1. Briefly explain/define the following terms. Try to explain what do these terms really mean?

- Genotype:

- Phenotype:

- Heterozygous:

- Homozygous:

2. In a cross between two parents (the P generation) where one is a homozygous short pea plant (tt) and the other is a homozygous tall pea plant (TT) you find that all of the offspring are tall (all the F₁ are tall).

   a. What allele is dominant? How do you know? Use a Punnett square to answer.

   b. If you mated two of these offspring pea plants together to make an F₂ generation, what types of offspring would you see, and in what proportion? Use a Punnett square to answer.
Lab 5: Heredity I (Simple Mendelian Genetics)

LAB SYNOPSIS:
- We will model the process of inheritance using a computer simulation
  - Mendel’s 1st law of genetics will be explored.
  - The use of Punnett squares to predict mating outcomes will be demonstrated.
  - Test-crosses will be done to determine unknown genotypes.
  - Hypotheses will be tested on cat color (orange vs. cream)

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.

Overview:

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 (see below)
- The Law Of Independent Assortment

Review:
- You are a diploid organism. Your somatic cells contain 2 sets of chromosomes. One set you inherited from your mom; the other set you inherited from your dad. Between these set are homologous pairs of chromosomes.

Homologous chromosomes- are similar in size, the order of genes and centromere position (represented here by the white magnetic links).
- Homologous chromosomes are not however identical because one was inherited from mom and the other from dad.

Alleles- Different forms of the same gene at a specific position on both homologous chromosomes.

Allele example:
- Tongue Rolling. Suppose the characteristic of tongue rolling is found on chromosome #8.

Genotype- The genetic constitution of an individual organism.
- Two alleles exist of tongue rolling (R and r)
  - R is the allele that codes for a protein that allows you to roll your tongue.
  - r is the allele that codes for a protein that does not allow you to roll your tongue.

Recall: You inherit one allele on your mom’s #8 chromosome and one allele on your dad’s #8 chromosome. For any one person 3 genotypes are possible (RR, Rr or rr). Fig. 2. What will be the appearance of these 3 possible genotypes? i.e. what will be their phenotype?
**Dominant trait**- A trait that masks the appearance of a recessive trait. Dominant traits are usually identified by a upper case letter. Ex. R

**Recessive trait**- A trait that is masked by a dominant trait. Recessive traits are usually identified by a lower case letter. Ex. r

The ability to roll your tongue is dominant to not being able to roll your tongue. (R is dominant to r)

**Phenotype**- The observable expression of a genetic characteristic.

Since rolling your tongue is dominant to not being able to roll your tongue, only two possible traits exist (able to roll your tongue or not able to roll your tongue).

<table>
<thead>
<tr>
<th>Three Genotypes</th>
<th>Two Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous dominant</td>
<td>(RR) Can roll tongue</td>
</tr>
<tr>
<td>Homozygous recessive</td>
<td>(rr) Cannot roll tongue</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>(Rr) Can roll tongue</td>
</tr>
</tbody>
</table>

**Mendel’s Law of Segregation**- Alleles segregate from one another during the formation of gametes.

Recall: Meiosis: 1 diploid cell \( \rightarrow \) 4 haploid cells

Meiosis separates homologous pairs of chromosomes. This process segregates (separates) alleles from one another such that each gamete just contains one allele.

Example: Following meiosis a heterozygous (Rr) would produce 4 cells. \( \frac{1}{2} \) would be (R), \( \frac{1}{2} \) would be (r) Fig. 3.

Recall: Random Fertilization means any one of these 4 gametes can fertilize any one of another individual’s 4 gametes. A **Punnett square** can be used to predict the outcome of mating crosses.

Example: Using a Punnett square to predict outcome of a cross between 2 heterozygotes for tongue rolling. (Rr x Rr) “P generation”- These are the parental generation.

Punnett square predictions of offspring or the F\(_1\) generation: F\(_1\)- first filial generation.

F\(_1\) Genotypic ratio (1:2:1) meaning \( \frac{1}{4} \) will be RR: \( \frac{1}{2} \) will be Rr: \( \frac{1}{4} \) will be rr

F\(_1\) Phenotypic ratio (3:1) meaning \( \frac{3}{4} \) will be able to roll their tongue: \( \frac{1}{4} \) will not be able to roll their tongue.
CatLab®

We will model mating crosses using a genetics simulation program called 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 1. The major genes that control coat color, fur patterns and tail length in domestic cats.

<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^H 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>

Exercise 1: A Tour of CatLab

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.4)

The menu bar along the top consists of:

File  Cat  Options  About

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.
***Practice*** Selecting your female cat

Under the Cat menu, select Add Cat. Select Female, Has a tail, and Not all-white by checking the buttons next to those choices. Click Next.

Select No white areas. Click Next.

Select Mackerel and Orange. Note the cat graphic with the chosen features and the text description. Click Finish.

Your cat is now listed on the cat list. Click on the cat’s number at the left.

From the Cat menu tab, select “Display Phenotype”. Graphic, text description, and image will be displayed.

***Practice*** Selecting your male cat

Select Add by clicking [+ ] in the toolbar. Select Male, Lacks a tail, and All-white. Click Finish.

Click on the number 1 of the first cat, and then click [P] (Set as Parent) in toolbar.

Click on the number 2 cat and then select it as a parent as well. Mom and Dad appear on the left.

In the toolbar, click [L] (Litter Size). Make your choice and click OK.

To mate the selected cats, click M in the toolbar. A box appears with graphics of the litter. Click OK.

The litter is then added to the Cat list.

Click the Phenotype tab on the right side and then click on the different cats to see what they look like. Select other cats as parents and mate them to continue your investigation.

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!

Options Menu (and Toolbar Shortcuts):

- Chi-Square [\( \chi^2 \)] statistical test option. (see below)
- 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

***Practice*** When you are ready to clear your list of cats and begin the next exercise, click [N] in the toolbar. And “Empty”

Exercise 2: Exploring One-Trait Crosses: Dense or Not?

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 purpose of this exercise is to examine the inheritance of this one gene. We will try to pay particular attention to the way that alleles segregate in this cross to confirm Mendel’s idea of segregation.

The alleles for this color density gene may be denoted as:

D: dense, in this case black  d: dilute, in this case gray

Based on these symbols, which is the dominant trait and which is the recessive trait?
Procedure:
1. Make two cats as follows: Note 1 we know the phenotype for those showing the recessive trait. They have to be homozygous recessive. Note 2 the only characteristic that differs is “color density”
   #1 Black: Female → Tailed → Not all-white → No white areas → No tabby stripes → black
   #2 Gray: Male → Tailed → Not all-white → No white areas → No tabby stripes → gray

2. What are the genotypes of these 2 cats? Refer to table 1 and fill in the table below:
   Example: For tail type; since the black female’s phenotype is “has a tail” she must be homozygous recessive for that characteristic. Thus her “has a tail” genotype is (mm)

<table>
<thead>
<tr>
<th>Cat #1 phenotype</th>
<th>Cat #1 genotype</th>
<th>Cat #2 phenotype</th>
<th>Cat #2 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>XX</td>
<td>Male</td>
<td>XY</td>
</tr>
<tr>
<td>Has a tail</td>
<td></td>
<td>Has a tail</td>
<td></td>
</tr>
<tr>
<td>Not all-white</td>
<td></td>
<td>Not all-white</td>
<td></td>
</tr>
<tr>
<td>No white areas</td>
<td></td>
<td>No white areas</td>
<td></td>
</tr>
<tr>
<td>No tabby stripes</td>
<td></td>
<td>No tabby stripes</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td>or Gray</td>
<td></td>
</tr>
</tbody>
</table>

   Note: Other than gender and color density all phenotypes are the same and are homozygous recessive.
   Note: The female’s color density phenotype is black. We do not know her color density genotype. She could be Dd or DD.

3. What phenotypes of kittens, and in what proportions, would you expect to obtain from these parents?
   Draw two Punnett squares to support your predictions. (you should only need to do this for color density)

   ![Punnett Square]

   Mate cat #1 and cat #2 [M] and obtain 5 litters (20 kittens). Record their colors (black or gray) in Table 2.

   **Table 2.** Phenotypes of kittens from a cross between dense (black) and dilute (gray) cats.

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

   Total number of black kittens: _________   Total number of gray kittens: _________
Circle the Punnett square from question #3 that your data supports?

5. Recall the mom black cat could have the genotype **Dd** or **DD**. Based on your results, what genotype must the parent female black cat be? ________.

6. Any gray kittens from this mating have to be what genotype? ________.

7. Any black kittens from this mating have to be what genotype? ________. **NOTE** you have created a heterozygous cat using this method. You will use the same method in exercise 4 below.

8. Now, select a **black female** and a **black male** from among the group of 20 kittens to be the next parents [P], but **DO NOT** mate them yet! Record the identifying numbers of these new “parents-to-be”:
   
   New black mom will be cat # _____ and her color density phenotype is _________. and her color density genotype has to be ________.
   
   New black dad will be cat # _____ and his color density phenotype is _________. and his color density genotype has to be ________.

9. Answer these questions about the transmission of genetic information from parents to offspring:
   
   a. What biological process takes 1 diploid cell and ultimately produces four haploid gametes (eggs or sperms). Recall gametes carry half of the genetic information present in the parents’ genotypes?

   b. During meiosis, alleles separate so that new mother’s egg and new father’s sperm, will only contain one allele. So….

      The mom will produce haploid eggs with either _____ or ____. Remember gametes have only one allele!

      The dad will produce haploid sperm with either _____ or ____. Remember gametes have only one allele!

10. Based on your answers to question #9 above, **draw one** Punnett square to predict the outcome of a cross between your two new “parents-to-be”.

    ![Punnett Square Diagram]

    Based on Punnett square, **What are the expected proportions of the genotypes and phenotypes in the offspring? i.e. Their genotype and how many black kittens to how many gray kittens?**
11. Explain how your Punnett square above illustrates the mathematical law of segregation that Mendel came up with nearly 150 years ago.

12. Now use Catlab to mate your two black “parents-to-be” and obtain 5 litters (20 kittens). Record the number of kittens of each color in Table 3. (cats #23-42)

**Table 3. Phenotypes of kittens obtained in a cross between two black cats.**

<table>
<thead>
<tr>
<th>Color density phenotype</th>
<th>Observed Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>Gray</td>
<td></td>
</tr>
</tbody>
</table>

**Monohybrid cross**- A cross between two individuals heterozygous for one trait. Example, Aa x Aa.

The cross you just did had to have been a monohybrid cross.

You should be aware that from the monohybrid cross of two heterozygous parents (Dd x Dd), two phenotypic classes of offspring are expected; the chance of a black kitten occurring is \( \frac{3}{4} \) and the chance of a gray kitten occurring is \( \frac{1}{4} \). This is the same as saying that in a reasonable sample of offspring, the ratio of dominant black to recessive gray will be a good fit of a 3:1 ratio. In the case of 20 kittens, you would expect 15 of them to be black and 5 of them to be gray.

13. Does your data appear to fit the expected 3:1 ratio? What might be the cause of deviation from the expected values?

**Chi-square test**- A statistical test to determine if results are significant or just 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. We have 20 kittens from our matings, so this test is usable.

Use a Chi-square test to see if your results support the expected 3:1 ratio. (of 3 black cats to 1 gray cat)

14. Perform a Chi-square test on your data from table 3.

   Click on the \( \chi^2 \) in the toolbar.

   Enter 2 as the number of classes (phenotypes) you want to test. Click OK.

   From table 3, enter your observed number of black kittens in the box for Class 1, and the observed number of gray kittens in the box for Class 2. Then enter your expected ratios (3 for Class 1, 1 for Class 2). Click Calculate and record results below.

   \[ \chi^2 = \phantom{0} \]

   Critical Chi-square value = \phantom{0} 

   If the calculated Chi-square is less than the “critical Chi-square value”, then your data fits the expected ratio and any deviations from 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 of 3 black cats to 1 gray cat?

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

Exercise 3: Using a Test-Cross to Determine Genotype

In cats, one gene controls the appearance of an All-White Coat. The different alleles (W or w) for this gene produce phenotypes in which the coat is either all-white (unpigmented) or in which the coat has pigmented areas.

Note: The color of “Not all-white” cats is not a single phenotype. You should note that the phenotype “not all-white” can cover a range of colors and patterns; a solid-black cat, an orange mackerel, a gray and cream cat, and a tuxedo (black-and-white) cat are all examples of “Not all-white” cats!

The purpose of this exercise is to examine the inheritance of the all-white gene using a test-cross.

Test-Cross- a cross between an individual of unknown genotype with an individual of known genotype. Used to determine the unknown’s genotype.

An all-white cat’s genotype can be either WW or Ww. How can we know? We can determine which genotype is present by crossing this cat with a cat whose genotype for that characteristic is known.

Review table 1. What is the genotype for the only cat whose “All-white” phenotype we know? _______

Procedure:
The alleles for this gene may be denoted as:

\[W: \text{ all-white} \quad w: \text{ not all-white}\]

1. Make two cats with the following phenotypes:
   - #1 all-white female (Tailed, All-white) This cat might be Ww or WW.
   - #2 not all-white male (Has a tail, Not all-white, No white areas, and then whatever else you like). Remember any other color or striping types is hidden if the All-white trait (W) is inherited.

2. What is/are the possible all-white genotype(s) for the “all-white” female cat (#1)? _______ or _______.

3. What is the All-white genotype for the “not all-white” male cat #2? ___________.

4. What phenotypes and genotypes of kittens, and in what proportions, would you expect to obtain from these parents? Draw Punnett squares to support your predictions. Hint: you will need to do two Punnett squares
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 colors (all-white or not all-white) in Table 4.

Table 4. Phenotypes of kittens from a cross between all-white and not all-white cats.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Color “all-white” or other</th>
<th>Cat #</th>
<th>Color “all-white” or other</th>
<th>Cat #</th>
<th>Color “all-white” or other</th>
<th>Cat #</th>
<th>Color “all-white” or other</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>

Total number of All-white kittens: __________       Total number of Not all-white kittens: __________

Circle your Punnett square from question 4 that supports this data.

6. Recall the mom “all-white” cat could have the genotype **Ww or WW**. In light of your results, what genotype must the parent female “All-white” cat be? __________.

7. Any “All-white” kittens from this mating have to be what genotype? __________.

8. Any not “All-white” kittens from this mating have to be what genotype? __________.

9. Now, select an “all-white” female and a not “all-white” male from among the group of 20 kittens to be the next parents [P], but **DO NOT mate them yet**! Record the identifying numbers of these new “parents-to-be”:

New “all-white” mom will be cat # _____ and her whiteness phenotype is ________________ and her whiteness genotype has to be __________.

New not “all-white” dad will be cat # _____ and his pigment/pattern phenotype is ________________ and his whiteness genotype has to be __________.

10. Draw **one Punnett square** to predict the outcome of a cross between your two new “parents-to-be”. What are the expected proportions of phenotypes in the offspring?

```markdown
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```
11. Now mate your two “parents-to-be” and obtain 5 litters (20 kittens). Record the number of kittens of each color in Table 5. (cats #23-42)

Table 5. Phenotypes of kittens obtained in a cross between all-white and pigmented cats.

<table>
<thead>
<tr>
<th>Color</th>
<th>Observed Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-white</td>
<td></td>
</tr>
<tr>
<td>Not all-white</td>
<td></td>
</tr>
</tbody>
</table>

Perform a Chi-square test on your data.
Click on the $\chi^2$ in the toolbar.
Enter 2 as the number of classes (phenotypes) you want to test. Click OK.
From table 5, enter your observed number of all-white kittens in the box for Class 1, and the observed number of not all-white kittens in the box for Class 2. Then enter your expected ratios you determined from your Punnett square in question #10. Click Calculate and record results below.

$Chi$-square = __________  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 3:1 are due to random chance.

12. Does your data fit the expected ratio? Why or why not?

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

**Exercise 4: Testing Hypotheses**

In this exercise you will be working with the Manx phenotype. This phenotype involves the presence or absence of a tail. The Manx phenotype is controlled by a single gene with two alleles: M and m.

There is a lethal combination of alleles when inherited results in the death of the embryo (thus no kittens of that genotype are born). The purpose of this exercise is for you to develop and test two hypotheses regarding cat tails.

1. Which is the dominant trait determining tail length?
2. Which combination of alleles results in the lethal condition? Is it MM, Mm, or mm?

Consider these things:
- If Manx (no tail) is dominant to having a tail, than any Manx cat from a Manx X tailed mating must be heterozygous.
- If having a tail is dominant to Manx, than any tailed cat from a Manx X tailed mating must be heterozygous.
- Two such heterozygous cats can then be crossed to see if they yield offspring of the other tail phenotype with an expected phenotypic ratio of 1:2:1 and a phenotypic ratio of 3:1.
- Deviations from these expected ratios should allow you to determine which combination of alleles is lethal.

When making your cats, the only important phenotype is that of study (Manx or tailed) ignore all other phenotypes.
Procedure:

1. Based on Table 1 at the start of CatLab which, Manx or tailed is the dominant trait? _____________.
   Note: you will be confirming this below.
   Based on dominance and recessive, which cat phenotype do you KNOW the genotype of? ___________
   And which cat phenotype do you NOT KNOW the genotype of? __________________

2. State your hypothesis about which combination of alleles (MM) (Mm) or (mm) you think is lethal.

3. Make predictions (if/then statements) about what you expect to see if your hypothesis is correct.
   How are you going to test your hypothesis? What matings will you carry out? Hint: think about all of the possible matings and outcomes and record your ideas. Draw Punnett squares to help clarify the expected ratios from matings. What are the ratios expected if only certain genotypes live?
   Note: Just like real life cat births, CatLab does not tell you what kittens do not survive. You need to determine this based on the expected ratios vs. observed ratios.

4. Test your hypothesis using CatLab. You should attempt to create heterozygous cats as you did in exercise 2. Recall how you did that?
   If you then mated two heterozygotes, what would be the expected ratios of Manx vs. tailed cats? Do several matings (at least 10). Keep track of the number of offspring that are Manx vs. tailed. Use Chi-square test to determine which of the allele combinations was most probably lethal.
   Include any data on a separate sheet of paper.

5. Which combination of alleles is lethal (MM) (Mm) or (mm)? Was your hypothesis supported? What evidence is there to support or refute your hypothesis?