Drosophila PreLab

Introduction

The fruit fly is an excellent organism for genetic studies because it has simple food requirements, occupies little space, is hardy, completes its life cycle in about 12 days at room temperature, produces large numbers of offspring, can be immobilized easily for examination, and has many types of hereditary variations that can be observed with low-power magnification. Fruit flies have a small number of chromosomes (four pairs). Much research about the genetics of fruit flies during the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes. In this lab, we'll be doing virtual fly matings to study how traits are inherited.

Life cycle of the fruit fly

The eggs are small, oval shaped, and can be seen with the naked eye. The eggs hatch into larvae after about a day. The wormlike larva eats almost continuously, and its black mouth parts can easily be seen moving back and forth even when the larva itself is less distinct. The larva sheds its skin twice as it increases in size. When a mature larva in a lab culture is about to become a pupa, the last larval covering then becomes harder and darker, forming the pupal case. Through this case the later stages of metamorphosis to an adult fly can be observed. In particular, the eyes, the wings, and the legs become readily visible. When metamorphosis is complete, the adult flies emerge from the pupal case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of an adult fly. They live a month or more and then die. A female does not mate for about ten to twelve hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. To ensure a controlled mating, it is necessary to use females that have not mated before.

Genetic notation:

In fruit fly genetics, the normal fly is called a "wild type" and any fly exhibiting a phenotypic mutation is called a "mutant". Mutant flies are given names that generally denote the type of mutation the fly exhibits. For example, the mutant "ebony" has a much darker body than the wild type fly. Each mutation is also given a letter code. Thus, in the case of ebony, the code is a lower case e. The wild type fly is denoted by a superscript $^+$ over the mutant letter code. For example, e^+ denotes a wild type fly for the ebony body trait - meaning it has normal body color (not ebony). The above description is for a gene located on an autosome (a non-sex chromosome). Of course, fruit flies also have sex chromosomes and they contain a subset of genes as well. If the gene is located on a sex chromosome, we use a slightly different notation. Under normal diploid conditions a female fruit fly has two X chromosomes, a male has an X and a Y chromosome. Sex-linked genes are located on one of the sex chromosomes (usually the X chromosome). Thus, the genotypic

notation for a mutant gene for white eye color on the X chromosome would look like:

$X^w X^w$ = white-eyed female

$X^{w+}X^w$ = wild type heterozygote female

$X^w Y$ = white-eyed male

$X^{w+} Y$ = wild type male

It may take a while to get used to this notation, but it's well worth the effort as latter classes will undoubtedly use this notation.

To get use to the idea of phenotypic mutations, you will be given several strains of mutant flies. In your lab notebook, describe their morphology paying particular attention to eye color, body color, and wing shape. You should begin with a wild type fly so that you will have some basis for comparison. You may want to draw a picture.

Determining the Type of Inheritance in a Cross

So now you know what a wild type fly and several mutant flies look like phenotypically, but that really doesn't tell us what their genotypes are (Why not?). In order to determine the genotypes, we must look at the ratios of phenotypes of the offspring. You will be given several bottles of fruit flies. Each bottle represents the F_1 (first filial) generation of a cross set up two weeks ago. Recall that there must be a P (parental) generation before you can have a F_1 generation. The idea here is to work back to the parental genotypes from the ratios of the F_1 phenotypes you are given today. This requires a bit of detective work, since there are several possible parental crosses.

In the first cross you are given there is a single mutation. Anesthetize the flies using ether (your instructor will demonstrate). Observe them carefully under the dissecting microscope and record your observations in your lab notebook. The first step is to identify the sex and type of fly — mutant or wild type. Once you have determined the type of mutation, you should write out all possible crosses that could have produced these offspring. Remember, a single trait can be inherited on an autosome or on a sex chromosome. Is your mutation on an autosome or sex chromosome? How would you tell?

Assume you identify 100 flies and record the following data for the offspring of an unknown cross involving a single trait.

	Male	Female	Total
Wild type	40	30	70
mutant	15	15	30

What are the possible crosses that could have produced this pattern of offspring? If the mutation was inherited as a simple autosomal recessive, then we might suspect the parental generation was either:

1. w w X w⁺w⁺
2. w⁺w X w⁺w
3. w⁺w X w w
4. w⁺w X w⁺w⁺

Remember a w represents the mutant trait and a w⁺ represents the wild type.

Which of these 4 possibilities would give you the same approximate pattern of offspring you actually observed? Which of these 4 possibilities can be ruled out? Take parental cross 1 above for example. In a cross between a homozygous wild type and a homozygous mutant type you would expect to get all heterozygous wild type offspring. Since heterozygous offspring all appear phenotypically to be wild type flies, you could now rule this cross our because you actually observed 30 mutant flies in you F_1 population.

Similarly, if this mutation was inherited as a sex-linked trait, you might predict one of the following parental crosses:

1. X^wX^w x X^wY
2. $X^{w+}X^{w+}$ x $X^{w+}Y$
3. $X^{w+}X^w$ x X^wY
4. $X^{w+}X^w$ x $X^{w+}Y$

Again you can do each cross and quickly rule out the ones that do not fit with your observed pattern of offspring. Assume you have ruled out all crosses but a parental cross between two heterozygotes for a trait located on an autosome (i.e. a parental cross of w⁺w x w⁺w). In this cross you would expect to see a phenotypic ratio of 3 wild type for every 1 mutant type regardless of the sex of the fly. Does this expected outcome fit with your observed data? It sure looks like it, but how can you be sure?

To test your hypothesis that the observed ratio of 70:30 is the same as the expected ratio of 3:1, we can use a statistic called the Chi square statistic.

Chi square statistic:

Statistics can be used to determine if differences among groups are **significant**. The statistical test most frequently used to determine whether data obtained experimentally provide a good fit to the expected data is the chi-square test. This test can be used to determine if deviations from the expected values are due to chance alone, or to some other circumstance.

To determine if the observed data fall within acceptable limits, a chi-square (X^2) analysis is performed to test the validity of the **null hypothesis** (that there is no statistically significant difference between the observed and expected data). If the chi-square analysis indicates that the observed data vary too much from the expected data, an **alternate hypothesis** is accepted.

The formula for chi-square is:

$$\chi^2 = \Sigma \frac{(o\text{-}e)^2}{e}$$

where o = observed number of individuals
e = expected number of individuals
Σ = the sum of the values (in this case, the differences, squared, divided by the number expected)

In our case, we can use the actual observed number of flies of each type as our *observed* values. We can find the *expected* number of flies of each type for a 3:1 ratio by using the same number of flies (100 flies) and dividing by 4 to give us *expected* values of 75:25 for a 3:1 ratio of 100 flies.

Calculate the chi square statistic x^2 by completing the following steps:

1. For each *observed* number in the table subtract the corresponding *expected* number ($O - E$).
2. Square the difference [$(O - E)^2$].
3. Divide the squares obtained for each cell in the table by the *expected* number for that cell [$(O - E)^2 / E$].
4. Sum all the values for $(O - E)^2 / E$. This is the chi square statistic.

For our example, the calculation would be:

	Observed	Expected	$(O - E)$	$(O - E)^2$	$(O - E)^2/E$
Wild type	70	75	5	25	0.31
Mutant type	30	25	5	25	1.00

totals	100	100			

$$x^2 = 1.31$$

Having now obtained our chi square statistic $x^2 = 1.31$, we look up in a table of the Chi Square X^2 distribution the probability attached to it. Before we can do this, however, we need to know the *degrees of freedom*. When a comparison is made between one sample and another, a simple rule is that the degrees of freedom equal (number of columns minus one) x (number of rows minus one) not counting the totals for rows or columns. For our data this gives (2-1) x (2-1) = 1. Entering the Chi square distribution table with 1 degree of freedom and reading along the row we find our value of x^2 (1.31) lies between 0.455 and 2.706. The corresponding probability is 0.5<P<0.1. This is well below the conventionally accepted significance level of 0.05 or 5%, so the null hypothesis that the two distributions are the same is verified. In other words, when the computed x^2 statistic exceeds the critical value in the table for a 0.05 probability level, then we can reject the null hypothesis of equal distributions. Since our x^2 statistic (1.31) did not exceed the critical value for 0.05 probability level (3.841) we can accept the null hypothesis that a ratio of 70:30 is the same as a 75:25 ratio (within 5% error).

Chi Square distribution table

Df	0.5	0.10	0.05	0.02	0.01	0.001
1	0.455	2.706	3.841	5.412	6.635	10.827
2	1.386	4.605	5.991	7.824	9.210	13.815
3	2.366	6.251	7.815	9.837	11.345	16.268
4	3.357	7.779	9.488	11.668	13.277	18.465
5	4.351	9.236	11.070	13.388	15.086	20.517

To put this into context, it means that we do have a 3:1 ratio of wild type to mutant offspring and that these offspring could have come from a cross between two heterozygotes.

Pre-Lab Questions:
Complete the answers to these questions on a separate sheet of paper and be prepared to hand it in at the very beginning of lab.

1. Why are fruit flies excellent organisms for genetic studies?

2. What is a null hypothesis?

3. Why is the chi-square test needed?

4. How do you determine degrees of freedom?

5. What is a p-value, and what does it mean?

6. You are given a monohybrid cross between a wild type fruit fly and a mutant fly called "apterous" (ap) which lacks wings. If both flies are homozygous, how would you denote the cross described? In other words, write out the parental genotypes in the form used for fruit fly genetics.

7. In the cross described in question #6, how would you write the cross if both parents were heterozygous?

8. What would the expected phenotypic ratio of offspring from the cross is question #1? Show the cross and the results.

9. What would be the expected phenotypic ratio of offspring in question #2? Show the cross.