1. You breed a homozygous pea plant that produces round yellow peas (RRYY) to a homozygous pea plant that produces wrinkled green peas (rryy). (RRYY) x (rryy)
Assume that the round allele is dominant to the wrinkled allele, and that the yellow allele is dominant to the green allele.
   a. What will the genotype of all of the offspring be?

   b. What will the phenotype of all of the offspring be?

2. Homozygous dominant chickens are black (Č Č). Homozygous recessive chickens are white (Č Ĉ).
   a. If feather color in chickens is an incompletely dominant trait, what would heterozygous (Č Ĉ) chickens look like?

   b. If feather color in chickens is a co-dominant trait, what would heterozygous (Č Ĉ) chickens look like?

3. The following symbols are commonly used in human pedigrees. Next to each symbol, write what it stands for. Read the lab or look in your text for the answers.

   [Pedigree symbols shown]

   If you have not yet covered pedigrees in class or you want some additional practice before lab, Google “pedigree practice” or how to analyze a pedigree.
Lab 6: Heredity II (Mendelian & Non-Mendelian Genetics)

LAB SYNOPSIS:
- We will model the process of inheritance using a computer simulation
  - Mendel’s 2nd law of genetics will be explored.
    - A dihybrid cross will be done using a cat model
  - Non-Mendelian genetics will be explored.
    - Co-dominance will be compared to Incomplete dominance.
    - X-linked traits will be explored (in calico cats)
  - Human pedigrees will be used to trace genes through generations

OBJECTIVES: After successfully completing this lab, a student will be able to:
- Correctly use the terms genotype and phenotype to describe an organism.
- Predict genotype and phenotype ratios from given crosses.
- Explain the results of crosses using the concepts of segregation and independent assortment.
- Be able to deduce genotypes and inheritance patterns from simple pedigrees.

Overview of Mendel’s 2nd Law of Genetics:

Genetics- Study of how genes are passed from parent to offspring.
Mendelian Genetics- Genetics that follow Mendel’s 2 laws of genetics.
- The Law Of Segregation (last week)
- The Law Of Independent Assortment (see below)

During the last lab, we examined Mendel’s first law, The Law Of Segregation.
The Law Of Segregation- Alleles segregate from one another during the formation of gametes.
This law explains how two alleles for a single trait segregate from one another in the formation of gametes.
For example, how is the single trait that determines flower color passed from parent to offspring?
purple flowers (P) are dominant to white (p) (Fig. 1).

Today’s lab examines Mendel’s second law and how two or more different traits, on separate homologous chromosomes independently assort in the formation of gametes.

The Law Of Independent Assortment- each pair of alleles segregates independently during gamete formation; applies when genes for two traits are located on different pairs of homologous chromosomes.
For example, how are two traits (seed color) and (seed shape) which are found on different homologous chromosomes passed from parent to offspring?
yellow seeds (Y) are dominant to green (y)
round seeds (R) are dominant to wrinkled (r)
**Dihybrid cross** - a cross between individuals who are heterozygous for 2 traits.

Mendel first crossed **RRYY** with **rryy** making dihybrids, i.e. dihybrids are heterozygous for both seed color and seed shape (RrYy). He then performed a dihybrid cross and showed that the inheritance of these two traits were independent of one another. In other words, the color that a pea is has no effect on what shape it is, the genes for color and shape are inherited independently of one another (Fig. 2).

We have already seen the genetic basis for this. Recall: during metaphase I of meiosis, the homologous pairs line up and assort chromosomes originally inherited from mom vs. from dad randomly.

**A Review of CatLab®**

With CatLab, you can mate domestic cats selected on the basis of coat color and pattern, and the presence or absence of a normal tail (Table 1). Genetically valid litters of kittens are produced, and subsequent matings can be performed.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Allele symbol and Trait</th>
<th>Allele symbol and Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whiteness</td>
<td>(W) All-white</td>
<td>(w) Not all-white (ex. gray or orange)</td>
</tr>
<tr>
<td>White areas</td>
<td>(S) Extensive white areas</td>
<td>(s) No white areas</td>
</tr>
<tr>
<td>Color density</td>
<td>(D) Dense color (black, orange)</td>
<td>(d) Dilute color (gray, cream)</td>
</tr>
<tr>
<td>Stripping</td>
<td>(A) Agouti (striped bodies)</td>
<td>(a) Non-agouti (unstriped bodies)</td>
</tr>
<tr>
<td>Stripe type</td>
<td>(T) Mackerel tabby (stripes)</td>
<td>(t) Blotched tabby (curved &amp; whorled stripes)</td>
</tr>
<tr>
<td>Color</td>
<td>X^O Orange</td>
<td>X^B Non-orange (usually black)</td>
</tr>
<tr>
<td>Tail type</td>
<td>(M) Tail absent (Manx)</td>
<td>(m) Normal tail present</td>
</tr>
</tbody>
</table>

In case you have forgotten how to use CatLab, read through the following section to re-familiarize yourself with the program. If you remember how to use CatLab, you can skip ahead to Exercise 1.

In this exercise, you will learn how to use CatLab. Using the lab netbook, open CatLab by clicking on the Start menu and then on “All Programs”. Click OK on the title screen to display the simulation screen and the program’s controls.

A left panel lists the cats you add and their offspring. The right panel contains help screens. Either panel can be moved to enlarge the other. (Fig. 3)

The menu bar along the top consists of:
**File  Cat  Options  About**

*Figure 2. Dihybrid Cross (RrYy X RrYy) showing a 9:3:3:1 phenotypic ratio*

*Figure 3. CatLab*
Use the options under each menu to select and mate the cats for your investigations. The icons under the menu bar are shortcuts to use once you are familiar with the operation of the program.

Cat Menu (and Toolbar Shortcuts):

- **Add a cat [+]** with the characteristics of your choice.
- **Set as Parent [P]** allows you to select a male and a female for mating.
- **Set Litter Size [L]** controls the litter size (4 to 7) or allows a random size between 4 and 7.
- **Mate Cats [M]** produces a litter from the selected parents.
- **Display Phenotype** shows a simple graphic of the selected cat’s characteristics and an image of its phenotype.

Options Menu (and Toolbar Shortcuts):

- **Chi-Square \(\chi^2\)** statistical test option.
- **Toolbar** displays a row of icons used as shortcuts when using the program.
- **Status Bar** describes the option selected in the Menu Bar or by clicking an icon button.
- **Sound** turns sound off or on. Leave it on unless directed otherwise by your instructor. 😊

Other Toolbar Shortcuts:

- **[N]**: Start new investigation with option to carry forward selected cats.
- **[Printer icon]**: Print the cat list

Note: The characteristics in each column can be sorted by clicking the column title: Parents, Sex, etc. This will make it easier for you to count phenotypes of interest, but remember to only count offspring (and not parents) for each cross!

**Exercise 1: Exploring Crosses of Two Traits: Yipes, Stripes!**

Recall: In cats, one gene controls the density (intensity) of the fur color. The different alleles of this gene produce phenotypes in which the color is dense (as seen, for example, in black cats) or dilute (as seen in gray cats). The alleles for this color density gene may be denoted as:

- **D**: dense, in this case **Orange**
- **d**: dilute, in this case **Cream**

A second gene in cats controls the pattern or arrangement of stripes on tabby cats. Mackerel tabbies have parallel stripes on the cat’s flank, while blotched tabbies have swirling stripes. The alleles for this striping pattern gene may be denoted as:

- **T**: Mackerel
- **t**: Blotched

The purpose of this exercise is to study the inheritance of two separate traits **color density & striping**.

**Procedure:**

1. Select two cats as follows:
   - #1 Black mackerel female (Tailed → Not all-white → No white areas → **Mackerel stripes** → **Orange**)
   - #2 Gray blotched male (Tailed → Not all-white → No white areas → **Blotched stripes** → **Cream**)


2. Refer to table 1; What are the possible genotypes for the female cat (#1)? Recall she is showing the dominate traits, so she might be homozygous dominate or she might be heterozygous for each.

For striping ___ ___ or ___ ___. For fur color density ___ ___ or ___ ___

3. Refer to table 1; What is the possible genotype for the male cat (#2)? Recall he is showing the recessive traits so he has to be homozygous recessive for both.

For striping ___ ___ For fur color density ___ ___

4. Set the litter size to a constant value of 4. Mate cat #1 and cat #2 and obtain 5 litters (20 kittens). Record their phenotypes (color + striping pattern) in Table 2. Use abbreviations (OM = Orange mackerel, CB = Cream blotched) if it will help keep your data easier to read.

Table 2. Phenotypes of kittens from a cross between an orange w/ black mackerel striped female with a cream w/grey blotched striped male.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Color/Pattern</th>
<th>Cat #</th>
<th>Color/Pattern</th>
<th>Cat #</th>
<th>Color/Pattern</th>
<th>Cat #</th>
<th>Color/Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Mom</td>
<td>7</td>
<td>13</td>
<td>18</td>
<td>2 Dad</td>
<td>8</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>15</td>
<td>20</td>
<td>4</td>
<td>10</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>17</td>
<td>22</td>
<td>6</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Now, select two of the orange with black mackerel striped cats (one male, one female) from among the group of 20 kittens. Make these parents [P] but DO NOT mate them yet! Record the identifying numbers and genotypes (hint: due to male being homozygous recessive, there is only one possible genotype!) of these new parents-to-be:

New mother will be cat # _____ and her genotype for color density is ___ ___ and for striping is ___ ___

New father will be cat # _____ and his genotype for color density is ___ ___ and for striping is ___ ___

6. Using your answers above answer the following, which relate to the transmission of genetic information from parents to offspring.

a. For each of the mother’s haploid eggs and the father’s haploid sperm, which alleles will be present? Recall each gamete will have one allele for color density and one allele for striping.

The mother will produce haploid eggs with either ___ ___ or ___ ___ or ___ ___ or ___ ___

The father will produce haploid sperm with either ___ ___ or ___ ___ or ___ ___ or ___ ___

6
7. Draw a Punnett square to predict the outcome of a cross between these two orange mackerel parents-to-be. What are the expected proportions of phenotypes in the offspring?

This is a dihybrid cross

![Punnett square]

Mendel determined a dihybrid cross would result in a phenotypic ratio of 9:3:3:1
Does your Punnett square support this?

8. Now mate your two orange mackerel parents-to-be and obtain 8 litters (32 kittens cats #23-54). Record the number of kittens of each color and pattern in Table 3.

<table>
<thead>
<tr>
<th>Color</th>
<th>Observed Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange mackerel</td>
<td></td>
</tr>
<tr>
<td>Orange blotched</td>
<td></td>
</tr>
<tr>
<td>Cream mackerel</td>
<td></td>
</tr>
<tr>
<td>Cream blotched</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Phenotypes of kittens obtained.**

**Dihybrid cross**- A cross between two individuals heterozygous for two genes. Example, AaQq x AaQq
The cross you just did had to have been a dihybrid cross.

A dihybrid cross of two heterozygous parents (DdTt x DdTt), produces four phenotypic classes of offspring; the chance of an orange mackerel kitten occurring is 9/16, the chance of an orange blotched kitten occurring is 3/16, the chance of a cream mackerel kitten occurring is 3/16, and the chance of a cream blotched kitten occurring is 1/16. This is the same as saying that in a reasonable sample of offspring, the ratio of orange mackerel to orange blotched to cream mackerel to cream blotched will be a good fit of a 9:3:3:1 ratio. In the case of 32 kittens, you would expect 18 of them to be orange mackerel, 6 of them to be orange blotched, 6 of them to be cream mackerel, and 2 of them to be cream blotched.

9. Does your data appear to fit the expected 9:3:3:1 ratio? What would be the cause of deviation from the expected values?
Recall: You can use a statistical test called a Chi-square test to test the goodness of fit of your observed data to the expected 9:3:3:1 ratio. This test compares an observed sample to the proportions expected in a test population to see if any deviations are due to random chance or to some other factor. It should be noted that the Chi-square test is not reliable when the numbers in any category (class) are less than 5.

10. Perform a Chi-square test on your data. Click on the $\chi^2$ in the toolbar. Enter 4 as the number of classes (phenotypes) you want to test. Click OK.

Enter your observed numbers of each kitten color/pattern combination in the appropriate boxes (Class 1 – Class 4). Then enter your expected ratios. Click Calculate and record results below.

\[ \text{Chi-square} = \] 

\[ \text{Critical Chi-square value} = \]

If the calculated Chi-square is less than the “critical Chi-square value”, then your data fits the expected ratio and any deviations from 9:3:3:1 are due to random chance.

What did the results of the Chi-square test tell you about your data? i.e. did results fall within the expected ratio?

11. Click on [N] in the toolbar to clear all of the data.

### Exercise 2: Exploring Non-Mendelian Inheritance: Not-So-Simple Dominance

Mendel tracked genes that show simple dominance/recessive. The dominant allele completely masks the expression of the recessive allele. In other words, pea flowers are either purple (dominant) or white (recessive). However, some genes have alleles that vary in their degrees of dominance.

**Incomplete dominance** - Heterozygotes have a phenotype intermediate to those of the homozygotes (a blending of alleles).

For example, in snapdragons, if homozygous red flowered plants are crossed with homozygous white flowered plants the resulting plants are pink flowered (Fig. 4).

**Co-Dominant** - Heterozygotes phenotype expresses both alleles separately.

An example of codominant is the AB form of human blood type. If an individual who is homozygous for type A blood (AA) mates with an individual who is homozygous for type B blood (BB) the offspring will be heterozygous with type AB blood. Both alleles are being expressed (Fig. 5).

Cats can have different degrees of white spotting on their coats-some have no white, some have some white (e.g. feet and face), and some have extensive white (a white cat with a black spot is really a black cat with a very very large white spot!). In this exercise, you will examine the inheritance pattern of this trait.
Procedure:

1. Create two cats as follows:
   - #1 female (Tailed → Not all-white → **No white areas** → Mackerel stripes → and then any color)
   - #2 male (Tailed → Not all-white → **Extensive white areas** → Mackerel stripes → and then any color)

2. Refer to table 1 and assign symbols for the two different alleles (no white areas vs. extensive white)

3. Using your symbols from #2, answer the following:
   - **The No white areas** genotype of the female cat is: _________
   - **The Extensive white areas** genotype of the male cat is: _________ or _________

4. What phenotypes of kittens, and in what proportions, would you expect to obtain from these parents? Draw Punnett squares to support your predictions.

5. Now test your predictions. Set the litter size to a constant value of 4. Mate cat #1 and cat #2 and obtain 5 litters (20 kittens). Record their phenotypes in Table 2.

   **Table 2. Phenotypes of kittens from a cross between cats with varying degrees of white areas.**

<table>
<thead>
<tr>
<th>Degree of White Areas</th>
<th>Observed Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Some</td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td></td>
</tr>
</tbody>
</table>

6. The kittens with some white areas are what genotype? _________.

7. Do you think this is an example of incomplete dominance or co-dominance? Explain your reasoning.
8. Predict the outcome of a cross between two cats with some white areas. Draw a Punnett square to support your prediction.

9. Carry out the cross, picking any two cats with some white spotting. Record your results and conclusions in the space below.

10. Click on [N] in the toolbar to clear all of the data.

**Exercise 3: Exploring Non-Mendelian Inheritance: X-Linked Inheritance**

Mendel was lucky in that the traits that he studied in pea plants followed simple dominant/recessive. Had they not, he probably would not have been able to develop his two laws of genetics. Not all genes are inherited in such a fashion (recall codominant and incomplete dominance). Pea plants also do not show differences in sex. Pea plants are hermaphroditic; their flowers have both male and female structures. Many other organisms are dioecious, they have separate male vs. female individuals.

**Sex chromosomes**- Chromosomes responsible for determining the sex of an individual. Humans have 1 pair of sex chromosomes and 22 pairs of autosomes.

**Autosomes**- Chromosomes that are not involved in sex determination. Humans have 22 pairs of autosomes. Cats have 18 pairs of autosomes and 1 pair of sex chromosomes (38 total chromosomes).

Chromosomal sex determination occurs in some plants, protists and animals. The **XX/XY system** determines sex in mammals. XY develop into males and XX develop into females. To prevent females from producing twice as many proteins from the X chromosomes, **X-inactivation** occurs.

**X-inactivation**- is a process by which one of the two copies of the X chromosome present in female mammals is inactivated.

X-inactivation happens during early embryo development. This results in patches of a female’s bodies that expresses genes from her mother’s X chromosome separate from those inherited from her father’s X chromosome.

In cats, this X-inactivation is seen in the calico phenotype. Fur color, orange and black (cream and gray in the dilute form) is carried on the X chromosome. There are 3 possible genotypes and phenotypes for female cats:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>X&lt;sup&gt;O&lt;/sup&gt;X&lt;sup&gt;O&lt;/sup&gt;</td>
<td>orange female, or cream female in the diluted form</td>
</tr>
<tr>
<td>X&lt;sup&gt;B&lt;/sup&gt;X&lt;sup&gt;B&lt;/sup&gt;</td>
<td>black female, or gray female in the diluted form</td>
</tr>
<tr>
<td>X&lt;sup&gt;O&lt;/sup&gt;X&lt;sup&gt;B&lt;/sup&gt;</td>
<td>Calico cats: orange and black patches, or cream and gray patched female in the diluted form</td>
</tr>
</tbody>
</table>
Hemizygous- Having one or more genes that have no allele counterparts. Applies to genes from a male's sex chromosomes.

Male cats only have one X chromosome. Their Y chromosome does not have a corresponding allele for fur color. Thus only 2 genotypes and 2 phenotypes are seen, there are no heterozygous forms.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X^OY$</td>
<td>orange male, or cream male in the diluted form</td>
</tr>
<tr>
<td>$X^B Y$</td>
<td>black male, or gray male in the diluted form</td>
</tr>
</tbody>
</table>

We will study the inheritance of the fur color on the X chromosome below.

**Procedure:**

Select two cats as follows:
#1 Cream Blotched Female (Tailed $\rightarrow$ Not all-white $\rightarrow$ No white areas $\rightarrow$ Blotched $\rightarrow$ Cream)
#2 Gray Blotched Male (Tailed $\rightarrow$ Not all-white $\rightarrow$ No white areas $\rightarrow$ Blotched $\rightarrow$ Gray)

Review the above descriptions and table 1:
1. What is the X-linked fur color genotype for the “cream” female cat (#1)? __________
2. What is the X-linked fur for the “gray” male cat #2? __________.
3. For each of the mother’s haploid eggs and the father’s haploid sperm, which alleles will be present?
   - The mother will produce haploid eggs with either _____ or _____.
   - The father will produce haploid sperm with either _____ or _____.
4. Set the litter size to a constant value of 4. Mate cat #1 and cat #2 and obtain 5 litters (20 kittens).
5. What phenotype(s) did you observe for the female kittens?
6. What phenotype(s) did you observe for the male kittens?
7. Click on[N] in the toolbar to clear all of the data.
8. Now select two cats as follows:
   #1 gray blotched female (not all-white, tailed, and anything else)
   #2 cream blotched male (not all-white, tailed, and anything else)
9. What is the genotype of cat #1? __________.
10. What is the genotype of cat #2? __________.
11. Set the litter size to a constant value of 4. Mate cat #1 and cat #2 and obtain 5 litters (20 kittens).
12. What phenotype(s) did you observe for the female kittens?
13. What phenotype(s) did you observe for the male kittens?

14. How are your results from this second cross different from those of the first cross? Why? Explain these results in terms of alleles being carried on the X chromosome.

15. What is the genotype of all of the female kittens produced in both crosses? ________

**Exercise 4: Human Pedigrees**

**Pedigree analysis** - A type of genetic analysis in which a trait is traced through several generations of a family to determine how the trait is inherited. The information is displayed in a pedigree chart using standard symbols (figure 1)

![Pedigree symbols](image)

**Figure 1.** Common styles and lines in a pedigree.

Pedigrees are often used to determine the mode of inheritance (dominant, recessive, sex-linked, etc.) of genetic traits. Pedigree fig. 2A shows the inheritance of an autosomal dominant trait for widow’s peak. Pedigree fig. 2B shows the inheritance of an autosomal recessive trait for earlobe attachment.

![Pedigree diagrams](image)

**Figure 2.** A. Pedigree of autosomal dominant trait. B. Pedigree of autosomal recessive trait.
Practice:
Pedigrees are like puzzles. Like puzzles you need to practice practice practice. Use a pencil with a good eraser as you attempt to solve the pedigrees below.

-------------------------

Galactosaemia:
People with galactosaemia are unable to digest milk sugar (galactose). Recall the solid black are those individuals who have galactoseamia. Galactosaemia is inherited as an autosomal recessive trait (gg).

1. Label the pedigrees with individuals that might be either homozygous recessive, dominant or heterozygous.

2. From the evidence of the pedigrees above, circle which pedigree shows beyond doubt that galactosaemia is inherited as an \textit{autosomal recessive} condition. Explain your reasoning.

-------------------------

\textbf{X-Linked Recessive Chromosomal Disorders Vs. Simple Autosomal Recessive Disorders}

The X chromosome carries genes for things other than sex determination, including color-blindness, forms of male pattern baldness, and hemophilia.

Red-green color-blindness is a \textit{sex linked recessive trait} caused by an allele carried on the X chromosome. Homozygous recessive females and hemizygous males cannot distinguish between red and green hues. In the US, this condition affects between 5 and 10\% of males, compared to about 0.5\% of females.

Cystic fibrosis is an autosomal recessive disorder blocking normal function of a membrane transport protein. This causes tissues of the lung, intestine and pancreas to become clogged with thick mucus.

Procedure:

1. Examine the two pedigrees below (Figure 3A &B). One shows a \textit{sex linked recessive} disorder the other is an \textit{autosomal recessive} pedigree.

2. Analyze each pedigree. Write down the genotypes that should give the indicated phenotypes you are certain of. Recall individuals who are showing the disorder are homozygous recessive or hemizygous males if sex-linked (these are the knows). Those without the conditions can be homozygous dominant or heterozygous (these are the unknowns you need to solve for).
3. Which of the above pedigrees demonstrates the inheritance of the X-linked recessive disorder red-green color-blindness? And which is for the autosomal recessive disorder cystic fibrosis?

4. How can you tell? What evidence is there to support your hypothesis? In other words, explain what conclusions you can infer based on the information presented in the pedigrees.