

Using an Investigative Approach to a Laboratory Exercise on the Genetics of Indian Corn

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Objectives

1. Understand Mendel's first and second laws of inheritance.
2. Observe examples of Mendel's monohybrid and dihybrid crosses and perform a chi-square test on his results.
3. Be able to recognize a monohybrid cross and a dihybrid cross.
4. Know the function of a test cross and be able to recognize a test cross.
5. State a hypothesis for the genotype of an ear of Indian corn. Test the hypothesis and perform a chi-square test on your results.

1. Mendel's Monohybrid Cross

Observation: When Mendel crossed red-flowered pea plants with white-flowered pea plants, the F_1 generation was all red-flowered. When plants from the F_1 generation were crossed with each other, the white-flowered plants were one-fourth of the F_2 generation.

Problem: What can explain the observation?

Hypothesis: Mendel's hypothesis was the basis for his first law, the law of segregation. Each pea plant carries pairs of alleles for flower color. The P (parental) generation red plants have two red alleles and the white plants have two white alleles. The alleles segregate when gametes are formed so offspring receive one allele from each parent. The allele for red flowers (A) is dominant over the allele for white flowers (a). Therefore, the F_1 generation plants (Aa) will all have red flowers. Crossing the F_1 generation with itself will yield offspring that are $3/4$ red-flowered and $1/4$ white-flowered.

Prediction: If a true breeding red flowered pea plant is crossed with a white flowered pea plant, then all offspring will be red flowered. If these red flowered offspring pea plants are self-crossed, then $3/4$ of the offspring will be red flowered and $1/4$ will be white flowered.

Experiment

P generation: Cross red-flowered plants with white-flowered plants.

F₁ (filial) generation: All plants have red flowers. Cross the F₁ generation with itself.

F₂ generation: 705 plants have red flowers and 224 have white flowers.

Conclusion: Mendel's hypothesis states that the ratio of plants in the F₂ generation will be 3/4 red-flowered to 1/4 white-flowered. Are his results close enough to the ones expected by his hypothesis? Can you accept his hypothesis? Of the total number of plants (705 + 224 = 929), 3/4 should be red-flowered. Thus, based on the hypothesis, the number expected to be red is 697 (929 x 3/4). The number expected to be white is 232 (929 x 1/4). Mendel observed 705 red and 224 white flowered plants. Is the difference between his observed results and the expected results due to chance or is his hypothesis incorrect? To determine if the observed results are close enough to the expected results, we must submit them to a chi-square analysis. Chi-square is a statistical test that can be used to determine whether results are close enough to those expected by a hypothesis. Chi-square tells you how many times out of 100 a deviation from the expected results is due to chance alone. It is the probability (expressed in percent) that chance alone has caused the deviation from the expected results. If chance has caused the difference between observed (O) and expected (E) results, then the results support the hypothesis.

Chi-square (χ^2) is calculated using the following formula:

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

	O	E	(O-E)	(O-E) ²	$\frac{(O-E)^2}{E}$
Red flowers	705	697	8	64	0.09
White flowers	224	232	-8	64	0.28
$\chi^2 =$					0.37

Table 1. Chi-square analysis for Mendel's cross between red-flowered pea plants and white-flowered pea plants.

What does the chi-square value signify? To interpret the chi-square value:

1. Find the degrees of freedom. Degrees of freedom are equal to the number of groups minus 1. In this example, there are two groups (red flowers and white flowers), so there is 1 degree of freedom.
2. Look at the chi-square table (Table 2). Find the closest probability (p) value associated with your chi-square and degrees of freedom. The degree of freedom is 1. The chi-square value is 0.37. Therefore, the probability lies between 0.50 and 0.70. Probability is a percentage. There is a 50% to 70% probability that the deviation from the expected results is due to chance alone.
3. The criterion for accepting or rejecting the hypothesis is $p > 0.05$. If the probability or p value is greater than 0.05, the hypothesis is accepted. Since the p value of Mendel's experiment is between 0.50 and 0.70, his hypothesis is accepted.

Degrees of Freedom	Probability (p)									
	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28
Hyothesis	Accept								Reject	

Table 2. Table of chi-square values.

2. Mendel’s dihybrid cross

Observation: When Mendel crossed round yellow-seeded pea plants with wrinkled green-seeded plants, the wrinkled green traits were lost in the F₁ generation but reappeared in the F₂ generation. The offspring in the F₂ generation had four phenotypes in the following proportion: Most had round yellow seeds, some had round green seeds or wrinkled yellow seeds, and a very few had wrinkled green seeds.

Problem: What can explain the observation?

Hypothesis: Mendel’s hypothesis was the basis for his second law, the law of independent assortment. The allele for round seeds (*A*) is dominant to the allele for wrinkled seeds (*a*), and the allele for yellow seeds (*B*) is dominant to the allele for green seeds (*b*). Alleles for texture and color are on different chromosomes. Since the alleles assort themselves independently when gametes are formed, crossing the F₁ generation with itself will yield offspring in the F₂ generation that have the following ratio:

- 9 round yellow seeds
- 3 round green seeds
- 3 wrinkled yellow seeds
- 1 wrinkled green seeds

	<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>
<i>AB</i>	<i>AABB</i>	<i>AABb</i>	<i>AaBB</i>	<i>AaBb</i>
<i>Ab</i>	<i>AABb</i>	<i>Aabb</i>	<i>AaBb</i>	<i>Aabb</i>
<i>aB</i>	<i>AaBB</i>	<i>AaBb</i>	<i>aaBB</i>	<i>aaBb</i>
<i>ab</i>	<i>AaBb</i>	<i>Aabb</i>	<i>aaBb</i>	<i>aabb</i>

Table 3. Dihybrid cross. *A* is the allele for round seeds and *a* is the allele for wrinkled seeds. *B* is the allele for yellow seeds and *b* is the allele for green seeds. The F₁ generation plants produce gametes with four possible allele combinations *AB*, *Ab*, *aB*, *ab*.

Prediction: If an F₁ pea plant that has dominant round and yellow seeds is self-crossed, the F₂ generation will have a ratio of 9:3:3:1. 9 round, yellow seeds; 3 round, green seeds; 3 wrinkled, yellow seeds; and 1 wrinkled, green seeds.

Experiment

P generation: Cross round, yellow-seeded plants with wrinkled, green-seeded plants.
 F₁ generation: All plants have round yellow seeds. Cross the F₁ generation with itself.
 F₂ generation: 315 have round yellow seeds
 108 have round green seeds
 101 have wrinkled yellow seeds
 32 have wrinkled green seeds

Conclusion: The next step is to submit Mendel’s results to a chi-square analysis. See Table 2. The total number of seeds is obtained by adding the number of seeds of each type: 315 + 108 + 101 + 32 = 556. Then the expected results are calculated. Of the 16 possible offspring, 9/16 of 556 or 313 should be round and yellow, 3/16 of 556 or 104 should be round and green, 3/16 of 556 or 104 should be wrinkled and yellow, and 1/16 of 556 or 35 should be wrinkled and green. Finish calculating the chi-square value to determine if Mendel’s hypothesis is supported by the results. Can you accept Mendel’s hypothesis?

	O	E	(O-E)	(O-E) ²	$\frac{(O-E)^2}{E}$
Round, yellow	315	313			
Round, green	108	104			
Wrinkled, yellow	101	104			
Wrinkled, green	32	35			
$\chi^2 =$					

Table 4. Mendel’s F₂ generation.

3. The Testcross

Mendel designed the test cross to determine whether an organism that exhibits a dominant trait has one or two alleles for the trait. The organism is crossed with one that has two alleles for the recessive trait. Since alleles segregate when gametes are formed, if an unknown organism has two dominant alleles and is test crossed with one that has two recessive alleles, then all offspring will exhibit the dominant trait. If the organism has one dominant and one recessive allele and is test

crossed with an organism that has two recessive alleles, then the offspring will be in a 1:1 ratio of dominant to recessive alleles for that trait. It does not matter how many traits you consider. If an organism has one dominant and one recessive allele for a trait, each trait will be in a 1:1 ratio of dominant to recessive alleles of offspring for the that trait. Therefore, a monohybrid test cross would give a 1:1 ratio of offspring for the trait. See Table 5. A dihybrid test cross would give a 1:1:1:1 ratio of offspring for the two traits or four phenotypes. See Table 6.

	<i>A</i>	<i>a</i>
<i>a</i>	<i>Aa</i>	<i>aa</i>

Table 5. Monohybrid testcross. The F₁ generation is crossed with a white-flowered plant. The F₁ generation produces *A* and *a* alleles in a 1:1 ratio. The white-flowered plant produces only an *a* allele.

	<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>
<i>ab</i>	<i>AaBb</i>	<i>Aabb</i>	<i>aaBb</i>	<i>aabb</i>

Table 6. Dihybrid testcross. The F₁ generation crossed with a white-flowered, green-seeded plant. The F₁ generation produces *AB*, *Ab*, *aB* and *ab* alleles in a 1:1:1:1 ratio. The white-flowered, green-seeded plant produces only an *ab* allele.

4. Determination of the Genotype of Indian Corn

You will be given an ear of Indian corn of unknown heredity. Each ear represents one of four possible crosses:

F₂ monohybrid cross: The result of an F₁ hybrid (*Aa*) self-cross (*Aa* x *Aa*).

F₂ dihybrid cross: The result of an F₁ hybrid (*AaBb*) self-cross (*AaBb* x *AaBb*) with alleles on separate chromosomes.

F₁ monohybrid test cross: The result of a testcross of an F₁ hybrid (*Aa*) with the recessive parent (*Aa* x *aa*).

F₁ dihybrid test cross: The result of a testcross of an F₁ hybrid (*AaBb*) with the recessive parent (*AaBb* x *aabb*) with alleles on separate chromosomes.

Make a hypothesis about the genotype of your ear. You will collect data and submit the data to a chi-square analysis to see if the data support your hypothesis. Every kernel on the Indian corn is a seed, the result of a fusion of a male gamete (pollen nucleus) carrying one allele and a female gamete (ovum) carrying one allele.

Observation: Look for kernel color and/or texture. How many different kinds of kernels are present on your Indian corn cob? List the different kernel phenotypes and notice their proportion in the space below.

Problem: What can explain your observation? What is the genotype of the parent generation of your ear of Indian corn?

Hypothesis: Your hypothesis answers the question posed by the problem. State the hypothesis you will test.

Experiment

1. Gather data by determining both the expected and observed numbers of kernels. Enter in the space below.
2. Calculate the chi-square value using the formula. Complete all calculations to three digits. Round off your answer to two digits. Determine the degree of freedom and locate the value closest to your calculated chi-square on that degrees of freedom row. Move up the column to determine the p value. See Table 7.

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

	O	E	(O-E)	(O-E) ²	$\frac{(O-E)^2}{E}$
$\chi^2 =$					

Table 7. Chi-square value for Indian corn.

Conclusion: The results either support or fail to support your hypothesis. State your conclusion about your hypothesis. If the p value for the calculated chi-square is greater than 0.05, accept your hypothesis.

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