Mendel's Peas Exercise 2

EFFECTS OF SAMPLE SIZE ON EXPERIMENTAL RESULTS

Goal

In this exercise you will use StarGenetics, a genetics experiment simulator, to compare the observed phenotypic ratios and corresponding genotypic ratios to those predicted using a one-gene Mendelian model of inheritance, and study the effect of sample size on experimental results, including the use of the chi-square test (optional).

Prerequisite knowledge

Before completing this exercise, students should be able to:

1. Determine whether a phenotype is dominant or recessive relative to another phenotype given a set of results.
2. Infer and assign genotypes of individual organisms using proper nomenclature of alleles.
3. Use Punnett Squares to predict and confirm expected genotypic and phenotypic ratios.

Learning objectives

After completing this exercise, you will be able to:

1. Determine if an organism is true breeding for a trait given a set of results.
2. Recognize differences between observed and expected phenotypic ratios.
3. Explain the effect of sample size on observed phenotypic ratios.
4. Define an appropriate null hypothesis for a chi-square test (Advanced).
5. Perform a chi-square statistical test and compare the calculated test statistic to the minimum chi-square value needed to reject your null hypothesis with at least 95% confidence (Advanced).
6. Assess whether your data lend support to a proposed model of inheritance using the results of your chi-square statistical analysis (Advanced).

Getting started with StarGenetics

- To access StarGenetics, please navigate to: **http://star.mit.edu/genetics/**.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- Click on **File → New** in the drop-down menu in the upper left hand corner.
- Click on the **Mendel's Peas Exercise 2** file.

You maintain a strain of <u>true-breeding</u> green-pod plants (*Green Plant*) when one day, many generations into propagating this strain, you notice that one of the plants has yellow pods (*Yellow Plant*). You hypothesize that a new mutation in the true-breeding *Green Plant* strain has resulted in the new yellow pod color.

1 First, we will analyze the F1 offspring of the *Yellow Plant* to determine whether it is true breeding for the yellow-pod phenotype.

a) What is/are the pod color phenotype(s) of F1 plants arising from a self-cross of the *Yellow Plant*? Self-cross the *Yellow Plant* to analyze a total of 1000 progeny (100 matings, 10 plants per mating) and record the pod color phenotype(s) of the F1 progeny.
- Self-cross the *Yellow Plant* by dragging this plant to the **Mating site** twice, to act as <u>both</u> the ovum and pollen donor, and then click on the **Mate** button to generate F1 offspring.

- To add additional offspring to a cross, click on the **Add more matings** button and select the appropriate number of matings (or crosses) you would like to perform in the pop-up window to add the appropriate number of additional offspring. Repeat until you have added the desired number of offspring.
- Rename this experiment by clicking the box with the current experiment name at the top of the **Active experiment** window and enter the new experiment name: "*Yellow selfed*" when prompted.

Answer

F1 phenotype(s):

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b) What is/are the pod color phenotype(s) of F2 plants derived from the *Yellow Plant*?

i. Self-cross an individual F1 plant of your choice from the "*Yellow selfed*" experiment (from Question 1a) and record the pod color phenotype(s) of the F2 progeny.
- To start a new mating experiment, click on the **New experiment** button. Your current experiment will automatically be saved for you.
- You can mate individual plants you have generated in previous saved experiments by dragging the desired plant from the **Saved experiments** window to the **Mating site**.
- Self-cross an individual of your choice from the F1 progeny generated in the "*Yellow selfed*" experiment within the **Saved experiments** window and record the phenotypes of the F2 progeny.
- Rename this experiment "*F1 cross 1*".

Answer

Name of 1st F1 plant selfed:

F2 Phenotype(s):

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ii. Self-cross a <u>second</u> F1 plant of your choice from the "*Yellow selfed*" experiment and record the pod color phenotype(s) of the F2 progeny.
- Rename this experiment *"F1 cross 2"*.

Answer

Name of 2nd F1 plant selfed:

F2 Phenotype(s):

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c) What is/are the pod color phenotype(s) of F3 plants derived from the *Yellow Plant*?

i. Self cross an F2 individual plant of your choice from the "*F1 cross 1*" experiment and record the pod color phenotype(s) of the F3 progeny.
- Rename this experiment "*F2 cross 1*".

Answer

Name of 1st F2 plant selfed:

F3 Phenotype(s):

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ii. Self cross an F2 individual plant from the "*F1 cross 2*" experiment and record the pod color phenotype(s) of the F3 progeny.
- Rename this experiment "*F2 cross 2*".

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Answer
Name of 2ⁿᵈ F2 Plant selfed:
F3 Phenotype(s):
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d) Based on your findings, is the newly identified *Yellow Plant* true breeding? Explain.

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Answer



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2 Is the yellow-pod phenotype dominant or recessive relative to the green-pod phenotype? Explain.
  • Cross the true-breeding *Green Plant* to the *Yellow Plant*.
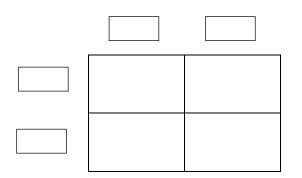 • Rename this experiment "*Green x Yellow*".

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Answer




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3 What are the genotypes of the true-breeding *Green Plant*, the *Yellow Plant,* and an F1 individual from the *Green x Yellow* experiment?
  • Use the letter 'Y' to refer to the pod color gene: the uppercase ('Y') and lowercase ('y') versions of this letter to refer to the allele associated with the dominant and recessive phenotypes, respectively.

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Answer
Green Plant:
Yellow Plant:
F1:
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4 Next, we would like to determine if the pod-color phenotype is controlled by a single gene, where one pod-color phenotype is completely dominant to another. We call this pattern of inheritance <u>one-gene Mendelian inheritance</u> because it follows the same pattern of inheritance that Mendel observed for the traits he characterized in garden peas. Assuming that pod color is inherited in this manner, what are the expected genotypic and phenotypic ratios when an F1 individual from the *Green x Yellow* experiment is <u>**self-crossed**</u>? Fill in the Punnett Square below to support your answer.

Answer

Expected phenotypic ratio:

Expected genotypic ratio:

5 Now, let's test the hypothesis that pod color is inherited in a one-gene Mendelian inheritance pattern. Self-cross one of the F1 individuals resulting from the *Green x Yellow* cross to generate a total of 10 F2 plants (1 mating). What do you observe?

Answer

Number of plants with green pods:

Number of plants with yellow pods:

% of plants with green pods:

% of plants with yellow pods:

Observed phenotypic ratio:

6 Continue to self cross this F1 individual to increase the number of total progeny to 1000 F2 plants (an additional 99 matings). What do you observe now?

Answer

Number of plants with green pods:

Number of plants with yellow pods:

% of plants with green pods:

% of plants with yellow pods:

Observed phenotypic ratio:

7 We will now compare the phenotypic ratios resulting from the F2 progeny in the case of 10 and 1000 plants to determine the effect of sample size on our results.

What happens to the ratio as the number of progeny (or sample size) increases from 10 to 1000? Which of the two F2 sample size results in a phenotypic ratio that is closest to the 'expected' phenotypic ratio you predicted in Question 4?

Answer

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8 (Optional) For this question, you share your results with the rest of the class by recording your ratios of green- to yellow-pod plants for both the 10 and 1000 progeny sample sizes on the board. After all students or student groups have finished recording their data, describe how the green:yellow ratios vary between students (or groups) in the experiments with 10 progeny versus 1000 progeny. Why is it better to use large sample sizes when performing experiments?

Answer

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As you most likely observed, a lot of variability is observed in the results of genetic crosses. Even when examining a larger sample size (1000 offspring), you may have noticed that the results you observe differ somewhat from what you expected. We will now use the chi-square (χ^2) statistical test to determine if the difference between your expected and observed results can be explained by variability due to chance (null hypothesis). In genetics, we often use the chi-square statistical test to determine if our data lends support to a particular inheritance pattern that we believe explains how our trait of interest is inherited.

The chi-square statistic takes into consideration the size of your experimental sample and the amount of variation between the expected and observed results. It is defined as:

$$\chi^2 = \Sigma \, \frac{(Number\ Observed - Number\ Expected)^2}{Number\ Expected}$$

The following questions will walk you through the process of: 1) performing a chi-square statistical analysis on your set of expected and observed data, and 2) analyzing the results of this test to determine if your observed data lend support to your null hypothesis.

9 What is the null hypothesis in this situation?

Answer

The Null Hypothesis is/are: (select ALL which are correct)

      (a) the experimental data are consistent with the one-gene Mendelian inheritance pattern

      (b) the experimental data are consistent with a two-gene Mendelian inheritance pattern

      (c) the experimental data are inconsistent with the one-gene Mendelian inheritance pattern

      (d) none of the above

10 You will now perform a χ^2 statistical test to determine if you can be at least 95% confident that the observed results lend support to your null hypothesis, despite there being a variation between your observed (Question 6) and expected (Question 4) results.

a) Determine the **chi-square (χ^2)** value of the variance between expected and observed F2 yellow- and green-pod plants using the data you obtained when generating 1000 individual F2 progeny from the self-cross of a **Green x Yellow F1** plant in Question 6.

In the table below, under the "Expected" column, write the numbers you would have expected for each phenotypic category for 1000 progeny based on your expected phenotypic ratio (Question 4). Next, under the "Observed" column, fill in the number of progeny you actually observed for each phenotypic category (Question 6). Then, perform the calculations indicated to complete the chart and sum all the values within the right column ($(O-E)^2/E$) to determine the χ^2 value.

   • In the chi-square statistical test, you must use values in your calculations, NOT frequencies or percentages. This means that you need to indicate the expected number of progeny for each phenotypic category before proceeding with the calculations. Make sure that the expected numbers add up to 1000.

Answer

	Expected (E)	Observed (O)	O-E	$(O-E)^2$	$(O-E)^2/E$
Green Pod Plants					
Yellow Pod Plants					
Total			----------------	-------------------	$\chi^2=$

b) What is the number of degrees of freedom?
   • The degrees of freedom is defined as the **number of phenotypic classes -1**

Answer

c) What is the minimum χ^2 value needed to <u>reject</u> the null hypothesis for your number of degrees of freedom and using a *p* value of 0.05? This represents the minimum χ^2 value for which we can be at least

95% confident that the differences between the observed and the expected results are NOT consistent with your null hypothesis. Use the chi-square table on the last page to answer this question.

Answer

d) Using your data and a *p* value of 0.05 as your cut off, can you reject the null hypothesis? Or, does the chi-square test lend support to your null hypothesis? Explain.

Answer

e) Based on your data, what can you infer about the inheritance of the pod-color phenotype in these plants? Explain.

Answer

Reference: Chi-Square Table

		Degrees of Freedom							
		1	2	3	4	5	6	7	8
P value	Confidence Interval	χ^2 value							
0.05	95%	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	99%	6.64	9.32	11.34	13.28	15.09	16.81	18.48	20.09

The number of degrees of freedom is equal to the number of distinct phenotypic classes minus one.