eyes with females that were homozygous wild type for both traits. This would be the $P_1$ generation. The resulting $F_1$ generation would all be heterozygous each trait although wild type in phenotype, as shown by the Punnett square below.

**TABLE 29. Punnett square showing genotypes of the $P_1$ eggs and sperm as well as the genotype of the $F_1$ generation of a dihybrid cross.**

| | sperm |
|---|---|
| **eggs** | **vg ro** |
| + + | + + / vg ro |

Because chromosomes assort independently during meiosis to form gametes, equal numbers of "+ +", "vg +", "+ ro", and "vg ro" allele pairs are expected in the eggs and sperm produced by $F_1$ adults. The F2 generation resulting from pairing F1 males and females would have all possible combinations of the gamete genotypes, as in the following Punnett square:

**TABLE 30. Punnett square showing genotypes of the $F_1$ eggs and sperm as well as genotypes of the $F_2$ generation of a dihybrid cross.**

| | sperm | | | |
|---|---|---|---|---|
| **eggs** | **+ +** | **vg +** | **+ ro** | **vg ro** |
| + + | + + / + + | + + / vg + | + + / + ro | + + / vg ro |
| *vg* + | + + / vg + | vg + / vg + | + + / vg ro | vg + / vg ro |
| + *ro* | + + / + ro | + + / vg ro | + ro / + ro | + ro / vg ro |
| *vg ro* | + + / vg ro | vg + / vg ro | + ro / vg ro | vg ro / vg ro |

The $F_2$ generation is expected to have a 9 wild type: 3 vestigial-winged: 3 rosy-eyed: 1 vestigial-winged rosy-eyed phenotypic ratio.

## Sex-linked crosses

Traits found on the sex-determining chromosomes are sex-linked. Because fruit flies have an X-Y sex determination system similar to humans, a slightly different notation form is generally used to keep track of sex-linked traits in a Punnett square, etc. Here we not only need to keep track of the alleles, but also the sex chromosomes. Forked bristles (fr) is an example of a sex-linked trait that is found on the "X" chromosome. If we were to mate a homozygous forked-bristled female with a wild type male, the Punnett square for predicting the resulting $F_1$ generation would look like the following:

**TABLE 31. Punnett square showing genotypes of the $P_1$ eggs and sperm as well as the genotype of the $F_1$ generation of a sex-linked cross.**

| | sperm | |
|---|---|---|
| **eggs** | **$\frac{+}{}$** | **Y** |
| $\frac{fr}{}$ | $\frac{+}{fr}$ | $\frac{Y}{fr}$ |

As in humans, offspring that contain the Y chromosome ("Y" in the above notation) are males and those lacking the Y are females. Note the male and female $F_1$ phenotypes are opposite of those of the $P_1$ generation. Mating these $F_1$ males and females would yield the following:

**TABLE 32. Punnett square showing genotypes of the $F_1$ eggs and sperm as well as the genotype of the $F_2$ generation of a sex-linked cross.**

| | sperm | |
|---|---|---|
| **eggs** | **$\frac{fr}{}$** | **$\frac{Y}{}$** |
| **$\frac{+}{}$** | $\frac{+}{fr}$ | $\frac{+}{Y}$ |
| $\frac{fr}{}$ | $\frac{fr}{fr}$ | $\frac{fr}{Y}$ |

In this case, we expect half of the $F_2$ males to be wild type and the other half to have forked bristles. The females would also have a 1 forked bristle: 1 wild type phenotype ratio. These are the results of beginning with $P_1$ forked-bristled females and wild type males; how would the results change if the $P_1$ phenotypes were reversed?

## Linked autosomal traits

Traits that occur on the same chromosome are not assorted independently, like they would if they occurred on different chromosomes. The dominant, wrinkled wings (W) allele and rosy eyes (ro) are linked autosomal traits. If P1 homozygous wrinkled-winged and rosy-eyed males were paired with wild type females, the Punnett square of this cross would be:

**TABLE 33. Punnett square showing genotypes of the $P_1$ eggs and sperm as well as the genotype of the $F_1$ generation of a cross involving 2 linked autosomal alleles.**

| | sperm |
|---|---|
| **eggs** | $\frac{W\ ro}{}$ |
| $\frac{+\ +}{}$ | $\frac{+\ +}{W\ ro}$ |

The $F_1$ generation resulting from this cross would all be heterozygous for the two traits and have red (wild type) eyes and wrinkled wings. So far this is no different than if the traits occurred on separate chromosomes. What is the expected phenotype ratio in the $F_2$ generation?

If females of these $F_1$ individuals were **back-crossed** to their $P_1$ mutant genotype the resulting offspring would be expected to have the following genotypes:

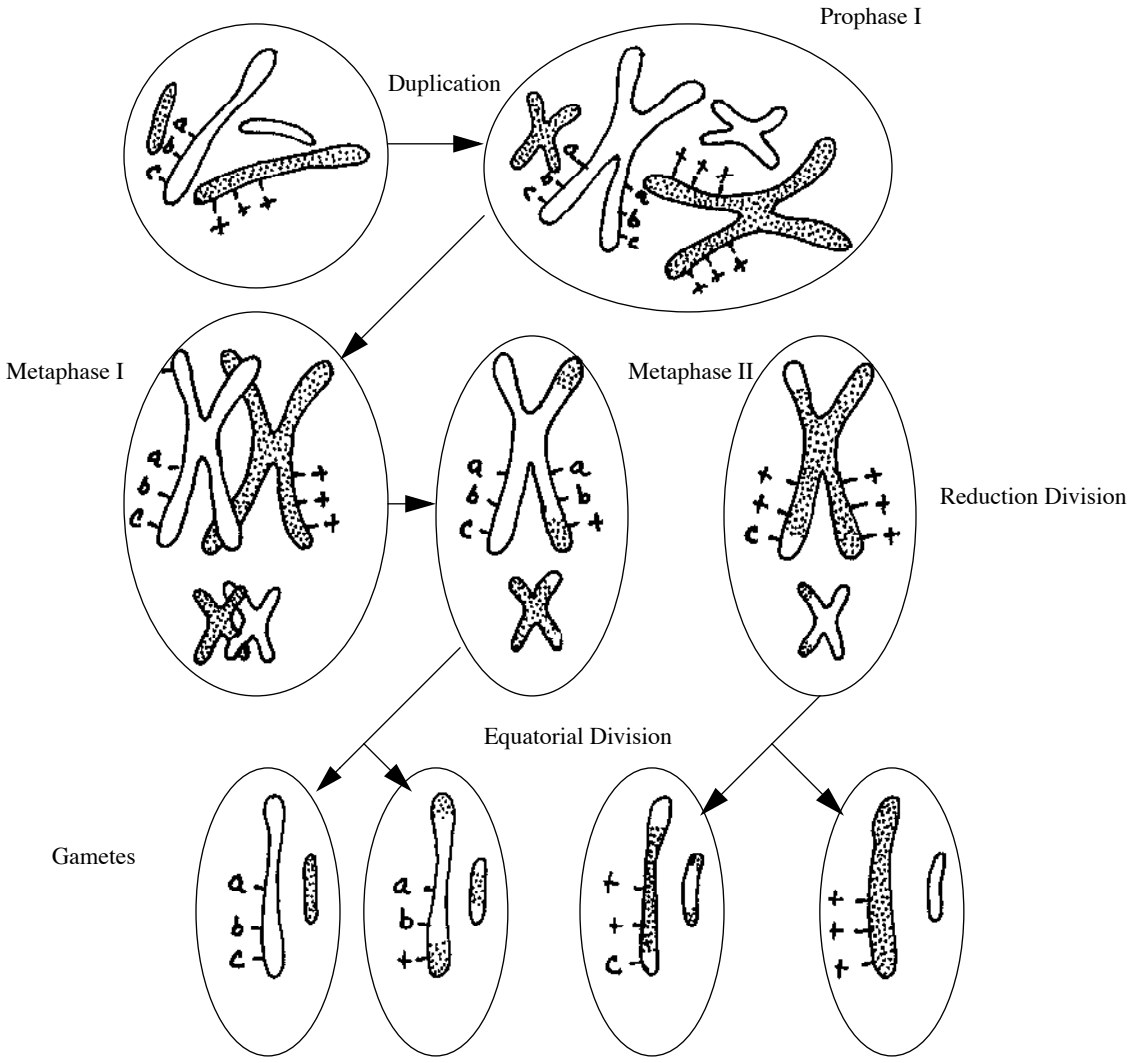
**TABLE 34. Punnett square showing the results of $F_1$ females back-crossed to the $P_1$ mutant strain males with 2 linked autosomal traits.**

| | sperm |
|---|---|
| **eggs** | $\frac{W\ ro}{}$ |
| $\frac{+\ +}{}$ | $\frac{+\ +}{W\ ro}$ |
| $\frac{W\ ro}{}$ | $\frac{W\ ro}{W\ ro}$ |

As seen in the above table, a 1 wrinkled-winged: 1 wrinkled-winged rosy-eyed phenotype ratio is expected from this cross. However, the observed results from this cross differ significantly from what is expected. If one were to make this cross with real flies, they would find that a small percentage of the resulting offspring would have either just rosy eyes or be completely wild type. This is the result of alleles being **recombined** due to **crossing over** of homologous chromosomes during metaphase I of meiosis. In fruit flies, crossing over occurs only in female gametogenesis.

The figure below shows where crossing over occurs and its effect on linked traits. There is also an excellent discussion of crossing over in Campbell (1996; pp. 266–269) along with how back crosses are used with linked traits for gene mapping. Please read over that discussion carefully.

**FIGURE 34. Summary of meiosis with cross-overs. Follow the movement of the recessive traits "a", "b", and "c" along with the dominant wild type alleles ("+") for each and note the influence of crossing over on the gamete genotype.**




## *Statistical analysis - Chi-Square*

Observed results from a cross rarely come out exactly how they were predicted, and the question is: Are the differences between the predicted and observed results significantly different? The **chi-square** ($X^2$) goodness-of-fit test is a simple tool used in comparing observed and predicted results to answer that question. Put simply, the chi-square tests whether or

not the differences observed are due to chance. If the chi-square analysis showed the differences might only be due to chance, then we would state that the observed and predicted results were not significantly different. However, if observed and predicted results differ greatly, the chi-square would show there is a statistically significant difference between them.

The first step in the chi-square analysis is constructing the appropriate hypotheses. Suppose we intend to flip a coin 100 times and count the number of heads and tails obtained. Assuming the coin was not off balance, we would predict to get 50 heads and 50 tails. Now let's say after flipping the coin 100 times we observed 60 heads and 40 tails. The **null hypothesis** in this case is: there is no difference between the predicted and observed results. The **alternative hypothesis** is: there is a difference between the predicted and observed results. The chi-square is used to see if the observed 60:40 head-to-tail count is different enough from the predicted 50:50 count to reject the null hypothesis. If we can reject the null hypothesis, then we must accept the alternative hypothesis; otherwise the null hypothesis is accepted.

Calculating the chi-square is relatively simple, it is:

$$\chi^2 = \sum \frac{(O-P)^2}{P}$$

where Σ is the sum, O is the observed value, and P is the predicted value.

Let's substitute values from our coin experiment in to this formula:

$$\chi^2 = \frac{(60-50)^2}{50} + \frac{(40-50)^2}{50} = 4$$

Once the chi-square value has been calculated, then we must interpret our results by looking up this value on a table showing chi-square values and their associated probabilities, such as the following:

**TABLE 35. Upper percentage points of the chi-square distribution. From Gill (1978).**

| d.f. | p | | | | | |
|---|---|---|---|---|---|---|
| | **0.3** | **0.2** | **0.1** | **0.05** | **0.01** | **0.001** |
| *1* | 1.07 | 1.64 | 2.71 | 3.84 | 6.64 | 10.8 |
| *2* | 2.41 | 3.22 | 4.60 | 5.99 | 9.21 | 13.8 |
| *3* | 3.66 | 4.64 | 6.25 | 7.82 | 11.3 | 16.3 |
| *4* | 4.88 | 5.99 | 7.78 | 9.49 | 13.3 | 18.5 |
| *5* | 6.06 | 7.29 | 9.24 | 11.1 | 15.1 | 20.5 |

The percentage points of this table are probability levels (**p**) that the difference between the predicted and observed results are due to chance. Researchers generally use $p < 0.05$ as the arbitrary cut off between chance and non-chance—that is, if p exceeds 0.05, then the difference is said to be due to reasons other than chance. Scientists set such a high significance level in order not to be confused by small differences or small sample sizes, and that any differences they do find are real. The **degrees of freedom** (d.f.)—or freedom to vary—is the number of categories minus one. In our coin tossing example we had only two categories, therefore we have only one degree of freedom.

The **decision rule** for accepting or rejecting the null hypothesis is: If $X^2_{(1\ d.f.)} > 3.84$, then we reject the null hypothesis and accept the alternative hypothesis, which states that the observed results differ significantly from the predicted ones; otherwise we accept the null hypothesis. If you follow across the d.f. = 1 row, you will see that the $X^2$ we calculated (4)

lies between the 0.05 and 0.01 probability levels, therefore it exceeds the 0.05 probability and we can conclude that either the coin was unbalanced or something was unbiased about our flipping method.

The chi-square goodness-of-fit test is an excellent tool for exploring genetics. For example, let's say we were interested in the inheritance pattern of two traits—eye color and hair color— in a hypothetical mammal. First we might want to test the dominance patterns for common alleles of these traits. After mating brown-eyed males with green-eyed females, let's say we obtained 48 offspring of which 38 were brown-eyed and 10 were green-eyed. Based on simple dominance, one would have predicted 3/4 of 48 (36) to be brown-eyed and 1/4 of 48 (12) to have green eyes. Are our observed results significantly different from those predicted? The chi-square calculated for this test is:

$$\chi^2 = \frac{(40-36)^2}{36} + \frac{(8-12)^2}{12} = 1.78$$

We have two categories, therefore only one degree of freedom. Looking this up on the chi-square table we find 1.78 is much less than the value associated with 0.05 probability level, thus we can't reject the null hypothesis of no significant difference between the observed and predicted outcomes. The data we obtained suggest a 3:1 ratio and simple dominant/recessive allele expression. For the sake of saving space, let's say we obtained similar results when testing hair color and found the black allele to be dominant over blond.

However, at this point, we are not sure whether or not the two traits are linked (i.e., they occur on the same chromosome). After mating several homozygous brown-eyed/black-haired males with a green-eyed/blond-haired females, and subsequently controlled matings of the $F_1$ generation; we would predict the $F_2$ generation to have a 9:3:3:1 expression of these traits if they were not linked, otherwise a 3:1 ratio if they were linked. Using the chi-square test, we could determine which ratio our observed data fit best.

## *References:*

Campbell, N. A. 1996. Biology, 4th ed. Benjamin/Cummings Pub. Co. Menlo Park, CA. 1206 pp.

Carolina Drosophila Manual. 1988. Carolina Biological Supply Co., Burlington, NC. 31 pp.

Gill, J. L., 1978. Design and analysis of experiments in the animal and medical sciences. Vol. 3. Iowa Univ. Press. Ames, IA. 173 pp.

## *Exercises:*

The following exercises are designed for students working in groups of 3 or 4. You will turn in assignments as a group, but each group member must participate in all aspects of each assignment.

## Real Flies

1. Each group should select one mating from each of the following categories:

**TABLE 36. Mating Categories. Groups must select one from each category.**

| Category | Mating | Generation(s) to Sort and Count |
|---|---|---|
| *A* | Apterous x Wild type | $F_1$ and $F_2$ |
| | Dumpy-winged x Wild type | $F_1$ and $F_2$ |
| | Ebony-bodied x Wild type | $F_1$ and $F_2$ |
| | Spineless-aristopedia x Wild type | $F_1$ and $F_2$ |
| *B* | White-eyed x Wild type | $F_1$ and $F_2$ |
| | Bar-eyed x Wild type | $F_1$ and $F_2$ |
| | Singed-bristled x Wild type | $F_1$ and $F_2$ |
| *C* | Sepia-eyed Apterous x Wild type | $F_1$ and $F_2$ |
| | Vestigial-winged Ebony -bodied x Wild type | $F_1$ and $F_2$ |
| | White-eyed Apterous x Wild type | $F_1$ and $F_2$ |
| *D* | Black-bodied Vestigial-winged x Wild type | $F_1$, $F_2$, and Backcross |
| | Sepia-eyed Ebony-bodied x Wild type | $F_1$, $F_2$, and Backcross |

Then obtain the five vials (1 Wild type + 1 of each phenotype to be mated) from the TAs needed to make these crosses. **Remember: You must use virgin females for these crosses.** Use females only from those vials that have been cleared within the last 10 hours. You may have to clear the flies first if the TAs have not already done this for you.

2. During the Open Lab periods of September 2–7, prepare vials and make the selected $P_1$ crosses from categories A, B, C, and D. Using a permanent marker and adhesive label, mark each vial as to the mating, date, and group as in the following example:

**FIGURE 35. Sample vial label.**

$P_1$
8 dp ♂ x 8 + ♀
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3. Check the vial after six days; you should see larvae burrowing through the medium. If the vial seems to have health growth, then clear all the parent flies from vial by tapping them into an empty foam-capped vial, freezing them, then disposing them into the Fly Morgue.

4. Check the vials 14–16 days after the initial set up date; now you should begin to see $F_1$ adults. If you have a dozen or more adults, then anesthetize them and dump them onto a sorting card. Sort the adults according to their sex and phenotype, then count the number in each pile. Record your results on the data sheet provided. **For matings involving Categories A, B, C, and D:** If you have only one phenotype in the $F_1$ generation, then remove 6–8 males and 6–8 females (these do **not** need to be virgins) and place them into a freshly prepared vial containing medium for rearing the $F_2$ generation. Label the new vials with the $P_1$ and $F_1$ phenotypes, date, and group.

**FIGURE 36. Sample vial label for mating $F_1$ flies.**

**$P_1$: se ap ♂ x + ♀**
**$F_1$: 8 $^+$/ ♂ x 8 $^+$/ ♀**

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Check the vials after 6 days and remove the $F_1$ adults. Check the vials again 14–16 days after you started the $F_1$ crosses. You should have several dozen adult $F_2$ generation flies. If so, anesthetize, sort, and count them over the next 10-day period. Record your results on the data sheets provided.

5. **For Category D matings:** In addition to mating $F_1$ individuals, also remove 6 or 8 virgin $F_1$ females and back cross them to homozygous recessive $P_1$ males. Check these vials after 6 days and remove the $F_1$ females and $P_1$ males. Check the vials again 14–16 days after you started the cross. You should have several dozen adult flies. If so, anesthetize, sort, and count them over the next 10-day period. Record your results on the data sheet provided.

**FIGURE 37. Sample vial label for back-crossing female $F_1$ to male $P_1$ flies.**

**$P_1$: se ap ♂ x + ♀**
**8 se ap ♂ x 8 $F_1$ $^+$/ ♀**

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## Virtual Flies

6. Visit the Virtual Fly Lab on the World Wide Web at: http://vflylab.calstatela.edu/edesktop/VirtApps/VflyLab/, read through the instructions, then click on the design flies button. [Note: the Virtual Fly Lab will be demonstrated in lab.]
7. Do at least 3–4 different fruit fly pairings (one for each member of your group) and follow them through to the $F_2$ generation. Show the results of $F_1$ and $F_2$ generations in a table, then do a chi-square analysis on the $F_2$ generation to select the ratio that best explains your findings.

## The Report

8. Each group must submit a report that includes the following information on each real and virtual fruit fly cross made: the observed results, the predicted (=expected) results, a chi-square analysis on all $F_2$ generation or back-cross results, and a paragraph or two explaining the results.

## *Genetics Data & Analysis—Category A Cross*

Group_____

$P_1$ Phenotypes: Male_____ Female_____

Date $P_1$ Mated: _____ Date $P_1$ Removed:_____

**TABLE 37. Use this table to record counts of $F_1$ offspring phenotypes from Category A mating.**

| Date Counted | $F_1$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

$F_1$ Phenotypes: Males_____ Females_____

Date $F_1$ Mated:_____ Date $F_1$ Removed:_____

**TABLE 38. Use this table to record counts of $F_2$ offspring phenotypes from Category A mating.**

| Date Counted | $F_2$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

Null Hypothesis: Alternative Hypothesis:

Decision Rule: Show calculations for $X^2$:

Degrees of Freedom_____ Test statistic at p = 0.05_____

State the Decision:

## *Genetics Data & Analysis—Category B Cross*

Group_____

$P_1$ Phenotypes: Male_____ Female_____

Date $P_1$ Mated: _____ Date $P_1$ Removed:_____

**TABLE 39. Use this table to record counts of $F_1$ offspring phenotypes from Category B mating.**

| Date Counted | $F_1$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

$F_1$ Phenotypes: Males_____ Females_____

Date $F_1$ Mated:_____ Date $F_1$ Removed:_____

**TABLE 40. Use this table to record counts of $F_2$ offspring phenotypes from Category B mating.**

| Date Counted | $F_2$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

Null Hypothesis:

Alternative Hypothesis:

Decision Rule:

Show calculations for $X^2$:

Degrees of Freedom_____

Test statistic at p = 0.05_____

State the Decision:

## *Genetics Data & Analysis—Category C Cross*

Group_____

$P_1$ Phenotypes: Male_____ Female_____

Date $P_1$ Mated: _____ Date $P_1$ Removed:_____

**TABLE 41. Use this table to record counts of $F_1$ offspring phenotypes from Category C mating.**

| Date Counted | $F_1$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

$F_1$ Phenotypes: Males_____ Females_____

Date $F_1$ Mated:_____ Date $F_1$ Removed:_____

**TABLE 42. Use this table to record counts of $F_2$ offspring phenotypes from Category C mating.**

| Date Counted | $F_2$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

Null Hypothesis: Alternative Hypothesis:

Decision Rule: Show calculations for $X^2$:

Degrees of Freedom_____ Test statistic at p = 0.05_____

State the Decision:

## *Genetics Data & Analysis—Category D Cross*

Group_____

$P_1$ Phenotypes: Male_____ Female_____

Date $P_1$ Mated: _____ Date $P_1$ Removed:_____

**TABLE 43. Use this table to record counts of $F_1$ offspring phenotypes from Category D mating.**

| Date Counted | $F_1$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

$F_1$ Phenotypes: Males_____ Females_____

Date $F_1$ Mated:_____ Date $F_1$ removed:_____

**TABLE 44. Use this table to record counts of $F_2$ offspring phenotypes from Category D mating.**

| Date Counted | $F_2$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

Null Hypothesis: Alternative Hypothesis:

Decision Rule: Show calculations for $X^2$:

Degrees of Freedom_____ Test statistic at p = 0.05_____

State the Decision:

## *Genetics Data & Analysis—Category D Back-Cross*

Group_____

$P_1$ Phenotypes: Male_____ Female_____

Date $P_1$ Mated: _____ Date $P_1$ Removed:_____

**TABLE 45. Use this table to record counts of $F_1$ offspring phenotypes from Category D mating.**

| Date Counted | $F_1$ Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

$F_1$ Female Phenotype_____ $P_1$ Male Phenotype_____

Date Back-Cross Mated:_____ Date Back-Cross Adults Removed:_____

**TABLE 46. Use this table to record counts of back-cross offspring phenotypes from Category D mating.**

| Date Counted | Back-Cross Phenotypes and Counts | | | | Totals & Grand Total |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| **Total Observed** | | | | | |
| **Expected Number** | | | | | |

Null Hypothesis: Alternative Hypothesis:

Decision Rule: Show calculations for $X^2$:

Degrees of Freedom_____ Test statistic at p = 0.05_____

State the Decision:

Show calculations for estimating relative distance between loci: