# The Amazing Maize Investigating Dihybrid Crosses 

## About this Lesson

This activity can be used to supplement a unit on genetics. It reviews Mendel's work with peas and focuses on the law of independent assortment. The students collect data and calculate the phenotypic ratio of a corn cob representing a dihybrid cross. They then use a chi-square analysis to statistically analyze the results.

This lesson is included in the LTF Biology Module 3.

## Objectives

Students will:

- Collect and analyze the results of a dihybrid cross in maize (corn)
- Statistically analyze and compare the observed results with the predicted results

Level
Biology
Common Core State Standards for Science Content
LTF Science lessons will be aligned with the next generation of multi-state science standards that are currently in development. These standards are said to be developed around the anchor document, A Framework for K-12 Science Education, which was produced by the National Research Council. Where applicable, the LTF Science lessons are also aligned to the Common Core Standards for Mathematical Content as well as the Common Core Literacy Standards for Science and Technical Subjects.

| Code | Standard | Level of <br> Thinking | Depth of <br> Knowledge |
| :--- | :--- | :---: | :---: |
| (LITERACY) <br> RST.9-10.3 | Follow precisely a multistep procedure when <br> carrying out experiments, taking measurements, <br> or performing technical tasks, attending to spe- <br> cial cases or exceptions defined in the text. | Apply | II |
| (MATH) <br> S-ID.4 | Use data from a sample survey to estimate <br> a population mean or proportion; develop a <br> margin of error through the use of simulation <br> models for random sampling. | Apply | II |
| (Literacy) <br> RST.9-10.7 | Translate quantitative or technical information <br> expressed in words in a text into visual form <br> (e.g., a table or chart) and translate information <br> expressed visually or mathematically (e.g., in an <br> equation) into words. | Apply | II |


| Code | Standard | Level of <br> Thinking | Depth of <br> Knowledge |
| :--- | :--- | :---: | :---: |
| (MATH) | Rearrange formulas to highlight a quantity of <br> interest, using the same reasoning as in solving <br> equations. For example, rearrange Ohm's law <br> A-CED. | Apply | II |

## Connections to AP

AP Biology: This lesson addresses concepts contained in Big Idea 3 in the revised AP Biology curriculum under the following sections: 3.A.3.b.1 and 3.A.3.b.3.
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## Materials and Resources

Each lab group will need the following:
calculator
marker, Vis-à-vis ${ }^{\circledR}$
2 push pins
corn, F2 ear

## Additional teacher materials:

2 corn, homozygous purple starchy corn, homozygous yellow sweet

## Assessments

The following assessments are located on the LTF website:

- Short Lesson Assessment: Amazing Maize
- Meiosis and Mendelian Genetics Assessment
- 2010 Biology Posttest, Free Response Question 1


## Teaching Suggestions

The ears of corn can be purchased from scientific supply companies such as Carolina Biological Supply or Ward's Natural Science Company.

When purchased, order eight of the homozygous purple smooth/starchy and change the label on four of them to the "F1 generation," as the phenotype will be the same. "SuSu" refers to the genotype as normal, which is what you want to order. There are other traits that can be examined and purchased from these companies.

To keep track of the kernels, students use two different colored push pins. One color is used to mark the first row counted. The second color is used to mark the row that is currently being counted. As a student finishes one row, the pin should be moved to the next row. The pins should be placed at the end of the corn cob and inserted carefully so as to avoid damaging the kernels of corn.

The best way to count kernels is to start at the beginning of a row and count the number of kernels with a given phenotype, record that information, and then return to the beginning of that same row to count the number of kernels with a second phenotype. Continue in this manner until all the phenotypes have been counted before moving on to the next row.
If this is your students' first exposure to calculating chi-square values, you will need to spend some class time explaining this type of statistical analysis.

## Acknowledgements

Vis-à-vis ${ }^{\circledR}$ and EXPO ${ }^{\circledR}$ are registered trademarks of Newell Rubbermaid, Inc.

## Answer Key

## Data and Observations



Figure A. Expected generational crosses

Answer Key (continued)

| Table 2. Phenotypes |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | Purple <br> Smooth | Purple Wrinkled | Yellow <br> Smooth | Yellow Wrinkled |
| Tally Sheet | $\begin{gathered} 25,31,28, \\ 24,19,27, \\ 27,25,21, \\ 20,22,33,22 \end{gathered}$ | $\begin{gathered} 11,10,4, \\ 9,9,5,5, \\ 5,11,7, \\ 6,2,11 \end{gathered}$ | $\begin{gathered} 10,5,9, \\ 10,13,6, \\ 8,9,3, \\ 11,13,6,6 \end{gathered}$ | $\begin{aligned} & 1,3,2, \\ & 4,5,7, \\ & 4,2,4 \\ & 2,2,1,4 \end{aligned}$ |
| Total number observed | 324 | 95 | 109 | 41 |
| Total number expected | $\begin{gathered} (9 / 16) \times 569 \\ =320 \end{gathered}$ | $\begin{gathered} (3 / 16) \times 569 \\ =107 \end{gathered}$ | $\begin{gathered} (3 / 16) \times 569 \\ =107 \end{gathered}$ | $\begin{gathered} (1 / 16) \times 569 \\ =36 \end{gathered}$ |
| Deviation | +4 | -12 | +2 | +5 |

## Conclusion Questions

1. The purple-smooth phenotype has kernels that are deep purple and the kernels appear to be smooth. The genotype is PPSS because it is the dominant genotype.

The yellow-wrinkled phenotype has kernels that are golden yellow and the kernels are
wrinkled. The genotype is ppss because it is the recessive genotype.
2. The phenotypes of purple and smooth are dominant alleles. These are the only phenotypes that appear in the F1 generation.
3. The sample corn cob had 569 kernels on it.

The total number expected for each phenotype is calculated by multiplying the total number of kernels counted on the cob by the expected fraction of each of the phenotypes.

Based on sample data, the numbers support the predicted 9:3:3:1 ratio. Class data can be combined to get more accurate results.
4. $\frac{\left(4^{2}\right)}{320}+\frac{\left(12^{2}\right)}{107}+\frac{\left(2^{2}\right)}{107}+\frac{\left(5^{2}\right)}{36}=2.13$
5. The chi-square value is less than 7.8 , which means that there is a 1 in 5 probability that the deviation observed is due to chance. If the experiment were repeated five times, one could expect the same results obtained here at least once. These are acceptable results even though they do not match the predicted results perfectly.

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## The Amazing Maize Investigating Dihybrid Crosses

Gregor Johan Mendel (1822-1884) was a monk who discovered the fundamental principles of genetic traits. His work was published in 1866 but was not noticed and recognized for its brilliance until the turn of the twentieth century. His work focused on plant breeding projects and, in particular, the garden pea.

Mendel worked with true-breeding strains, with each strain differing from each other in very distinct characteristics. This made it easy to identify the results in his experiments. Peas were a very appropriate choice of organisms for genetic study because Mendel could obtain large numbers of results in a relatively short period of time.

After studying and analyzing monohybrid crosses, Mendel began to study the results of crossing two traits at a time. It is a fortunate coincidence that he selected two traits that happened to be on different chromosomes. Mendel worked with pure-bred peas that were round and yellow and crossed them with pure-bred peas that were wrinkled and green. In the parental cross of round and yellow with that of wrinkled and green, the "P" generation, all of the offspring from the parental cross were yellow and round seeds. This meant that yellow and round alleles were dominant. Mendel then allowed these "F1" offspring, which were heterozygous for both traits, to self-pollinate and produce an "F2" generation. The F2 generation produced seeds that were yellow and round, and seeds that were green and wrinkled.

In the F2 generation, there were new combinations of phenotypes that were not present in either the P or the F 1 generation. Two new phenotypes were produced: yellow and wrinkled peas, and green and round peas. Mendel also found that the peas occurred in specific phenotypic ratios: 9/16 were round and yellow, $3 / 16$ were round and green, $3 / 16$ were wrinkled and yellow and, finally, $1 / 16$ were wrinkled and green (Table 1).

| Table 1. Mendel's Predicted Ratios and Results |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Phenotype of F2 | Ratio <br> Predicted | Number Predicted <br> Out of 556 | Number Actually <br> Observed | Deviation |  |
| Yellow and round | $9 / 16$ | 313 | 315 | 2 |  |
| Yellow and wrinkled | $3 / 16$ | 104 | 101 | 3 |  |
| Green and round | $3 / 16$ | 104 | 108 | 4 |  |
| Green and wrinkled | $1 / 16$ | 35 | 32 | 3 |  |

Mendel explained the results of the F2 generation by proposing that the traits underwent independent assortment. This means that when the F1 parents made gametes, or pollen and eggs, the traits, or genes, for seed coat color and texture segregated independently of one another (Figure 1).
This result is known as Mendel's law of independent assortment. It states that if an organism is heterozygous for two different genes that are unlinked, then those alleles will assort independently of one another during the formation of gametes.


Figure 1. Generational crosses for Mendel's peas

After the rediscovery of Mendel's work in 1900, many biologists began studying genetics. The two organisms of choice were the Drosophila fruit fly and maize, or corn. Barbara McClintock and her colleagues studied corn extensively at Cornell University.
Maize is an excellent choice for genetic study for the very same reasons that Mendel chose peas. There are a number of very distinct traits for the seed or corn kernel, and a large number of offspring are produced in each generation. The large number of offspring in corn makes it even more advantageous than peas. An ear of corn can average 500-600 seeds, whereas pea pods contain only 4 to 6 seeds. It is through McClintock's studies of genetics that transposons or "jumping genes" were discovered. McClintock received a belated Nobel Prize in 1983 for her discovery.
In this activity, you will use corn to examine two unlinked genes. These two genes determine the texture and the color of a corn kernel. The first gene determines whether a kernel of corn is phenotypically smooth or wrinkled. The allele that makes a kernel smooth does so because it stores starch in the seed, whereas the allele the makes a kernel wrinkled stores sugar instead of starch. When the starchy kernels dry out they remain full and plump, whereas the kernels that have a high sugar content wrinkle upon drying.
The second gene to be examined is color. The purple color found in corn is due to the deposition of anthocyanin (plant pigment) in the outer layer of the endosperm, or seed. If the pigment is not made, then the seed will typically have a yellow color.

## Purpose

The purpose of this activity is to illustrate Mendel's law of independent assortment through a dihybrid cross using maize.

## Materials

## Each lab group will need the following:

| calculator | 2 push pins |
| :--- | :---: |
| marker, Vis-à-vis ${ }^{\circledR}$ | corn, F2 ear |

## Procedure

Do not remove any seeds from any ear of corn.

1. Obtain two ears of corn that represent the parental generation. One ear should be purple and smooth, and the other should be yellow and wrinkled. Formulate a hypothesis predicting the phenotypic ratios that would result from a cross between two corn plants that are both heterozygous for purple and smooth. Record a hypothesis on your student answer page.
2. Obtain an F1 ear of corn. By looking at the F1 ear of corn, determine what phenotypes are dominant and what phenotypes are recessive.
3. Write out the genotype using " $P$ " for purple and yellow and " $S$ " for smooth and wrinkled for the $P$ generation and the F 1 generation on your student answer page.
4. Predict the phenotypic ratio in the F2 generation for a cross that is heterozygous for both color of seed coat and texture ( $\mathrm{PpSs} \times \mathrm{PpSs}$ ). Record your predicted genotypes in the F2 Punnett square space on your student answer page.
5. Obtain an F2 ear of corn. Look at the different phenotypes for the kernels of corn, and make sure that you can distinguish among kernels that are purple and smooth, purple and wrinkled, yellow and smooth, and yellow and wrinkled.
6. Put a colored push pin in the end of one row of corn kernels. This will be used to mark your starting position. Count all the kernels that are both purple and smooth in that row. Record that number in the space provided in the data table.
7. Repeat Step 6, this time counting all the kernels that are purple and wrinkled. Be sure to record the number in the space provided in the data table. Do this for the last two phenotypes.
8. Using another colored pin to mark your current position, move one row over and repeat Step 6 through Step 8. After each row is counted, move the row marker to the next row and continue until you return to your starting place.
9. Total the number of seeds found in each phenotype.
10. Calculate the total number expected for each phenotype by multiplying the total number of kernels counted on the cob by the expected fraction of each of the phenotype. For example, if you counted 500 kernels and expect to find $9 / 16$ of them to be purple and smooth, then the expected value for that phenotype would be $500 \times 9 / 16=281$.
11. Determine the deviation for each phenotype.
12. Calculate the chi-square value for your results to determine whether or not the results support your hypothesis.

## Hypothesis

## Data and Observations

Write out a summary of the expected P, F1, and F2 crosses.

P


F1 $\quad \times$ $\qquad$

F2


Figure 2. Expected generational crosses

## Data and Observations (continued)

|  | Table 2. Phenotypes <br> Purple <br> Smooth |  |  | Purple <br> Wrinkled |
| :--- | :---: | :---: | :---: | :---: |
| Tally Sheet |  |  | Yellow <br> Smooth | Yellow <br> Wrinkled |
| Total number <br> observed |  |  |  |  |
| Total number <br> expected |  |  |  |  |
| Deviation |  |  |  |  |

## Conclusion Questions

1. Describe the $P$ generation's phenotype, and write out their genotypes.
2. Which alleles are dominant? How do you know?
3. What is the total number of kernels of corn found on the cob? Explain how the predicted number of kernels for the various phenotypes is determined. How do the observed numbers of various phenotypes differ from the predicted numbers of various phenotypes?
4. Based on your data, determine a chi-square value for the phenotypes produced. Remember that the equation for chi-square is

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

5. What is the probability that your deviation is due to chance?

## Reference

| Table 3. Critical Values of $\chi^{\mathbf{2}}$ |  |  |  |  |  |  |
| :---: | :---: | :---: | ---: | ---: | ---: | :---: |
| Degrees of <br> Freedom | $p=0.9$ <br> (9 in 10) | $p=\mathbf{0 . 5}$ <br> $(\mathbf{1}$ in 2) | $p=\mathbf{0 . 2}$ <br> $(\mathbf{1}$ in 5) | $p=0.05$ <br> $(\mathbf{1}$ in 20) | $p=0.01$ <br> $(\mathbf{1}$ in 100) $)$ | $p=\mathbf{0 . 0 0 1}$ <br> $(\mathbf{1}$ in 1000) |
| 1 | 0.158 | 0.455 | 1.642 | 3.841 | 6.635 | 10.827 |
| 2 | 0.214 | 1.386 | 3.219 | 5.991 | 9.210 | 13.815 |
| 3 | 0.584 | 2.366 | 4.642 | 7.815 | 11.345 | 16.268 |
| 4 | 1.064 | 3.367 | 5.989 | 9.488 | 13.277 | 18.465 |
| 5 | 1.610 | 4.351 | 7.289 | 11.070 | 15.086 | 20.517 |
| 6 | 2.204 | 5.348 | 8.558 | 12.592 | 16.812 | 22.457 |
| 7 | 2.333 | 6.346 | 9.903 | 14.067 | 18.475 | 24.322 |
| 8 | 3.490 | 7.344 | 11.303 | 15.507 | 20.090 | 26.125 |
| 9 | 4.168 | 8.343 | 12.242 | 16.919 | 21.660 | 27.877 |
| 10 | 4.865 | 9.342 | 13.442 | 18.307 | 23.209 | 29.588 |

The value $p$ is the probability that the results could be due to chance alone. The numbers in parentheses below each value of $p$ restate $p$ in terms of chance, e.g., a 9 in 10 likelihood that the results could be due to chance alone.

