**AP Lab 7: The Mendelian Genetics of Corn**

**Objectives:**
In this laboratory investigation, you will: Use corn to study genetic crosses, recognize contrasting phenotypes, collect data from F2 ears of corn, and analyze the results from monohybrid and dihybrid crosses, study dominance, segregation, and independent assortment of alleles in corn, compare predicted results with obtained results from actual data, and analyze the data using Chi-square analysis techniques.

**Introduction:**
*Zea Maize,* commonly called corn in the United States, is one of the most important food crops. Because of its economic importance, the genetics of corn has been studied extensively. In sexual reproduction, two gametes fuse, bringing together homologous chromosomes from both parents. Corn has 10 pairs of chromosomes. One member of each pair is inherited from the “male” parent and the other is inherited from the “female” parent. For example, on Chromosome 2, there is a gene for plant height, and there are two forms or alleles of this gene, *D5,* a dominant allele for normal height and *d5,* a recessive allele for dwarf height. If both alleles on Chromosome 2 are for normal height (*D5/D5*), the phenotype (appearance) is normal height and the genotype is homozygous normal height. If the two alleles are different (*D5/d5*), the phenotype is normal height and the genotype is heterozygous for normal height. If the two alleles are for dwarf height (*d5/d5*), the phenotype is dwarf height and the genotype is homozygous dwarf height or simply homozygous recessive.

Given this information, we can now diagram and predict the results of crossing a homozygous normal height corn with a homozygous dwarf variety of corn.

<table>
<thead>
<tr>
<th><strong>P1</strong></th>
<th><strong>Gametes</strong></th>
<th><strong>F1</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>D5/D5</td>
<td>D5, d5</td>
<td>D5/d5</td>
</tr>
</tbody>
</table>

**P1** designates the parents of this cross and **F1** (first *filial,* a word that refers to siblings) designates their offspring. All of the gametes produced by one parent carry the dominant allele and all of the gametes produced by the other parent carry the recessive allele. (For now, we will ignore discussion about mutations and other unlikely but possible events), so there is only one possible combination of alleles for the **F1:** all have the phenotype normal height and the genotype heterozygous normal height. If the **F1** are crossed, we obtain different results.

<table>
<thead>
<tr>
<th><strong>F1 cross</strong></th>
<th><strong>Gametes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>D5/d5</td>
<td>D5, d5</td>
</tr>
</tbody>
</table>

We can use a Punnett Square to determine how the gametes will recombine in the next, or F2 generation.

<table>
<thead>
<tr>
<th>Gametes</th>
<th>D5</th>
<th>d5</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5</td>
<td>D5/D5</td>
<td>D5/d5</td>
</tr>
<tr>
<td>d5</td>
<td>D5/d5</td>
<td>d5/d5</td>
</tr>
</tbody>
</table>

Thus, in the F2, there are four possible combinations of gametes:
1 for homozygous normal height (phenotype normal height)
2 for heterozygous normal height (phenotype normal height)
1 for homozygous dwarf height (phenotype dwarf height)
The genotypic ratio is 1:2:1; the phenotypic ratio is 3 normal height: 1 dwarf height.
Determining Expected Counts:
With this information, we can calculate the expected number of each phenotype. Suppose we perform the cross and harvest 1000 F$_2$ corn grains. How many of these grains should we expect to germinate into plants of normal height? How many dwarf plants should we expect? The expected ratio is 3:1. Since the total of 3+1 is 4, we first determine $\frac{1}{4}$ of the whole (1000/4=250). The expected count for 1000 grains is calculated as 3(250):1(250) or 750 normal height to 250 dwarf plants in the F$_2$.

Background:
In these activities, you will investigate several phenotypes of corn that are expressed in the seed. You will be given ears of corn for your investigation. Each seed on an ear of corn results from a separate fertilization event. Further, the seeds on the ear are the F$_2$ from a cross that began with two parental varieties of corn with contrasting phenotypes.

To successfully complete these activities, you must have good background knowledge of genetics. Think about the following questions PLUS ANY other questions YOU come up with:
1. How will I know which allele is dominant and which allele is recessive?
2. Am I dealing with a monohybrid or a dihybrid cross? How will this affect my results?
3. How will I know what the F$_2$ seed should look like? What phenotype ratio will I expect in the F$_2$?
4. How will I know that my results are valid?

Exercise A: P$_1$ and F$_1$
Your group will be given two Corn Parental Cross Cards, A and B, that show a cross of two parental varieties of corn with contrasting phenotypes as well as the F$_1$ corn resulting from the cross. Two of you will work on the cross shown on card A and two will work with the cross shown on card B.
All the P$_1$ corn varieties are homozygous for all the genotypes you will be studying.
Examine the cross that is shown on your Corn Parental Cross Card. Collect information and complete the steps 1-5 below.
1. Will you treat this as a monohybrid cross or a dihybrid cross? Explain your answers.

___________________________________________________________________________

___________________________________________________________________________

___________________________________________________________________________

2. Decide on a one-word description of the phenotype of each of your P$_1$ corn varieties.
Phenotype of (Choose one—Circle it) A-1 or B-1________________________________________
Phenotype of (Choose one—Circle it) A-2 or B-2________________________________________

3. Decide what symbols you will use to represent the alleles by which these phenotypes are inherited. Record the symbols in the blanks below.

________ is the symbol for the allele ____________________________

________ is the symbol for the allele ____________________________

4. Which allele is dominant? Explain how you know. ______________________________________

___________________________________________________________________________

___________________________________________________________________________

5. What is the genotype of the F$_1$? ____________________________
**Exercise B: F₁ Cross and F₂**

Using the information you recorded in Exercise A and your knowledge of genetics, draw in the space below a Punnett Square showing the results of crossing your F₁ to obtain an F₂. Below the Punnett Square, give the phenotypes and their expected ratios for the F₂.

Phenotypes and expected ratios for the F₂:

Your teacher will now give you an F₂ ear of corn from the cross you diagramed above. Working in pairs, count and record in Table 1 (below) the number of grains of each phenotype. One person should call out the phenotypes while the other records them in the table. To make the count, mark the beginning of one row of grains and count and record the phenotypes of each grain in that row. Continue counting, marking the beginning of each row as you count. (You will count ____ row(s)) When finished counting, total your results. Then obtain and record the class totals for the same cross.

<table>
<thead>
<tr>
<th>Table 1: F₂ Phenotype Count for __________ x __________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype</td>
</tr>
<tr>
<td>Team Count</td>
</tr>
<tr>
<td>Totals:</td>
</tr>
<tr>
<td>Team total for all phenotypes counted:</td>
</tr>
<tr>
<td>Class count totals:</td>
</tr>
<tr>
<td>Call total for all phenotypes counted:</td>
</tr>
</tbody>
</table>

Questions:

1. What evidence do you have that the phenotypes you are investigating are actually being inherited? Could they be the result of environmental effects? ____________________________________________________________________________________________

2. If the phenotypes are under genetic control, are they inherited through a single gene, a few genes, or many genes? How do you know? ____________________________________________________________________________________________

3. State your hypothesis about the inheritance of the phenotypes. ____________________________________________________________________________________________
4. Using the class’s data from Table 1 and information from your Punnett Square, calculate the expected counts for the F\textsubscript{2} and record them below.
   Phenotype: ________ Expected count ________
   Phenotype: ________ Expected count ________

5. Compare the class’s actual counts to your calculated expected counts. Do the actual counts deviate from what the expected? If so, explain why. ____________________________
   ____________________________

6. Are the deviations from the expected results for the phenotypic ratio of the F\textsubscript{2} generation within the limits expected by chance? To answer this question, statistically analyze the data using Chi-square analysis. Refer to the **Chi-Square (χ\textsuperscript{2}) Test** section of Exercise D. In the space below, calculate the Chi-square statistic for the F\textsubscript{2} generation. Refer to Table 3 on page 10 to determine the probability that is associated with your χ\textsuperscript{2} statistic.

   ______ = χ\textsuperscript{2}
   ______ = % probability that the observed variation is due to random chance alone.

7. What does this probability mean? ____________________________________________
   ____________________________________________

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**Exercise C: A Different Cross**

As a team, look again at the Corn Parental Cross Cards, A and B. Remember that all of the P\textsubscript{1} seeds are homozygous for all of the genotypes that you are studying. This means that the P\textsubscript{1} shown on Card A are homozygous for the alleles involved in the cross shown on Card B and the P\textsubscript{1} shown on Card B are homozygous for the alleles involved in the cross shown on Card A. Suppose you performed a new cross using, as your P\textsubscript{1}, corn varieties A-1 and B-1. Working as a team, collect the following information about this cross as you complete the steps below.

1. Will you treat this as a monohybrid cross or a dihybrid cross? Explain your answer.

   ____________________________________________

2. Give the genotypes and phenotypes for the P\textsubscript{1} and F\textsubscript{1} of this cross.

   \[
   \begin{array}{ccc}
   \text{A-1} & \text{P}_1 & \text{B-1} \\
   \text{Genotype} & \times & \text{Genotype} \\
   \text{Phenotype} & \text{Phenotype} \\
   \end{array}
   \]

   F\textsubscript{1} Genotype: ____________ Phenotype: ____________
3. If the space below, construct a Punnett Square showing the results of crossing your F₁ to obtain an F₂. Below the Punnett Square, give the phenotypes and their expected ratios for the F₂ of this cross.

Phenotypes and expected ratios of the F₂: __________________________________________
______________________________________________________________________________
Your instructor will now give you an F₂ ear of this cross. Count and record the phenotypes in Table 2 below. Also, record count totals for the class.
Table 2: F₂ Phenotype Count for ___________ x ___________
<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Team Count</th>
<th>Totals:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Team total for all phenotypes counted:
Class count totals:
Call total for all phenotypes counted:

Questions
1. On the basis of the data recorded in Table 2, what new inferences can you make about the inheritance of the phenotypes? __________________________________________
______________________________________________________________________________

2. Calculate the expected counts for the F₂ and record them below:
   Phenotype: ___________ Expected Count ___________
   Phenotype: ___________ Expected Count ___________
   Phenotype: ___________ Expected Count ___________
   Phenotype: ___________ Expected Count ___________

3. Calculate the Chi-Square statistics for this new data set. What does the value tell you about the class’s data? __________________________________________
______________________________________________________________________________
Exercise D: Chi-Square ($\chi^2$) Test

Does your data, as recorded in the activities above, actually support your hypothesis about how the trait is inherited? Genetics, like gambling, deals with probabilities. When you flip a coin, you have the same chance of getting a head as a tail: one-to-one ratio. That does not mean that if you flip a coin 100 times you will always get 50 heads and 50 tails. You might get 53 heads and 47 tails. That is probability close enough to a one-to-one ratio that we would accept it without a second thought. But what if you got 61 heads and 39 tails? At what point do you begin to suspect that something other than chance is at work in determining the fall of your coin? Look back at your data. You were expecting a 3:1 or 9:3:3:1 phenotype ratio in the $F_2$. This assumes that chance (and chance only) has been operating in the assortment and recombination of alleles that gave rise to the $F_1$ and $F_2$ you have investigated. Thus, any variation of the observed results from the expected results are due to chance. This is known as the null hypothesis. Does your data actually support the null hypothesis?

The Chi-Square ($\chi^2$) test is a statistical test used to determine how well observed ratios fit expected ratios. The difference between the number observed and the number expected for a phenotype is squared and then divided by the number expected. This is repeated for each phenotype class. The $\chi^2$ value consists of the summation of these values for all classes. The formula for $\chi^2$ is:

$$\chi^2 = \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

The calculated value for $\chi^2$ is then compared to the values given in a statistical table, such as the one shown on the top of the next page.

<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>p= 99%</th>
<th>95%</th>
<th>80%</th>
<th>50%</th>
<th>20%</th>
<th>5%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000157</td>
<td>0.00393</td>
<td>0.0642</td>
<td>0.455</td>
<td>1.642</td>
<td>3.841</td>
<td>6.635</td>
</tr>
<tr>
<td>2</td>
<td>0.020</td>
<td>0.103</td>
<td>0.446</td>
<td>1.386</td>
<td>3.219</td>
<td>5.991</td>
<td>9.210</td>
</tr>
<tr>
<td>3</td>
<td>0.115</td>
<td>0.352</td>
<td>1.005</td>
<td>2.366</td>
<td>4.642</td>
<td>7.815</td>
<td>11.345</td>
</tr>
<tr>
<td>4</td>
<td>0.297</td>
<td>0.711</td>
<td>1.649</td>
<td>3.357</td>
<td>5.989</td>
<td>9.488</td>
<td>13.277</td>
</tr>
<tr>
<td>5</td>
<td>0.554</td>
<td>1.145</td>
<td>2.343</td>
<td>4.351</td>
<td>7.289</td>
<td>11.070</td>
<td>15.086</td>
</tr>
<tr>
<td>6</td>
<td>0.872</td>
<td>1.635</td>
<td>3.070</td>
<td>5.348</td>
<td>8.558</td>
<td>12.592</td>
<td>16.812</td>
</tr>
<tr>
<td>8</td>
<td>1.646</td>
<td>2.733</td>
<td>4.594</td>
<td>7.344</td>
<td>11.030</td>
<td>15.507</td>
<td>20.090</td>
</tr>
</tbody>
</table>

Accept-------------------------------------------- Reject--NH

In this table, note the column titled “Degrees of Freedom.” The degree of freedom is always one less than the number of different phenotypes possible. For a monohybrid $F_2$, in this experiment we have two possible phenotypes so there is $2-1 = 1$ degree of freedom. For a dihybrid $F_2$, there are four possible phenotypes combinations and 3 degrees of freedom. The numbers to the right of the Degree of Freedom column in the table are $\chi^2$ values. The percentages given at the top of each column represent the probability that the variation of the observed results from the expected results is due to chance. If the probability is greater than 5%, we accept the null hypothesis; that is, our data fits the expected ratios. The following are two examples, one for a monohybrid cross and the other for a dihybrid cross.
In the F$_2$ population of 100 *Drosophila* (fruit flies), there are 60 with normal wings and 40 with vestigial wings. (The expected ratio would be 75 normal wings and 25 vestigial wings). Therefore:

$$\chi^2 = \frac{(60-75)^2}{75} + \frac{(40-25)^2}{25} = 3 + 9 = 12.0$$

Looking at the table, in the row for 1 degree of freedom, for $\chi^2 = 12$, the probability is less than 1%. Therefore, these results do not support the expectation (or null hypothesis) of a 3:1 ratio, since the probability is less significant (less than 5%) that deviation from the expected ratio is due to chance.

List at least three possible sources of error that could explain why the data in this example do not fit the expected 3:1 ratio.

1. __________________________________________________________________________
2. __________________________________________________________________________
3. __________________________________________________________________________

Now consider the following data for F$_2$ *Drosophila* of a dihybrid cross of P$_1$ flies having normal wings and red eyes with flies having vestigial wings and sepia eyes. The alleles for normal wings and red eyes are dominant for *Drosophila*. The expected phenotypic ratio is 9 Normal Wings, Red Eyes; 3 Normal Wings, Sepia Eyes; 3 Vestigial Wings, Red Eyes; 1 Vestigial Wings; Sepia Eyes.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype</td>
<td>Normal Wings, Red Eyes</td>
<td>Normal Wings, Sepia Eyes</td>
<td>Vestigial Wings, Red Eyes</td>
<td>Vestigial Wings, Sepia Eyes</td>
<td>4</td>
</tr>
<tr>
<td>Phenotype Count</td>
<td>577</td>
<td>204</td>
<td>176</td>
<td>59</td>
<td>1016</td>
</tr>
<tr>
<td>Expected Numbers</td>
<td>571.5</td>
<td>190.5</td>
<td>190.5</td>
<td>63.5</td>
<td>1016</td>
</tr>
</tbody>
</table>

$$\chi^2 = \frac{(577-571.5)^2}{571.5} + \frac{(204-190.5)^2}{190.5} + \frac{(204-190.5)^2}{190.5} + \frac{(59-63.5)^2}{63.5}$$

The probability of 2.43 from the table for 3 degrees of freedom is greater than 30% but less than 50%. This means that a deviation as large or larger would be expected to occur purely by chance more than 30% of the time but less than 50% of the time. Such a deviation is NOT significant (because the probability is greater than 5%), so we accept the null hypothesis in favor of the 9:3:3:1 ratio. Note that the acceptance is provisional. Additional data could always cause us to reject the Null Hypothesis.