

Fruit Fly Exercise 1 – Level 2

Goal

In this exercise, you will use StarGenetics, a software tool that simulates mating experiments, to analyze the nature and mode of inheritance of specific genetic traits.

Learning Objectives

After completing this exercise, you will be able to:

1. Perform genetics experiments in the genetics cross simulator, StarGenetics.
2. Identify the phenotype of a fruit fly within StarGenetics.
3. Determine whether a phenotype is dominant or recessive relative to another phenotype through the analysis of results from genetic crosses.
4. Infer and assign genotypes of individual organisms using proper nomenclature of alleles.
5. Determine if individual organisms are homozygous or heterozygous for a given trait through the analysis of results from genetic crosses.
6. Use Punnett Squares to predict and confirm expected genotypic and phenotypic ratios.
7. Explain the effect of sample size on observed phenotypic ratios.
8. Generate an appropriate hypothesis to explain observed experimental results.
9. 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.
10. Assess whether your data lend support to a proposed model of inheritance using the results of your chi-square statistical analysis.

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 **Fruit Fly Exercise 1 – Level 2** file.

You have begun a research project in a genetics laboratory that studies the fruit fly *Drosophila melanogaster*. Your research advisor decides to give you an interesting mutant fly called **Mutant 1** that has just been discovered in one of the fly vials in the lab. In addition, you are also given two true-breeding wild-type flies (male and female). All 3 flies can be found within the **Strains box**.

1 First, describe the sex and phenotype of the mutant fly. Describe the phenotype as it compares to wild type.

- The sex and phenotype of each fly should be visually obvious. You can also obtain this information by clicking on the fly and looking at the **Properties** window.

Answer

2 You want to determine the genotype of **Mutant 1**. You are unsure whether the mutant allele confers a phenotype that is dominant or recessive to wild type. You are also not sure if **Mutant 1** is true-breeding or not. To determine its genotype, cross **Mutant 1** with one of the wild-type flies.

- Set up a cross between **Mutant 1** and a wild-type fly by dragging each to the **Mating Site** and by clicking on the **Mate** button.
- Each resulting offspring can be viewed within the **Individual** tab or a summary of the results is available on the **Summary** tab.

Answer

Number of F1 flies that look like the wild-type parent: _____

Number of F1 flies that look like the *grounded* parent: _____

Total number of progeny generated: _____

% of progeny that is wild type: _____

% of progeny that is mutant: _____

Circle the ratio that best fits the progeny that you observe.

3 WT : 1 mutant

1 WT : 1 mutant

1 WT : 3 mutant

3 Based on the results that you obtained in question 2, does the mutant allele seem to confer a phenotype that is dominant or recessive relative to the wild-type phenotype? Why?

Answer

4 Based on the results that you obtained in question 2, is the **Mutant 1** parent fly a true-breeding fly? What is the likely genotype of this **Mutant 1** fly? Please explain.

- You can use the **Punnett Square** tool to help determine genotypes for a given trait. In the **Punnett Square** tool, click on the different genotype options to see the resulting genotypic ratios.

Answer

5 Now you have determined some facts about the Mutant 1 allele and the trait that it causes. Given what you know, do you expect the mutant F1 flies to be homozygous or heterozygous for the allele that causes the Mutant 1 trait? According to your reasoning, if you mated two mutant F1 flies, what percentage of flies would you expect to be wild type versus mutant in the F2 progeny? Draw a Punnett square of this cross to justify your answer.

Answer

6 Now mate a mutant F1 female fly with a mutant F1 male fly. Out of the 50 F2 progeny, what percentage of flies are wild type and what percentage are mutant?

- You can collect individual flies that you have generated (for use in future experiments) by dragging them to the **Strains** box.
- To start a new mating experiment, click on the **New experiment** button.

Answer

7 From the cross in question 6, you notice that the percentage of wild-type and mutant progeny generated is not quite what you had predicted in question 5. Continue to mate the mutant F1 female fly with the mutant F1 male fly to produce 1000 progeny flies. Out of the 1000 F2 progeny, what percentage of flies are wild type and what percentage are mutant?

- To add additional flies to one cross click on the **Add more matings** button again after the initial mating between the two F1 mutant flies.

Answer

8 Record the percentages of wild-type and mutant flies that you obtained for both the 50 and the 1000 progeny sample sizes on the white board!

9 Do you notice a difference between the percentages of wild-type and mutant progeny generated when you observe 50 F2 flies versus 1000 F2 flies? Which of these two sample sizes, the smaller set of 50 flies or the larger set of 1000 flies, do you think offers a more accurate view of the inheritance pattern of the Mutant 1 trait? Explain your answer.

Answer

10 In the 1000 progeny flies generated in question 7, you are surprised by the percentages of wild-type and mutant flies that you observe.

a) Based on the Punnett square that you drew in question 5, do you expect to find a mutant F2 fly that, when crossed with a wild-type fly, produces only mutant progeny? See if you can find this fly by performing a few crosses. Are you surprised by your results? Explain your answer.
Hint: You do not need to cross all of the mutant F2 flies to answer this question, just try some.

Answer

b) You are still very puzzled by your results. Then, you remember that you noticed an unusually large number of underdeveloped dead embryos in your vial from the matings between the two F1 mutant flies (in question 6). Aha!
State a new hypothesis to explain your data. Explain your reasons for proposing this hypothesis.

Answer

11 Record your new hypothesis on the whiteboard to share with your classmates!

12 As you can see by comparing your data with that of your classmates, a lot of variability can be observed in the results from genetic crosses. You might have noticed that your results differ to some extent from what you expected, given the results you predicted in question 5. We will now use the chi-square test, a statistical analysis, to determine if our original hypothesis should or should not be rejected. The chi-square test takes into consideration the size of your progeny sample and the amount of variation (for instance, the number of different phenotypes) that is being assessed in any particular cross.

The formula for the chi-square test is:

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

This formula is easier to understand and apply when expressed as shown in the chart below.

a) Based on the Punnett square that you drew in question 5, fill in the numbers that you would have expected for each phenotypic category for 1000 progeny in the “Expected” column below and the numbers you actually observed in question 7 in the “Observed” column below. Then perform the calculations indicated to complete the chart.

	Observed	Expected	(O - E)	(O - E) ²	(O - E) ² / E
Mutant					
Wild type					
Total	1000	1000	-	-	$\chi^2 =$

You can use this calculated chi-square value (χ^2) to determine the probability (p value) that any deviation that you saw between the “Expected” and “Observed” data is merely due to chance. To do this, you’ll also need to determine the “Degrees of freedom” in your data. This is a measure of the amount of variation that existed in your experiment. There were only two different phenotypic classes (wild type and mutant) in your cross. You can calculate “Degrees of freedom” using the following formula:

$$\text{Degrees of freedom} = \text{Number of classes} - 1$$

Enter the results of your calculations below:

$$\text{Chi-square } (\chi^2) \text{ value} = \text{_____} \text{ Degrees of freedom} = \text{_____}$$

Now you can use the chi-square table (included at the end of this exercise) to determine the p value from this test.

$$\text{probability } (p) \text{ value} = \text{_____}$$

A p value of 0.05 or less indicates that your hypothesis was incorrect and that you should reject it. This p value of 0.05 means that any difference that you observed between your expected data and your observed data would only occur JUST BY CHANCE 5% of the time. Based on the p value you obtained, should you reject your original hypothesis (from question 5)? Explain.

Answer

b) Now repeat the chi-square test using the predictions from your new hypothesis (from question 11) and the data generated in question 7. Could your new hypothesis explain the observed data? Fill in the chi-square table below and explain your reasoning.

	Observed	Expected	(O - E)	(O - E) ²	(O - E) ² / E
Mutant					
Wild type					
Total	1000	1000	-	-	$\chi^2 =$

Chi-square (χ^2) value = _____ Degrees of freedom = _____ probability (p) value = _____

Answer

Reference: Chi-Square Table

		Degrees of Freedom							
		1	2	3	4	5	6	7	8
<i>P</i> 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.