Overview
One form of genetic modification that has been used for centuries is artificial selection, or selective breeding. This is the selection of individuals or populations possessing desirable traits to produce the next generation. This process has given rise to many of the plants and animals we encounter every day, including: different breeds of dogs, some good at hunting and retrieving game and others that are specifically bred for companionship and living in a house; and different breeds of cattle, some bred for milk production and others bred specifically for beef. Corn has been developed and is constantly being improved to produce higher yields in drought conditions, resistance to pests, and other advantageous characteristics through this process. Artificial selection is also important in that it played an important role in the development of the theory of natural selection.

An understanding of Mendelian inheritance and statistical analysis of the results of crosses is important in determining the genes of an organism and the ability to produce offspring with the desired traits.

In this lab, students will observe ears of corn from F2 plants produced by a dihybrid cross for the traits of kernel color and endosperm composition, which changes the shape of the kernel. The color blue is dominant to yellow and the starchy nonwrinkled endosperm is dominant to a sugary wrinkled endosperm. Students will hypothesize the genotypes of the parents (F1 generation). First looking at each trait independently, and then looking at the independent assortment or linkage between the two traits. These hypotheses will be used to make predictions that will be analyzed by collecting data and applying a chi-square test.

Kansas College and Career Ready Standards

Science

- **HS-LS3-2. Heredity: Inheritance and Variation of Traits**
  Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

- **HS-LS3-3. Heredity: Inheritance and Variation of Traits**
  Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

- **HS-LS4-3. Biological Evolution: Unity and Diversity**
  Apply concepts of statistics and probability to support explanations that organisms with an advantageous heritable trait tend to increase in proportion to organisms lacking this trait.
Kansas Corn: Corn Genetics and Statistical Analysis
Grade Level: High School

Math

- **S.ID.** Interpreting Categorical and Quantitative Data
  a. Summarize, represent, and interpret data on a single count or measurement variable.
  b. Summarize, represent, and interpret data on two categorical and quantitative variables.
- **S.IC.** Making Inferences and Justifying Conclusions
  a. Understand and evaluate random processes underlying statistical experiments.
  b. Make inferences and justify conclusions from sample surveys, experiments, and observational studies.
- **S.CP.** Conditional Probability and the Rules of Probability
  a. Understand independence and conditional probability and use them to interpret data.
  b. Use the rules of probability to compute probabilities of compound events in a uniform probability model.

Learning Objectives

- Students will develop a hypothesis of the genotypes of the parents in monohybrid and dihybrid crosses.
- Students will predict the outcome of their hypothesis using knowledge of inheritance and probability.
- Students will quantify the results of monohybrid and dihybrid crosses and compare the results with their prediction by performing chi-square analysis.

Materials

- Corn Genetics and Statistical Analysis PowerPoint (available at www.kscorn.com)
- Dihybrid Corn Ears from Carolina Biological, R and Su Alleles 9:3:3:1 (item #176600) one for each group
- Corn Genetics Student Handout (pg. S1-4 or available at www.kscorn.com)
- Transparency markers

Safety Considerations

Students should be cautioned to be careful when handling ears of corn. If handled with care, ears may last many years, but may be damaged if handled roughly. Kernels may occasionally fall off of the ears, but may be glued back into place.
Procedures for Instruction

Length of Time for Preparation: 15 minutes
Length of Time for Classroom Teaching: 2 hours

Preparation Procedure Lab Set-up
1. Group or pair students so that there are enough materials for each group.
2. Each lab group will need one dihybrid ear and a transparency marker.
3. Each member of the group will need a student handout.

This lab is recommended to follow the teaching of Mendelian inheritance as students will need basic knowledge of inheritance and probability as well as the vocabulary associated with this subject and experimental procedures.

Students should be familiar with the following terms: allele, gene, hybrid, dihybrid, purebred, P generation, F1 generation, F2 generation, homozygous, heterozygous, dominant, recessive, independent assortment, and linked genes.

Students should also be able to predict ratios of offspring from monohybrid and dihybrid crosses using Punnett squares and/or the rules of probability.

Background Information

A chi-square test is used to evaluate the proposed genotypes of the parent generation that produced the observable offspring. The proposed genotype is called the null hypothesis. Probability is used to predict the number of individuals with different traits that would be expected in the next generation if the hypothesis is correct. The equation for performing a chi-square test is shown in Figure 1.

Figure 1.

\[
\chi^2 = \sum \frac{(O - E)^2}{E}
\]

The chi-square value is compared with a critical value shown on the table in Figure 2.
Figure 2.

<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>Probability of exceeding critical value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.10 or 10%</td>
</tr>
<tr>
<td>1</td>
<td>2.706</td>
</tr>
<tr>
<td>2</td>
<td>4.605</td>
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<tr>
<td>3</td>
<td>6.251</td>
</tr>
<tr>
<td>4</td>
<td>7.779</td>
</tr>
</tbody>
</table>

Classroom Discussion

Introduce the topic and assess students for prior understanding.

- Have students observe ears of corn and record observations.
- Have each group report one observation to be recorded collaboratively as a class.
- Students should report that there are wrinkled and not wrinkled kernels as well as yellow and blue kernels.

Let students discuss their ideas, and guide the discussion without telling them if they are right or wrong.

- What characteristics do you see that are different from what you are used to seeing?
- How many different types of kernels do you see?
- Which traits seem to be more common and less common?
- Which allele for each trait do you think would be dominant or recessive? Why do you think so?
- If this is the F2 generation, what do you think the genotypes of the F1 generation would most likely be for color and smoothness, respectively?
- If your answer is correct, what ratios for each phenotype should be observed?
- Do the traits appear to be linked or inherited independently?
- What traits might corn producers want to select for when producing new varieties?
Kansas Corn: Corn Genetics and Statistical Analysis
Grade Level: High School

Procedure for Lab

Guided Introduction Monohybrid
1. Instruct students to focus on the trait of kernel color.
2. Have them record which allele, either blue or yellow, that they infer is the dominant and recessive allele.
3. Instruct students to form a hypothesis of the parents’ (F1 generation) genotypes that would produce the ear they observed in the ears (F2 generation).
4. Have them predict the ratio of phenotypes that this cross would produce.
5. Students then need to determine the number out of 100 that should express each phenotype.
6. Students should then select 4 or 5 rows to count 100 kernels, recording the number of each phenotype.
7. Guide the class through a chi-square analysis of their data to determine if their prediction and hypothesis used to make it is statistically reasonable.
8. If the chi-square test suggests a new hypothesis, have the students come up with a new one, predict and run a chi-square test to see how it fits.
9. Have each group report their hypothesis, prediction and chi-square results.

Smooth vs. Round
10. Instruct students to repeat the above procedure focusing on the wrinkled and not wrinkled kernel trait.
11. If the chi-square test suggests a new hypothesis, have the students come up with a new one, predict and run a chi-square test to see how it fits.
12. Have each group report out their hypothesis, prediction and chi-square results.

Dihybrid Cross
13. Now, ask students to determine if they predict the traits are inherited separately or if they might be linked or influencing each other in some way.
14. As a class, determine how the degrees of freedom will be different in this chi-square test. With four possible phenotypes, blue smooth, blue shriveled, yellow smooth, and yellow shriveled, there are now 3 degrees of freedom.
15. If the chi-square test suggests a new hypothesis, have the students come up with a new one, predict and run a chi-square test to see how it fits.
16. Have each group report their hypothesis, prediction and chi-square results.
Reflection and Conclusion
A lab group received an F2 ear of corn that appeared to have equal numbers of blue and yellow kernels.

1. What F1 genotypes would most likely produce this ear of corn?
   - Because blue is dominant, the convention would be to use B to represent the blue allele and b to represent yellow.
   - Heterozygous x homozygous recessive (Bb x bb) would be most likely to produce the colors seen on this ear of corn.

2. If this hypothesis is correct, how many kernels out of 100 should be blue? How many should be yellow?

   $\frac{1}{2} \times 100 = 50$ blue and $\frac{1}{2} \times 100 = 50$ yellow

3. If the kernels were counted and 44 were yellow and 56 were blue, conduct a chi-square test on your hypothesis.

\[
x^2 = \sum \frac{(O - E)^2}{E}
\]

\[
x^2 = \sum \frac{(44 - 50)^2}{50} + \frac{(56 - 50)^2}{50}
\]

\[
x^2 = \sum \frac{(-6)^2}{50} + \frac{(6)^2}{50}
\]

\[
x^2 = \sum \frac{36}{50} + \frac{36}{50}
\]

\[
x^2 = \sum 0.72 + 0.72
\]

\[
x^2 = 1.44
\]

There are only two possibilities for kernel color, meaning there is 1 degree of freedom.

The agreed upon percentage of a Type I error is 5%, giving a critical value for this test of 3.841.

\[
x^2 = 1.44 < 3.841
\]

Thus we fail to reject the null hypothesis.
Kansas Corn: Corn Genetics and Statistical Analysis
Grade Level: High School

Assessment
1. In performing a chi-square test for pest resistance that is found to be expressed in three forms, completely resistant, somewhat resistant, and not resistant, how many degrees of freedom should be used?

   3 possibilities - 1 = 2 degrees of freedom

2. If a homozygous blue corn plant, BB, was crossed with a homozygous yellow plant, bb, what should be the predicted numbers of blue and yellow kernels if 100 kernels were counted on an ear of the plant produced?

   All offspring would be heterozygous Bb, having blue kernels
   100 blue, 0 yellow

3. A lab group in another section hypothesized the parents were heterozygous for both kernel color and shape, BbSs. When they counted the kernels, they recorded the following results. Calculate the expected values for their hypothesis and perform a chi-square test on your hypothesis.

<table>
<thead>
<tr>
<th></th>
<th>Blue and Smooth</th>
<th>Blue and Shriveled</th>
<th>Yellow and Smooth</th>
<th>Yellow and Shriveled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>53</td>
<td>20</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Expected</td>
<td>56</td>
<td>19</td>
<td>19</td>
<td>6</td>
</tr>
</tbody>
</table>

\[
x^2 = \sum \frac{(53 - 56)^2}{56} + \frac{(20 - 19)^2}{19} + \frac{(22 - 19)^2}{19} + \frac{(5 - 6)^2}{6}
\]

\[
x^2 = \sum \frac{(-3)^2}{56} + \frac{(1)^2}{19} + \frac{(3)^2}{19} + \frac{(-1)^2}{6}
\]

\[
x^2 = \sum \frac{9}{56} + \frac{1}{19} + \frac{9}{19} + \frac{1}{6}
\]

\[
x^2 = \sum 0.1607 + 0.0526 + 0.4737 + 0.1667 = 0.8537
\]

Critical value for 3 degrees of freedom, and 5% probability = 7.815, so we fail to reject the null hypothesis.
Kansas Corn: Corn Genetics and Statistical Analysis
Grade Level: High School

Science and Agriculture Careers
To learn more about agriculture careers, visit www.agexplorer.com. You can also find career profiles at www.kscorn.com.

Sources
- https://newonlinecourses.science.psu.edu/stat414/node/147/
- https://www.biologycorner.com/worksheets/corn_chisq.html

Any educator electing to perform demonstrations is expected to follow NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops, which are available at http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are under a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.
Mendelian Inheritance and Chi-Square Analysis in Corn

A chi-square is a statistical test to determine how well the actual data fit a predicted outcome. If the chi-square test fits then the prediction is considered accurate enough that the hypothesis cannot be rejected. Observe the corn cob pictured below.

We will first look at the trait of kernel color.

Which trait do you think might be dominant? _____________, ... recessive ________________?

What cross do you think might have produced this corn cob? ____ x _____

(This is your null hypothesis)

If that is correct, what would be the probability of the kernels being blue? _____, ... yellow? _____

I randomly selected 100 kernels in the image below. If your null hypothesis is correct, how many kernels would be expected to be blue? ______, ... yellow? ______ (Write these numbers in the table below.)

<table>
<thead>
<tr>
<th></th>
<th>Blue</th>
<th>Yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected</td>
<td></td>
<td></td>
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</tbody>
</table>

Now, we will count the number of blue and yellow kernels and see how well they fit our prediction.
A chi-square test is used to determine the percent chance that the observed difference between the predicted and observed value is due to chance alone. The agreed upon limit for this value is 0.05 or 5%.

\[ x^2 = \sum \frac{(O - E)^2}{E} \]

Degrees of freedom – the number of possible outcomes – 1
In this cross, the kernels are either blue or yellow. 2 outcomes – 1 = 1 degree of freedom

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Do we reject the hypothesis? ________

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<tr>
<td>Observed</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>Expected</td>
<td>75</td>
<td>25</td>
</tr>
</tbody>
</table>
Now using an actual corncob, run a chi-square test for the same kernel color trait.

Hypothesis for parent genotypes: _____ x _____

Probability for prediction is blue _____, yellow _____.

Count 3 or 4 rows of kernels, recording the number of blue and yellow.

Multiply the total number counted by the predicted probability for each trait.

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![Chi-square formula](image)

Chi-square value  
\[ \chi^2 = \sum \frac{(O - E)^2}{E} \]

Perform a chi-square test.

Chi-square critical value = __________

Was your hypothesis rejected?
Dihybrid cross

You have probably noticed that the kernels also have a variety of textures, some being smooth while others are wrinkled. This is also controlled by a single gene (S) for starchy smooth or shriveled sugary endosperm. Observe your corncob and come up with a hypothesis for the genotypes of each parent for both kernel color and texture genes. ______ x _______ (Example BbSs x BbSs)

Using your hypothesis, determine the probability for each of the following trait combinations.

Blue and Smooth ______

Blue and Shriveled______

Yellow and Smooth ______

Yellow and Shriveled_____

Count 4 different rows of kernels, and record the number of each of the trait combinations.

Multiply the total number of kernels counted to determine the expected number of each phenotype.

How many degrees of freedom are there in this test?

<table>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expected</strong></td>
<td></td>
<td></td>
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Perform a chi-square test on the data above. There will be two more data sets to test.

Chi-square critical value = ___________ Degrees of freedom= ________

Does your data support your hypothesis? ________

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Chi-square value  
\[
\chi^2 = \sum \frac{(O - E)^2}{E}
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