

## Mendelian Inheritance in Corn

The study of genetics and inheritance is concerned with understanding the biological properties that are transmitted from parent to offspring. With knowledge of inheritance, plants and animals have been developed that are more productive and, in some cases, have more nutrients. Potential parents can now receive counseling regarding many human inheritable diseases. The timber industry can grow trees that yield more lumber in fewer growing seasons. Through genetic engineering bacteria can synthesize insulin and other human proteins.

In this laboratory you will study the results of some inheritance crosses similar to those performed by Mendel in his classical experiments on heredity in corn seed color and seed shape. In addition to studying the information about such inheritance patterns you will learn how to apply a statistical test to determine the validity of data obtained by such crosses.

### Exercise 1 - The Monohybrid Cross and Chi-Square Test

#### Materials Needed

Several ears of Monohybrid 3:1 corn (maize)

In a monohybrid cross, one pair of alleles is studied. If both parents are homozygous, with one parent homozygous dominant and the other parent homozygous recessive, the first generation will be heterozygous exhibiting the dominant phenotype. As Mendel demonstrated, 75% of the second generation ( $F_2$ ) offspring will have the dominant phenotype and 25% the recessive phenotype (a 3:1 ratio). This prediction can be validated using the chi-square statistical test.

In corn (*Zea mays*), for our purposes, the inheritance of the seed (kernel) color can be considered to be a single gene, with one pair of alleles (purple vs. yellow). The purple color allele is dominant, and the yellow allele is recessive. *Note: For genetic accuracy, the color-determining pigments are found in the aleurone coating of the endosperm, which can be colorless, purple, or red, and in the endosperm proper, which is yellow or white. Color production is subject to epistasis and modification by other genes.*

In this exercise you will perform a chi-square test on the second-generation offspring of a monohybrid cross.



Homozygous Dominant Parent



Homozygous Recessive Parent



Second Generation ( $f_2$ ) Offspring

#### Procedure

1. Obtain one (1) ear of monohybrid corn per student pair.
2. Count all the purple seeds (kernels) and all the yellow seeds on your ear of corn.
3. Record the numbers of purple and yellow seeds and the total number of seeds of your ear of corn on the Table 1 below. Also record your totals on the classroom whiteboard.

**Table 1 – Number of Seeds Counted for Monohybrid Cross**

	# Purple Seeds	# Yellow Seeds	Total # Seeds
Your ear of corn			
Class Total			

N = \_\_\_\_\_ (total number of ears of corn counted by the class).

What is your inheritance ratio for seed color in corn for this cross? \_\_\_\_\_.

4. You can now test the validity of your data using the chi-square test. The chi-square is a relatively simple mathematical calculation that can determine how well the observed experimental results (the actual count) correspond to the theoretical prediction (the expected results). The formula for the chi-square calculation is:

$$\chi^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2} =$$

$\chi^2$  = the symbol for the term, chi-square

o = observed count (number) ( $o_1$  = the purple seeds,  $o_2$  = yellow seeds)

e = the expected number according to the predicted ratio. Both  $e_1$  and  $e_2$  must be calculated for your particular sample.

5. To calculate the expected numbers ( $e_1$  and  $e_2$ ) for your monohybrid cross:
- You expect 3/4 of your sample (total # of seeds counted) to be purple.  
 $e_1$  = your sample (total #) multiplied by 3/4 (or 0.75) = \_\_\_\_\_  
 (Round off to the nearest whole number.)
  - You expect 1/4 of your sample (total #) to be yellow.  
 $e_2$  = your sample (total #) multiplied by 1/4 (or 0.25) = \_\_\_\_\_  
 (Round off to the nearest whole number.)
6. Once you have calculated your expected numbers ( $e_1$  and  $e_2$ ) for your corn seed sample, put your numbers in the chi-square formula and perform the calculations. **The chi square test instructions and table are at the end of this laboratory exercise.**
7. Record your Chi Square calculations, results and analysis on the Results and Analysis sheet provided below.

## Exercise 2 - The Dihybrid Cross and Chi-Square Test

### Materials Needed

Several ears of corn (maize) with 9:3:3:1 dihybrid cross.

Two pairs of alleles are studied in a dihybrid cross. If both parents are homozygous, with one parent homozygous dominant for both traits and the other parent homozygous recessive for both traits, the first generation will be heterozygous expressing the dominant phenotype for both traits. As Mendel demonstrated, there will be four different phenotypes in the second generation yielding a 9:3:3:1 ratio (9/16 with both dominant traits, 3/16 with one dominant and one recessive trait, 3/16 with the alternative dominant and recessive trait and 1/16 with both recessive traits). This predictions can be validated using the chi-square statistical test.

In corn (*Zea mays*), the inheritance of the seed color is on single gene, with one pair of alleles (purple vs. yellow). Seed shape (smooth vs. wrinkled) is on a second gene. Purple color is dominant and the yellow is recessive. Smooth textured seed shape is a dominant allele, and a wrinkled kernel shape is recessive. *Note: For genetic accuracy, smooth kernels have an endosperm rich in starch, which is inert. The wrinkled or shrunken kernels have a sugar-rich endosperm that loses water as the corn dries, resulting in the wrinkled appearance of the corn.*

In this exercise you will perform a chi-square test on the second-generation offspring of a dihybrid cross with 9:3:3:1 phenotypic ratio.



Dihybrid Cross

**Procedure – Dihybrid Corn with dominant and recessive alleles**

1. Obtain one (1) ear of dihybrid corn with a 9:3:3:1 ratio per group of 4 students.
2. Count all the purple/smooth, purple/wrinkled, yellow/smooth and yellow/wrinkled seeds on your corn.
3. Record the numbers of purple/smooth, purple/wrinkled, yellow/smooth and yellow/wrinkled seeds and the total number of seeds in each sample on Table 2 below. Also record your totals on the classroom whiteboard.

**Table 2 - Number of Seeds Counted for Dhybrid Cross**

<b>9:3:3:1 Corn</b>	# Purple/Smooth Seeds	# Purple/Wrinkled Seeds	# Yellow/Smooth Seeds	# Yellow/Wrinkled Seeds	Total # Seeds
Your ear of corn					
Class Total					

N = \_\_\_\_\_ (total number of ears of corn counted by the class).

What is your inheritance ratio for seed color and shape in corn for this cross? \_\_\_\_\_.

4. You can now test the validity of your data using the chi-square test. The chi-square is a relatively simple mathematical calculation which can determine how well the observed experimental results (the actual count) correspond to the theoretical prediction (the expected results). The formula for the chi-square calculation is:

$$\chi^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2} + \frac{(o_3 - e_3)^2}{e_3} + \frac{(o_4 - e_4)^2}{e_4} =$$

$\chi^2$  = the symbol for the term, chi-square

o = observed count (number) (o<sub>1</sub> = the purple/smooth, o<sub>2</sub> = purple/wrinkled, o<sub>3</sub> = yellow/smooth, and o<sub>4</sub> = yellow/wrinkled seeds)

e = the expected number according to the predicted ratio. All e<sub>1</sub>, e<sub>2</sub>, e<sub>3</sub>, and e<sub>4</sub> must be calculated for your particular sample.

5. To calculate the expected numbers (e<sub>1</sub>, e<sub>2</sub>, e<sub>3</sub>, and e<sub>4</sub>) for your **9:3:3:1 dihybrid cross**:
  - a. You expect 9/16 of your sample (total #) to be purple/smooth.  
e<sub>1</sub> = our sample (total #) multiplied by 9/16 (or 0.5625) = \_\_\_\_\_  
(Round off to the nearest whole number.)
  - b. You expect 3/16 of your sample (total #) to be purple/wrinkled.  
e<sub>2</sub> = your sample (total #) multiplied by 3/16 (or 0.1875) = \_\_\_\_\_  
(Round off to the nearest whole number.)
  - c. You expect 3/16 of your sample (total #) to be yellow/smooth.  
e<sub>3</sub> = our sample (total #) multiplied by 3/16 (or 0.1875) = \_\_\_\_\_  
(Round off to the nearest whole number.)
  - d. You expect 1/16 of your sample (total #) to be yellow/wrinkled.  
e<sub>4</sub> = our sample (total #) multiplied by 1/16 (or 0.0625) = \_\_\_\_\_  
(Round off to the nearest whole number.)
6. Once you have calculated your expected numbers (e<sub>1</sub> and e<sub>2</sub>) for your corn seed sample, put your numbers in the chi-square formula and perform the calculations. **The chi square test instructions and table are at the end of this laboratory exercise.**
7. Record your Chi Square calculations, results and analysis on the Results and Analysis sheet provided below.

### Exercise 3 - The Dihybrid Test Cross and Chi-Square Test

#### Materials Needed

Several ears of corn (maize) with 1:1:1:1 dihybrid test cross.

Two pairs of alleles are studied in a dihybrid test cross. When both parents are homozygous, with one parent homozygous dominant for both traits and the other parent homozygous recessive for both traits, the first generation will be heterozygous expressing the dominant phenotype for both traits. In the dihybrid test cross, the first generation, which exhibits the dominant phenotype for both traits, is crossed with the recessive parent. As Mendel demonstrated, there will be four different phenotypes expressed in the test cross offspring in equal proportions: (1/4 with both dominant traits, 1/4 with one dominant and one recessive trait, 1/4 with the alternative dominant and recessive trait and 1/4 with both recessive traits). The predicted ratio for a dihybrid test cross is 1:1:1:1. These predictions can be validated using the chi-square statistical test.

In corn (*Zea mays*), the inheritance of the seed color is on single gene, with one pair of alleles (purple vs. yellow). Seed shape (smooth vs. wrinkled) is on a second gene. Purple color is dominant and the yellow is recessive. Smooth textured seed shape is a dominant allele, and a wrinkled kernel shape is recessive. *Note: For genetic accuracy, smooth kernels have an endosperm rich in starch, which is inert. The wrinkled or shrunken kernels have a sugar-rich endosperm that loses water as the corn dries, resulting in the wrinkled appearance of the corn.*

In this exercise you will perform a chi-square test on a dihybrid test cross with a 1:1:1:1 phenotypic ratio.



Dihybrid Test Cross

#### Procedure – Dihybrid Test Cross

1. Obtain one (1) ear of dihybrid test cross corn with a 1:1:1:1 ratio per group of 4 students.
2. Count all the purple/smooth, purple/wrinkled, yellow/smooth and yellow/wrinkled seeds on your corn..
3. Record the numbers of purple/smooth, purple/wrinkled, yellow/smooth and yellow/wrinkled seeds and the total number of seeds in each sample on Table 3 below. Also record your totals on the classroom whiteboard.

**Table 3 – Number of Seeds Counted for Dihybrid Test Cross**

<b>1:1:1:1 Corn</b>	# Purple/Smooth Seeds	# Purple/Wrinkled Seeds	# Yellow/Smooth Seeds	# Yellow/Wrinkled Seeds	Total # Seeds
Your ear of corn					
Class Total					

N = \_\_\_\_\_ (total number of ears of corn counted by the class).

4. You can now test the validity of your data using the chi-square test. The chi-square is a relatively simple mathematical calculation which can determine how well the observed experimental results (the actual count) correspond to the theoretical prediction (the expected results). The formula for the chi-square calculation is:

$$\chi^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2} + \frac{(o_3 - e_3)^2}{e_3} + \frac{(o_4 - e_4)^2}{e_4} =$$

$\chi^2$  = the symbol for the term, chi-square

o = observed count (number) ( $o_1$  = the purple/smooth,  $o_2$  = purple/wrinkled,  $o_3$  = yellow/smooth, and  $o_4$  = yellow/wrinkled seeds)

e = the expected number according to the predicted ratio. All  $e_1$ ,  $e_2$ ,  $e_3$ , and  $e_4$  must be calculated for your particular sample.

What is your inheritance ratio for seed color and shape in corn for this cross? \_\_\_\_\_.

5. To calculate the expected numbers ( $e_1$ ,  $e_2$ ,  $e_3$ , and  $e_4$ ) for your **1:1:1:1 dihybrid cross**:
- You expect 1/4 of your sample (total #) to be purple/smooth.  
 $e_1$  = our sample (total #) multiplied by 1/4 (or 0.25) = \_\_\_\_\_  
 (Round off to the nearest whole number.)
  - You expect 1/4 of your sample (total #) to be purple/wrinkled.  
 $e_2$  = your sample (total #) multiplied by 1/4 (or 0.25) = \_\_\_\_\_  
 (Round off to the nearest whole number.)
  - You expect 1/4 of your sample (total #) to be yellow/smooth.  
 $e_3$  = our sample (total #) multiplied by 1/4 (or 0.25) = \_\_\_\_\_  
 (Round off to the nearest whole number.)
  - You expect 1/4 of your sample (total #) to be yellow/wrinkled.  
 $e_4$  = our sample (total #) multiplied by 1/4 (or 0.25) = \_\_\_\_\_  
 (Round off to the nearest whole number.)
6. Once you have calculated your expected numbers ( $e_1$ ,  $e_2$ ,  $e_3$ , and  $e_4$ ) for your corn seed sample, put your numbers in the chi-square formula and perform the calculations. **The chi square test instructions and table are at the end of this laboratory exercise.**
7. Record your Chi Square calculations, results and analysis on the Results and Analysis sheet provided below.

## Corn Genetics Results and Analysis

Name \_\_\_\_\_

Section \_\_\_\_\_

*Note: Include all of your math for the chi square calculations and your data from Tables 1, 2 and 3. Attach a separate sheet if necessary.*

### Exercise I – Monohybrid Cross

**Table 1 – Number of Seeds Counted for Monohybrid Cross**

	# Purple Seeds	# Yellow Seeds	Total # Seeds
Your ear of corn			
Class Total			

#### Individual Corn

What is the inheritance ratio for seed color for your individual ear of corn for this cross? \_\_\_\_\_.

1. Calculate the expected numbers ( $e_1$  and  $e_2$ ) for your ear of corn.
  - a.  $e_1 =$  \_\_\_\_\_ (Show your work.)
  - b.  $e_2 =$  \_\_\_\_\_ (Show your work.)
2. Determine the chi square number.  $\chi^2 =$  \_\_\_\_\_ (Show your work.)

Is the observation of the seed data significant (accept or reject your hypothesis) as indicated by the Chi-square? \_\_\_\_\_

#### Class Data

What is the inheritance ratio for seed color for class data for the monohybrid cross? \_\_\_\_\_.

1. Calculate the expected numbers ( $e_1$  and  $e_2$ ) for the class data.
  - a.  $e_1 =$  \_\_\_\_\_ (Show your work.)
  - b.  $e_2 =$  \_\_\_\_\_ (Show your work.)
2. Determine the chi square number.  $\chi^2 =$  \_\_\_\_\_ (Show your work.)

Is the observation of the seed data significant (accept or reject your hypothesis) as indicated by the Chi-square? \_\_\_\_\_

### Exercise 2 – Dihybrid Cross

**Table 2 - Number of Seeds Counted for Dhybrid Cross**

9:3:3:1 Corn	# Purple/Smooth Seeds	# Purple/Wrinkled Seeds	# Yellow/Smooth Seeds	# Yellow/Wrinkled Seeds	Total # Seeds
Your ear of corn					
Class Total					

#### Individual Corn

What is the inheritance ratio for seed color and seed shape for your individual ear of corn for the dihybrid cross? \_\_\_\_\_.

1. Calculate the expected numbers ( $e_1$ ,  $e_2$ ,  $e_3$  and  $e_4$ ) for your ear of corn.
  - a.  $e_1 =$  \_\_\_\_\_ (Show your work.)
  - b.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - c.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - d.  $e_2 =$  \_\_\_\_\_ (Show your work.)
2. Determine the chi square number.  $\chi^2 =$  \_\_\_\_\_ (Show your work.)

Is the observation of the seed data significant (accept or reject your hypothesis) as indicated by the Chi-square? \_\_\_\_\_

**Class Data**

What is the inheritance ratio for seed color and seed shape for the class data for the dihybrid corn for this cross? \_\_\_\_\_.

1. Calculate the expected numbers ( $e_1$ ,  $e_2$ ,  $e_3$  and  $e_4$ ) for your ear of corn.
  - a.  $e_1 =$  \_\_\_\_\_ (Show your work.)
  - b.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - c.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - d.  $e_2 =$  \_\_\_\_\_ (Show your work.)
2. Determine the chi square number.  $\chi^2 =$  \_\_\_\_\_ (Show your work.)

Is the observation of the seed data significant (accept or reject your hypothesis) as indicated by the Chi-square? \_\_\_\_\_

**Exercise 3 – Dihybrid Test Cross****Table 3 – Number of Seeds Counted for Dihybrid Test Cross**

<i>1:1:1:1 Corn</i>	# Purple/Smooth Seeds	# Purple/Wrinkled Seeds	# Yellow/Smooth Seeds	# Yellow/Wrinkled Seeds	Total # Seeds
Your ear of corn					
Class Total					

**Individual Corn**

What is the inheritance ratio for seed color and seed shape for your individual ear of corn for the dihybrid test cross? \_\_\_\_\_.

1. Calculate the expected numbers ( $e_1$ ,  $e_2$ ,  $e_3$  and  $e_4$ ) for your ear of corn.
  - a.  $e_1 =$  \_\_\_\_\_ (Show your work.)
  - b.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - c.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - d.  $e_2 =$  \_\_\_\_\_ (Show your work.)
2. Determine the chi square number.  $\chi^2 =$  \_\_\_\_\_ (Show your work.)

Is the observation of the seed data significant (accept or reject your hypothesis) as indicated by the Chi-square? \_\_\_\_\_

**Class Data**

What is the inheritance ratio for seed color and seed shape for the class data for the dihybrid test cross in corn? \_\_\_\_\_.

1. Calculate the expected numbers ( $e_1$ ,  $e_2$ ,  $e_3$  and  $e_4$ ) for your ear of corn.
  - a.  $e_1 =$  \_\_\_\_\_ (Show your work.)
  - b.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - c.  $e_2 =$  \_\_\_\_\_ (Show your work.)
  - d.  $e_2 =$  \_\_\_\_\_ (Show your work.)
2. Determine the chi square number.  $\chi^2 =$  \_\_\_\_\_ (Show your work.)

Is the observation of the seed data significant (accept or reject your hypothesis) as indicated by the Chi-square? \_\_\_\_\_

## Chi-Square Test

Any population contains all possible values of a variable, and ideally we would measure those values for the entire population. Usually, however, this is not possible so we sample some subset of the population, and draw inferences from the data. We then often need to test that the differences we see are part of the normal variation for the population or are statistically significant.

Chi-square is a statistical test commonly used to compare observed data with the expected data we would get if our hypothesis is correct. For example, if you expect 5 out of 10 offspring to have the recessive phenotype in a cross of a homozygous recessive with a heterozygote and you actually observe 6, it would be useful to be able to decide whether or not your data refutes or supports your hypothesis. In other words, were the differences between observed and predicted the result of chance or due to some other process? The chi-square test can help us decide. We use the chi-square test to test the "Null" hypothesis that states "there is no significant difference between the observed and expected data".

The chi-square value is a mathematical comparison of the data obtained in an experiment (our observed values) and the expected values. The chi-square value represents the degree to which the data fit the hypothesis on which the experiment was based. If chi-square is small, the data fit the hypothesis. If chi-square is large, the hypothesis is in doubt.

The formula for chi-square ( $\chi^2$ ) is:

$$\chi^2 = \sum(o-e)^2 / e$$

This means chi-square is the sum of the squares of the difference of observed less expected divided by the expected data.

In the example above, we have ten offspring and six of them are of the recessive phenotype. Calculate the chi-square as done in the following table:

	Recessive	Dominant
Observed (o)	6	4
Expected (e)	5	5
Deviation (o-e)	1	-1
Deviation squared (d <sup>2</sup> )	1	1
d <sup>2</sup> /e	1/5 = 0.2	1/5 = 0.2
$\chi^2 = \sum(o-e)^2 / e = 0.4$		

Determine the degrees of freedom (df) in your experiment. Degrees of freedom are the number of categories in the problem minus one. For this example there are two categories (recessive and dominant) so our degrees of freedom is 2 - 1 = 1.

Now decide on a relative standard for accepting or rejecting your Null hypothesis. In biological research it is common to use  $p < 0.05$  (or 5%) as the relative standard. The P value is the probability of rejecting the null hypothesis i.e. that there is no difference between the observed and expected values, and any deviation is due to chance alone. The lower the probability the less safe we are in accepting that the difference is due to chance alone, so we reject the null hypothesis.

In our example,  $\chi^2 = 0.4$ , read df = 1 line, this value lies between probability 0.70 and 0.50 - not significant, indicating that the deviation is most likely due to chance.

If your results are not significant, that doesn't mean they are insignificant. Assuming your experimental design was valid, you may not have disproved the null hypothesis but you have still obtained valid experimental results.

### Limitations of Chi-square

The chi-square test can only be applied to numerical values, not to percentages. If data is in the form of percentages you need to convert it to numerical data.

The chi-square test is not reliable if the expected value in any category is less than 5. Usually this means the sample size should be greater than 25.

### Chi-Square Distribution

Degrees of Freedom (df)	Probability (p)										
	0.95	0.90	0.85	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59
	<b>Not significant</b> Chi-square value is consistent with hypothesis								<b>Significant</b> Chi-square value is not consistent with hypothesis		

Materials for this lab were provided by Bellevue College Life Science Instructor Al Farrand. The Genetic Corn samples used in this exercise and Illustrations are from Carolina Biological Supply Company.