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## 8:10 Plant Genetics lab

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### Materials needed (per pair of students)

Ear of corn, monohybrid cross (purple/yellow seeds)

Ear of corn, test cross (purple/yellow seeds)

Ear of corn, dihybrid cross (purple/yellow/smooth/wrinkled seeds)

### Introduction

Gregor Mendel was the first scientist to really understand genetics. He worked with pea plants, and published his results in 1866, however it was not until after his death, in the early 1900s that scientists realized that his work applied to all sexually reproducing organisms, from moss plants to humans. Mendel was the first person able to accurately predict the outcome of a genetic cross. His initial experiments concentrated on only one characteristic at a time (for example the color of the seeds), which is called a **monohybrid cross**. Later he expanded his work to two characteristics in the same plants, a **dihybrid cross**. Mendel noticed that in every cross one trait was dominant. For example if a purple-flowered pea was crossed with a white-flowered pea, all the first generation (F<sub>1</sub>) had purple flowers. In the second generation, however, some white flowers were produced. Mendel was able to explain these results, even though it would be almost another 100 years before the role of DNA was discovered.

### Monohybrid cross

A strain of corn producing pure purple kernels (PP) is crossed with a strain producing pure yellow kernels (pp). Purple is dominant, and the entire F<sub>1</sub> generation results in purple corn kernels. Two of these F<sub>1</sub> offspring are crossed, and the F<sub>2</sub> generation shows both purple and yellow kernels.

- A) Count the number of purple and yellow kernels on one row of the F<sub>2</sub> ear, **without removing** the kernels. Record your results in the table below.
- B) Calculate the ratio of purple to yellow (to 2 decimal places).
- C) Record the total number of yellow and purple kernels on the whole ear (usually found on a small tag on the corn).
- D) Calculate the ratio for the whole ear of corn (to 2 decimal places).
- E) Record the data from other groups of students in your table.
- F) Total each column, then recalculate the ratios, using the results from the whole class.

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**RESULTS**

**MONOHYBRID CROSS**

One row			Whole ear		
Purple seeds	Yellow seeds	Ratio of Purple:Yellow	Purple seeds	Yellow seeds	Ratio of Purple:Yellow
<b>Total:</b>					

1) What are the expected genotypes of the P generation and the F1 generation?

Parents:  $PP \times pp$

F1 generation are all:  $Pp$

2) Show the Punnett square for the F1 x F1 cross that resulted in the F2 generation.

	X	P	p
P	PP	Pp	
p	Pp	pp	

3) Did the actual ratio of the phenotypes **exactly** match the predicted ratio? If not , why not?

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4) Did you get better results by counting a single row of kernels, or by counting the whole ear? Explain.

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#### Chi-square test

As you can see from the example above, the results in a genetics experiment often turn out a little different from the predicted (Punnett square) ratio. This is typically because the sample size is relatively small. It is important to check to see whether the difference between the observed and predicted results is just due to chance, or to a problem with the experiment. The Chi-square, or goodness-of-fit, test is used in this case.

For example, assume that in part A of this exercise you had a total of 332 purple seeds and 96 yellow, which is a ratio of 3.46 : 1. The predicted ratio is exactly 3.0 : 1. In this case a total of 428 seeds were counted, so 75% of this total should be purple ( $428 \times 0.75 = 321$ ), similarly 25% of the total should be yellow ( $428 \times 0.25 = 107$ ). Using the Chi-square test you can find out whether this difference between observed and expected is due to chance:

	<u>Purple seeds</u>	<u>Yellow seeds</u>
<b>Observed</b>	332	96
<b>Expected</b>	321	107
<b>Difference</b>	11	-11
<b>(Difference)<sup>2</sup></b>	121	121
<b>(Difference)<sup>2</sup> /Expected</b>	0.38	1.13

**Result:** Chi-square =  $0.38 + 1.13 = 1.51$

To find out if this value of Chi-square is significantly different from the expected result, you first work out how many **degrees of freedom** (df) you have in the experiment. The degrees of freedom is always one less than the number of different categories. So here we have two categories of plant (purple or yellow), so only one degree of freedom. The table below shows the chi-square distribution:

	<b>Probability</b>							
<b>df</b>	<b>0.90</b>	<b>0.50</b>	<b>0.30</b>	<b>0.20</b>	<b>0.10</b>	<b>0.05</b>	<b>0.01</b>	<b>0.001</b>
<b>1</b>	0.02	0.46	1.07	1.64	2.71	3.84	6.64	10.83
<b>2</b>	0.21	1.39	2.41	3.22	4.60	5.99	9.21	13.82
<b>3</b>	0.58	2.37	3.66	4.64	6.25	7.82	11.34	16.27
<b>4</b>	1.06	3.36	4.88	5.99	7.78	9.49	13.28	18.47
	<b>Non significant</b>					<b>Significant</b>		

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Our calculated chi-square of 1.51 is closest to 1.64 (with 1 degree of freedom), so this result is **not** significantly different from the expected.

Use your results from **one row** of corn in part A above, and go through the Chi-square test by filling in the table below.

	<u>Purple seeds</u>	<u>Yellow seeds</u>
<b>Observed</b>		
<b>Expected</b>		
<b>Difference</b>		
<b>(Difference)<sup>2</sup></b>		
<b>(Difference)<sup>2</sup> /Expected</b>		

**Result:** Chi-square =

5) Was your Chi-square result non-significant or significant?

6) What is the lowest Chi-square value that would be significant with 3 degrees of freedom (look at the table of distribution above)?

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7) Do you think that a plant breeder or an animal breeder would be more likely to need to use the Chi-square test? Explain your answer.

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**Test cross**

A test cross is used to determine an unknown genotype if the individual has a dominant phenotype. This always involves crossing with a known homozygous recessive, because any dominant alleles will mask the recessive alleles. For example, if the resulting offspring are all dominant phenotype, the unknown must be a homozygous dominant. If half of the offspring are dominant phenotype, and half are the recessive phenotype, the unknown must be heterozygous.

Count the number of purple and yellow kernels on one row of the test cross corn, without removing the kernels. Calculate the ratio of purple to yellow. Do the same for the whole ear, and be sure to collect the data from the other student groups as before.

**TEST CROSS**

F<sub>1</sub>

One row

Whole ear

Purple seeds	Yellow seeds	Ratio of Purple:Yellow	Purple seeds	Yellow seeds	Ratio of Purple:Yellow
<b>Total:</b>					

8) Was the unknown plant in the test cross homozygous dominant or heterozygous for kernel color? Explain your answer.

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**Dihybrid cross**

In Mendel's experiments, he found that if two heterozygous plants were crossed, the ratio of phenotypes in the offspring was 9:3:3:1. In this exercise, you will count for two separate traits: kernel color (purple or yellow) and kernel texture (smooth or wrinkled). Most of the ears of corn have tags that you can record below. Be sure to collect the data from the other student groups.

**DIHYBRID CROSS**

<u>Purple/round</u>	<u>Purple/wrinkled</u>	<u>Yellow/round</u>	<u>Yellow wrinkled</u>
<b>Total:</b>			

9) Convert the numbers in the "Total" row above to a ratio (it should be roughly 9:3:3:1 – keep your answer to two decimal places). What ratio did you get?

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10) Which ratio in this lab, the monohybrid or the dihybrid, is closer to the predicted ratio? Explain why.

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11) Give an example of when it would be important to tell if an organism is heterozygous or homozygous. In other words, in which situation is a Test cross used?

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12) If you found two plants of the same species, one with yellow flowers and one with blue flowers, how could you find out which gene was dominant?

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13) If you had a plant which had flowers in two colors, how could you tell if these colors were caused by a single gene (monohybrid) or two genes (dihybrid)?

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14) Charles Darwin, working at about the same time as Gregor Mendel, also did many breeding experiments, but unlike Mendel he was unable to make sense of them – particularly in the dihybrid cross. Darwin wrote “The offspring from the first cross between two pure breeds (pigeons) is quite uniform in character; but when these mongrels are crossed with one another hardly two of them are alike.” Explain why this is so (remember that the color and shape of a pigeon’s body is affected by many genes).

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