Mendel's Peas Exercise 3

1-GENE VS. 2-GENE INHERITANCE PATTERNS & THE EFFECT OF SAMPLE SIZE ON EXPERIMENTAL RESULTS

Goal

In this exercise you will use StarGenetics, a genetics experiment simulator, to explore the differences in the patterns of inheritance of a trait when it is controlled by either one or two genes, the effect of sample size on the variation between observed and expected results, and differentiate between these two possible models of inheritance using the chi-square statistical 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. Recognize differences between observed and expected phenotypic ratios.
2. Explain the effect of sample size on observed phenotypic ratios.
3. 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).
4. 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 get to 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 3** file.

You have two boxes full of plants in the lab. Each box contains a different plant species and both boxes contain a mix of tall and short plants that otherwise look the same. A fellow classmate previously determined that for one of these plant species, plant height is controlled by one gene (one gene case) and that for the other species, plant height is controlled by two different genes (two-gene case). Unfortunately, after labeling the two boxes with the name of the species and the corresponding inheritance pattern for plant height, the labels fell off and now you no longer know which species is associated with a one- or two-gene inheritance pattern. It is up to you to determine the inheritance pattern of plant height (one- or two-gene) for both species of plants.

To perform your experiments in StarGenetics, you have representative tall and short plants from both boxes, Box 1 and Box 2. The following strains are in your **Strains** box: *Box1-Tall*, *Box1-Short*, *Box2-Tall*, *Box2-Short*. Both boxes contain only true-breeding plants.

1 First, you must determine which plant height phenotype is dominant to the other for each species.

a) Is the tall phenotype dominant or recessive relative to the short phenotype for the plant species in Box 1? Explain.
- Cross *Box1-Short* with *Box1-Tall* by dragging each strain from the **Strains** box into the **Mating site** and clicking **Mate**. Analyze the resulting F1 progeny.
- Rename this cross as "*Box1 Short x Tall*"

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Answer



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b) Is the tall phenotype dominant or recessive to the short phenotype for the plant species in Box 2? Explain.
- Click **New experiment** to save your current experiment and start a new one.
- Cross *Box2-Short* with *Box2-Tall* by dragging each strain from the **Strains** box into the **Mating site** and clicking **Mate**. Analyze the resulting F1 progeny.
- Rename this experiment as: "*Box2 Short x Tall*".

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Answer



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2 a) What is the observed phenotypic ratio of tall and short plants in an F2 generation derived from the "*Box 1 Short x Tall*" cross?
- Self-cross an F1 individual generated in the "*Box1 Short x Tall*" experiment by dragging an individual plant of your choice from the **Saved experiments** window to the **Mating site** twice, to act as <u>both</u> the ovum and pollen donor, and then click the **Mate** button to generate F2 offspring.
- Record the phenotypes for the first <u>10 plants</u> produced (1 mating).
- Rename this experiment as: "*Box1 F1 self-cross*".

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Answer

Number of Tall Plants:

Number of Short Plants:

Phenotypic Ratio:
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b) Continue to self-cross your chosen F1 individual to produce a total of <u>1000 plants</u> (99 more matings). What phenotypic ratio with respect to plant height do you observe in this larger sample size?
- To add additional offspring to a cross, click on the **Add more matings** button and select the number of matings you would like to perform in the pop-up window to generate the appropriate number of additional offspring.

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Answer

Number of Tall Plants:

Number of Short Plants:
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Phenotypic Ratio:

3 a) What is the observed phenotypic ratio of tall and short plants in an F2 generation derived from the "*Box 2 Short x Tall*" cross?
- Self-cross an F1 individual generated in the "*Box2 Short x Tall*" experiment by dragging an individual plant of your choice from the **Saved experiments** window to the **Mating site** twice, to act as <u>both</u> the ovum and pollen donor, and then click the **Mate** button to generate F2 offspring.
- Record the phenotypes for the first <u>10 plants</u> produced (1 mating).
- Rename this experiment as: "*Box2 F1 self-cross*".

Answer

Number of Tall Plants:

Number of Short Plants:

Phenotypic Ratio:

b) Continue to self-cross your chosen F1 individual to produce a total of <u>1000 plants</u> (99 more matings). What phenotypic ratio with respect to plant height do you observe in this larger sample size?

Answer

Number of Tall Plants:

Number of Short Plants:

Phenotypic Ratio:

4 Did you notice any changes in the phenotypic ratios as you increased the number of plants that you analyzed in Questions 2 and 3 for Box 1 or for Box 2 plants, respectively? Explain.

Answer

5 We know that for one of the plant species, plant height is controlled by a single gene, while in the other species, plant height is controlled by two different genes. Now let's think about the genotypic and phenotypic ratios you would expect in the F1 progeny of a cross between a short and tall plant if plant height is controlled by either one gene or two genes.

a) <u>If plant height is controlled by ONE gene</u>: what are the genotypes of the parents and the genotype(s) and phenotype(s) of the F1 offspring when you cross a true-breeding tall parent with a true-breeding short parent?
- Use capital **A** and lower case **a** to represent the alleles encoding the dominant and recessive phenotypes, respectively, of the one gene that controls plant height in this scenario, *Gene A*.
- Remember, that you previously determined the dominant and recessive plant height phenotypes in Question 1.

Answer

Tall Parent genotype:

Short Parent genotype:

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F1 genotype(s):
F1 phenotype(s):
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b) <u>If plant height is controlled by TWO genes</u>: what are the genotypes of the parents and the genotype(s) and phenotype(s) of the F1 offspring when you cross a true-breeding tall parent with a true-breeding short parent?
  - Use capital **A** and lower case **a** to represent the alleles encoding the dominant and recessive phenotypes, respectively, of one of the genes controlling plant height, *Gene A*.
  - Use capital **B** and lower case **b** to represent the alleles encoding the dominant and recessive phenotypes, respectively, of the second gene that controls plant height, *Gene B*.
  - Remember, you determined the dominant and recessive plant height phenotypes in Question 1.

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Answer
Tall Parent:
Short Parent:
F1 genotype(s):
F1 phenotype(s):
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6 Now, let's predict what the genotypic and phenotypic ratios for the F2 progeny will be when you self-cross F1 progeny from plants in which height is controlled by one gene or two genes. Fill in the Punnett Squares and tables below with the expected genotypes and corresponding phenotypes of the F2 progeny for both cases.

Note: <u>You do not have to use all rows provided to you in the tables and only write each unique genotype ONCE</u>.

a) One gene: F1 self-cross

Answer

Genotype	Frequency	Phenotype
		Phenotypic ratio:

b) Two genes: F1 self-cross

Note: In the two-gene case, a coworker has determined that a plant exhibits the recessive phenotype if it is homozygous recessive for:

     i) *Gene A* (aa)
     ii) *Gene B* (bb)
     iii) BOTH *Gene A* AND *Gene B* (aabb)

This means that the plant must have at least one copy of the allele that confers the dominant phenotype for *Gene A* <u>AND</u> *Gene B* (A_B_) to exhibit the dominant plant height phenotype.

Answer

Genotype	Frequency	Phenotype
		Phenotypic ratio:

7 Recall that the pea plant species in Box 1 and Box 2 are genetically different from one another. In one case, height is controlled by one gene whereas, in the other case, height is controlled by two genes. Using the information that you obtained from your crosses between plants from either Box 1 or Box 2 so far, as well as your answers to Question 6, can you differentiate between the one-gene and two-gene scenarios with only 10 F2 offspring? How about 1000 F2 offspring? Explain.

Answer

Advanced

We will now perform chi-square statistical tests to distinguish between the one-gene and the two-gene modes of inheritance. 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 observed between the expected and observed results. It is defined as:

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

8 We will now use the chi-square statistical test to determine if the data you obtained when generating 1000 **Box 1** F2 plants lend support to the one-gene model of inheritance.

Null Hypothesis: The experimental data observed in F2 plants from Box 1 are consistent with a one-gene model of inheritance, and any variations between expected and observed values are due to chance.

a) Determine the **chi-square (χ²)** value of the variance between expected and observed F2 tall and short pea plants using the data you obtained when generating 1000 individual F2 progeny from the "***Box1 F1 self-cross***" experiment (Question 2b).

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 6a). Next, under the "Observed" column, fill in the number of progeny you actually observed for each phenotypic category (Question 2b). 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
Tall Plants					
Short Plants					
Total	1000	1000	----------------	-------------------	χ² =

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, can you reject the null hypothesis? Or does the chi-square test lend support to the null hypothesis, and therefore to the one-gene model of inheritance for the **Box 1 plant species**? Explain.

Answer

9 Now repeat this same chi-square analysis using the data you obtained when generating <u>1000 **Box 2**</u> F2 progeny.

Null Hypothesis: The experimental data observed in F2 plants from <u>Box 2</u> are consistent with <u>a one-gene model of inheritance</u>, and any variations between expected and observed values are due to chance.

a) Determine the **chi-square (χ^2)** value of the variance between expected and observed F2 tall and short plants. Use the data you obtained when generating 1000 individual F2 progeny from the "*Box2 F1 self-cross*" experiment (Question 3b).

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 6b). Next, under the "Observed" column, fill in the number of progeny you actually observed for each phenotypic

category (Question 3b). 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
Tall Plants					
Short Plants					
Total	1000	1000	-----------------	--------------------	χ^2 =

b) What is the number of degrees of freedom?

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?

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 the null hypothesis, and therefor to the one-gene model of inheritance for the **Box 2 plant species**? Explain.

Answer

10 Based on your data, what can you infer about the inheritance of plant height for Box 1 and Box 2 plant species? Which box contains the plants in which height is controlled by one gene, and which box contains the plants in which height is controlled by two genes? Explain.

- Remember that in this exercise there are <u>only</u> 2 possibilities, so if the box of plants does not follow the one-gene model, then it must follow the two-gene model.

Answer

Reference: Chi-Square Table

Degrees of Freedom

p value	Confidence Interval	1	2	3	4	5	6	7	8
					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.