Monohybrid Genetics with Corn Kit
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Monohybrid Genetics with Corn Kit

Overview
This kit is written primarily as a genetics lab for an introductory high school or middle school biology course. Students study the inheritance of grain color using ears of corn. Each grain on the ears is the F2 of a cross that began with a homozygous red corn crossed with a homozygous white corn. In addition to a detailed Student Guide, this manual also contains reproducible pages for a Chi-Square Test optional activity that is highly recommended for pre-AP® and College Preparatory classes.

Storing Your Kit
With reasonable care, this kit will last for many years. At the other extreme, the kit could be destroyed upon its first use. When not in use, store the ears of corn in the storage box, in a dark area, with the lid sealed. Although the ears have been fumigated, we recommend the use of an insect-repellant substance in the storage box. Students may be tempted to pick grains off the corn ears. This should be discouraged before it happens. The loss of a few grains is to be expected. In many cases, it is possible to glue lost grains back into their original positions.

Objectives
In this laboratory, students will
- use corn to study genetic crosses.
- recognize contrasting phenotypes.
- collect data from F2 ears of corn.
- study dominance, segregation, and independent assortment of alleles in corn.
- compare predicted results with results obtained from actual data.

Required Knowledge
Before doing this laboratory students should understand
- meiosis.
- the chromosomal theory of heredity.
- the basics of Mendelian genetics.
- the use of Punnett squares.
- the life cycle of organisms useful in genetics studies.

Expectations
At the completion of this laboratory, students should be able to
- collect and organize data from genetic crosses.
- predict patterns of inheritance given relevant data.
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Time Requirements

Exercise A (Parental Cross and F1) and Exercise B (Investigating the F2) can be completed in one 45-minute period, although students may have to answer the questions outside of class. Alternatively, Exercise A can be completed during 15–20 minutes of one class period and then Exercise B can be completed in one 45-minute period. The class total for all phenotypes counted in Table 1 should be at least 1,000. More is better. Comparisons of group totals to class totals can demonstrate the importance of sample size.

Preparation

Photocopy the Student Guide at the end of this manual for your class.

Student Self-Assessments

In the Background section, before beginning Exercise A, students are asked to compile a list of the things they know about Mendelian genetics. This can be done outside of class. We recommend that you collect these lists and examine them closely. They will give you some idea of your students’ background knowledge and level of ability. More importantly, these lists can reveal misconceptions in student knowledge that need to be addressed. Photocopy the lists before returning them to your students. Compare the initial self-assessments to the amended lists that students submit at the end of the laboratory exercise. This will help in assessing student progress.

Student Materials and Equipment

If you have a class of 32 students, have them work in pairs. For Exercise A, each group needs a Corn Parental Cross Card. For Exercise B, each group needs an F2 ear of corn and a transparency marker.

Troubleshooting

Before beginning the laboratory exercises, the class as a whole should agree upon the names and genetic symbols they will use during this exercise to describe the phenotypes they observe. Otherwise, student groups will encounter difficulty sharing and assessing data.

Background

Corn Life Cycle

The tassel at the top of the corn plant produces pollen. Pollen grains containing three haploid (n) nuclei are produced from microspores in the stamens of the tassel. One nucleus in each pollen grain becomes the pollen tube nucleus, while the other two become sperm nuclei.

The developing ears have rows of pistils that grow long, thin styles (silks) that grow out the ends of the husks that cover the ears. Within each pistil a megaspore gives rise to an embryo sac containing eight haploid nuclei. Two of these megaspore nuclei fuse to form the fusion nucleus (2n) and one becomes the egg nucleus (n). The other five nuclei become separated by cell walls and take no known part in the plant life cycle after the formation of the fusion nucleus and egg nucleus.

When a pollen grain falls on the silk, a pollen tube grows from the pollen grain through the silk to the embryo sac. One of the sperm nuclei (n) unites with the fusion nucleus (2n), forming the endosperm nucleus (3n). The other sperm nucleus (n) unites with the egg nucleus (n) to form a zygote (2n).
The zygote develops into an embryo that becomes dormant during the seed stage of the corn life cycle. The endosperm nucleus develops into a mass of tissue (the endosperm), which surrounds the embryo. The outer layer of cells of the endosperm is called the aleurone.

A corn “seed” is a fruit that consists of the embryo (2n), the endosperm (3n), and endosperm aleurone (3n), all enclosed in a pericarp (2n). The pericarp is a remnant of the pistil and is of maternal origin and genetics.

**Genetics of Corn**

In this lab, your students will investigate the inheritance of aleurone color, which involves triploid genetics. However, in these exercises, we treat the maternal pair of genes as a single allele. We do not feel we have done too much injustice to the true situation, because the maternal pair of alleles are duplicates (C/C or c/c) of the same allele.

The color of a corn grain is controlled by a large number of genes that determine the phenotypes of three tissues: the pericarp, the aleurone, and the endosperm proper (see Figure 1). In the corn your students will use, the pericarp is always colorless, but the aleurone may be colorless or red. Although several genes interact in producing aleurone color, it is the C alleles that undergo segregation and recombination in the ears that your students study. Conditions C/C and C/c give a red aleurone. The homozygous recessive (c/c) disrupts production of pigment (anthocyanin) and results in a colorless aleurone. Thus, if the aleurone has color, the grain will be red, and if the aleurone is colorless, the grain will be the color of the endosperm, which in this case is white. These phenotypes of red and white grain color are obvious and easy to score by beginners.

![Figure 1. The layers of a corn kernel involved in producing color phenotypes.](image)

**The Cross**

Students will study a monohybrid cross that begins with a red corn (C/C) that is crossed with a white corn (c/c).

<table>
<thead>
<tr>
<th>Generation</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>C/C × c/c</td>
<td>heterozygous Red</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>C/c</td>
<td>heterozygous Red</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; cross</td>
<td>C/c × C/c</td>
<td>phenotypes Red, White in a phenotype ratio of 3:1</td>
</tr>
</tbody>
</table>

**Transposable Elements**

In the 1940s, while studying the inheritance of speckled (as opposed to solid) color in corn grains, Barbara McClintock found that she could not map the locus of the responsible gene, because it moved about on the chromosome. These transposable
elements, as they are now called, are common in corn and other organisms. In corn, they can disrupt color production in the aleurone so that most of the grain is white with only specks or restricted areas of red. Our breeding program seeks to eliminate the effects of transposable elements on grain color, but examine your corn carefully. Any grain that clearly has at least one speck of red color should be scored as having the red phenotype, because it has at least one copy of the \( C \) allele.

### Exercise A: Parental Cross and \( F_1 \)

1. Decide on a one-word description of the phenotype of each of your \( P_1 \) corn varieties and record them here.
   - Phenotype Red (answer will vary but should be consistent for class)
   - Phenotype White (answer will vary but should be consistent for class)

2. Decide what symbols you will use to represent the alleles by which these phenotypes are inherited. Record the symbols here.
   - \( C \) is the symbol for the allele red (answer will vary but should be consistent for class);
   - \( c \) is the symbol for the allele white (answer will vary but should be consistent for class)

3. Which allele is dominant? Explain how you know.
   - The allele for red is dominant because it is the trait expressed in the heterozygous \( F_1 \).

4. What is the genotype of the \( F_1 \)?
   - \( C/c \) or heterozygous

5. Using the information you recorded above and your knowledge of genetics, draw in the space below a Punnett square showing the results of crossing the \( F_1 \) to obtain an \( F_2 \).

<table>
<thead>
<tr>
<th>Gametes</th>
<th>( C )</th>
<th>( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C )</td>
<td>( C/C )</td>
<td>( C/c )</td>
</tr>
<tr>
<td>( c )</td>
<td>( C/c )</td>
<td>( c/c )</td>
</tr>
</tbody>
</table>

6. From your completed Punnett square, give the phenotypes and their expected ratios for the \( F_2 \).
   - 3 Red to 1 White

7. State, as completely as you can, your hypothesis about the inheritance of the phenotypes. Using your hypothesis, predict what you expect to find in the \( F_2 \).
   - The traits are inherited through two alleles at one gene locus. The allele for red grain color is dominant to the allele for white grain color. One of the parents was homozygous red and the other was homozygous white. Their offspring, the \( F_1 \), are heterozygous and express the red phenotype. Prediction: In the \( F_2 \) there will be approximately three times more red grains than white grains.
Exercise B: Investigating the F₂

Note: All answers will depend on the data that is collected. Data from sample counts are given below for comparison.

Table 1: F₂ Phenotype Count for Red × White

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Team Count</th>
<th>Class Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red</td>
<td>White</td>
</tr>
<tr>
<td>Team</td>
<td>Total 120</td>
<td>Total 44</td>
</tr>
<tr>
<td></td>
<td>Team total for all phenotypes counted 164</td>
<td>Class total for all phenotypes counted 2377</td>
</tr>
<tr>
<td>Class</td>
<td>Total 1811</td>
<td>Total 566</td>
</tr>
</tbody>
</table>

8. The expected ratio is 3:1 for a total of 4. Therefore: $2377 \div 4 = 594.25$, which we round to 594. Thus: $3(594) = 1782$ and $1(594) = 594$

Phenotype Red expected count 1782
Phenotype White expected count 594

9. Compare the class actual counts from Table 1 to your calculated expected counts. Are the actual counts different from the expected counts? If so, explain why.

They are different, but close. Since the recombination of gametes is a chance event, some variation of actual counts from expected counts is normal.

10. Do these results tend to confirm or disprove your hypothesis as stated in 7 above? Explain your answer.

The results tend to confirm the hypothesis. Explanations will vary.
Further Activities

1. Students who do not relate the events in meiosis and fertilization to the segregation and recombination of alleles can have difficulty understanding genetics. Such students may benefit from making posters of meiosis showing chromosomes with the alleles they are studying attached. This visualization may help them understand what can otherwise seem to be a confusing set of rules.

2. Mendelian genetics involves chance events. Because some students equate chance with chaos, they can have trouble understanding how chance can ever produce an orderly sequence. These students can benefit from some simple probability studies. For example, let the head of a penny represent a dominant allele for a trait and the tail represent the corresponding recessive allele. Students could use red and white as the phenotypes, or anything else. Pairs of students flip two pennies to represent the crossing of F1 hybrids. The results (H/H, H/h, or h/h) give the genotype of the F2. Each pair of students can flip their coins 100 times, then determine a class total. This activity can help students to better understand the role of probability in genetics.

3. Use coin flipping to teach the concept of $\chi^2$. Ask students, “If you flipped a coin 10 times, how many heads and tails should you get?” Students know they should get 5 of each result. They also know they may not. Have every student in the class flip a coin 10 times, record the results, and share them with the class. Ask them why (presumably) some class members got results different from the theoretical results. Next, pose this problem to the class: “Suppose that you did not know that there is an equal probability of getting heads and tails. How could you decide whether the results you get represent random deviations from 50:50 or not?” Students may suggest good ideas such as repeating the test many times. Have students conduct a $\chi^2$ analysis of their coin-flipping results. This will help them learn how to calculate expected results, the value of $\chi^2$, determine the degrees of freedom, and so on.

4. The biochemical pathways through which the C alleles are expressed are known in some detail. Students can research this on the Internet.
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Background Knowledge

In these activities, you will investigate phenotypes of corn that are expressed in the seed. You will be given ears of corn for your investigation. Each seed on an ear results from a separate fertilization event and thus represents an offspring. Further, the seeds on the ear are the F2 from a cross that began with two parental varieties of corn with contrasting phenotypes.

To successfully complete these activities, you must have a good background knowledge of genetics. On a sheet of notebook paper, write the things you know about genetics that you think will help you complete these activities. Include at least six different facts that you know about genetics and explain how this knowledge will help you. Here are some questions to help you get started, but do not be limited by these questions. Keep the paper as a reference during the rest of this lab. Add notes and make modifications as needed.

1. What are homologous chromosomes and what do they have to do with genes and alleles?
2. What are gametes?
3. What is a phenotype? What is a genotype?
4. How will I know which allele is dominant? How will I know which allele is recessive?
5. What is a Punnett square? How is a Punnett square used?
6. How will I know what the F2 seeds will look like? What phenotype ratio will I expect in the F2?

Exercise A: Parental Cross and F1

You will be given a Corn Parental Cross Card that shows a cross of two parental varieties of corn with contrasting phenotypes, and the F1 corn resulting from the cross. P1 and P2 designate the parents of this cross, and F1 (first filial, a word that refers to siblings) designates their offspring.

The parental corn varieties are homozygous for the genotypes you will be studying.

Examine the cross that is shown on your Corn Parental Cross Card and complete the following activities and questions.

1. Decide on a one-word description of the phenotype of each of your P1 corn varieties and record them here.

Phenotype ________________________________

Phenotype ________________________________
2. Decide what symbols you will use to represent the alleles by which these phenotypes are inherited. Record the symbols here.

_____ is the symbol for the allele ____________________________

_____ is the symbol for the allele ____________________________

3. Which allele is dominant? Explain how you know.

_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________

4. What is the genotype of the F₁? ____________________________________________________________

5. Using the information you recorded above and your knowledge of genetics, draw in the space below a Punnett square showing the results of crossing the F₁ to obtain an F₂.

6. From your completed Punnett square, give the phenotypes and their expected ratios for the F₂.

_______________________________________________________________________________________
7. State, as completely as you can, your hypothesis about the inheritance of the phenotypes. Using your hypothesis, predict what you expect to find in the F₂.

_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________
_______________________________________________________________________________________

Exercise B: Investigating the F₂

You will now test your hypothesis and prediction made in 7, above. Your teacher will give you an F₂ ear of corn from the cross you diagramed above. Working in pairs, count and record in Table 1 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.

When finished counting, total your results. Then obtain and record the class totals.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Team Count</th>
<th>Class Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Team total for all phenotypes counted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Class total for all phenotypes counted</td>
</tr>
</tbody>
</table>
8. How do the Class Counts in Table 1 compare to the expected counts for this cross? You can determine the expected counts using the phenotype ratios you recorded in 6 above. Let us suppose you determined that there would be 3 of phenotype A for every 1 of phenotype B (a 3:1 ratio), and the Class Total for all phenotypes counted is 1,000. Because the total of $3 + 1$ is 4, we first determine $\frac{1}{4}$ of the total for all phenotypes: $1,000 \div 4 = 250$ (rounded to the nearest whole). We can now calculate the expected counts as $3(250) = 750$ for phenotype A and $1(250) = 250$ for phenotype B. Use your phenotype ratios from 6 above and the Class Total for all phenotypes counted from Table 1 to calculate the expected counts for your cross, and record them here.

Phenotype _____________________ expected count ______________

Phenotype _____________________ expected count ______________

9. Compare the class actual counts from Table 1 to your calculated expected counts. Are the actual counts different from the expected counts? If so, explain why.

_______________________________________________________________________________________

_______________________________________________________________________________________

_______________________________________________________________________________________

_______________________________________________________________________________________

10. Do these results tend to confirm or disprove your hypothesis as stated in 7, above? Explain your answer.

_______________________________________________________________________________________

_______________________________________________________________________________________

_______________________________________________________________________________________

_______________________________________________________________________________________

**Final Activity**

Refer to the Background Knowledge sheet that you produced at the beginning of this lab. Make any needed additions, corrections, or deletions to it that you feel are necessary. Has your knowledge of genetics changed as a result of doing this lab? Explain your answer.

_______________________________________________________________________________________

_______________________________________________________________________________________

_______________________________________________________________________________________

_______________________________________________________________________________________
The Chi-Square Test

Does your data as recorded in Table 1 actually support your hypothesis about how the phenotypes are inherited? Genetics, like gambling, deals with probabilities. When you flip a coin, you have the same chance of getting a head as a tail: a 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 probably close enough to a one-to-one ratio that you would accept it without a second thought, but what if you get 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 the coin? Look back at your data. You were expecting a 3:1 phenotype ratio in the F2. This assumes that chance (and chance only) has been operating in the assortment and recombination of alleles that gave rise to the F1 and F2 you have investigated. Thus, any variation of the observed results from the expected results is 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. The $\chi^2$ value consists of the summation of these values for all phenotypes. For a monohybrid cross, there are two phenotypes. Thus, $\chi^2$ for a monohybrid cross is calculated in this way:

$$\chi^2 = \frac{(\text{observed phenotype 1} - \text{expected phenotype 1})^2}{\text{expected phenotype 1}} + \frac{(\text{observed phenotype 2} - \text{expected phenotype 2})^2}{\text{expected phenotype 2}}$$

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

<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>

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 the monohybrid F2 corn there are two possible phenotypes. So, $2 - 1 = 1$ degree of freedom. The numbers to the right of the “Degrees of Freedom” heading 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 value is greater than 5%, we accept the null hypothesis; that is, our data fits the expected ratios. Following is an example to show you how this works.
In an F2 population of 1016 *Drosophila* (fruit flies), there are 781 with normal wings and 235 with vestigial wings (expected numbers are 762 with normal wings and 254 with vestigial wings). Then,

\[ \chi^2 = \frac{(781 - 762)^2}{762} + \frac{(235 - 254)^2}{254} = 0.47 + 1.42 = 1.89 \]

Looking at the chi-square table for 1 degree of freedom and \( \chi^2 = 1.89 \), the probability is greater than 5% but less than 20%. This is greater than 5%. Therefore, we accept the null hypothesis that the variation of actual counts from the expected counts is due to chance. Notice that acceptance of the null hypothesis is provisional. It is always possible that additional data would cause us to reject it.

Using the formula for \( \chi^2 \) and the values in Table 2, calculate \( \chi^2 \) for your data and determine the probability that the observed variation is due to chance. Do the results support your hypothesis about how the phenotypes are inherited? Explain your answer.