Experiment 8
(Lab Periods 9 and 12)

Genetics and the analysis of inheritance

Genetics is the study of inheritance that traces its scientific beginning to the work undertaken by Gregor Mendel. It was Mendel who proposed the concept of what we now know as genes, although he did not use this term, in order to explain the inheritance of different traits in the pea plants that he worked with. His proposal was that each physical trait of an organism is the result of the action of a separate gene and that different forms of a gene (known as alleles) give rise to different forms of a trait, such as differences in flower color that he studied. In addition to the concept of genes, Mendel proposed certain 'laws' about the action of these genes. The first was the Law of Segregation. This Law was based on Mendel's explanation of inheritance, which proposed that every individual received two copies of each gene, one from each parent. When that individual in turn passed on it's genes, only one copy of each gene would be passed to each offspring. Mendel proposed that the two copies segregated to different gametes at this stage such that each gamete only contained a single copy. The two copies were restored in the offspring by the fusion of two gametes, each carrying a single copy. Mendel's second Law concerned the interaction of different genes and stated that when two genes segregate, the segregation of the first gene (first pair of alleles) will be independent of the segregation of the second pair of alleles. This means that we can study the inheritance of the two traits separately.

We now know that although Mendel gave us the first insight into the correct mechanism of inheritance, his proposals were much simpler than the reality. With respect to the second law, we now know that there are many exceptions to Mendel's two laws. For example genes can be on the same chromosome so that they do not segregate independently but stay together, or remain "linked". Mendel never noticed an exception since the traits he happened to study were not linked. In addition, the concept of individual traits being determined by separate genes is now known to be an oversimplification. In reality, each trait is influenced by the action of many different genes and every gene influences many different traits. However, in many cases we are able to study how a particular gene influences a specific trait, even though many other genes also play an important role. In this lab, you should keep in mind when examining specific traits that although you are only considering the action of a single gene it is not the only gene affecting that trait.

In this lab you will follow the inheritance of several traits in the experimental organism *Drosophila melanogaster*, or the fruit fly. This is one of the most common lab organisms, particularly for genetic studies, since fruit flies grow quickly (a new generation emerges in about 2 weeks), produce many offspring and are easy to maintain. In addition, there is now a great deal known about Drosophila biology, including the sequence of the entire genome that was completed in the year 2000, so there is an excellent foundation to build upon. This experiment will be divided into two sections. In the first part you will familiarize yourself with the traits you are studying and then set up a cross. These crosses will sit for 3 weeks until the final lab period (the week before the second exam) at which time you will count the number of offspring with the different traits and analyze the inheritance of each.

You are going to study the inheritance of genes that influence two separate traits in Drosophila, wing shape and eye color. In natural populations of Drosophila the flies have flat wings that grow straight back. This is what we refer to as the 'wild type' appearance (or wild type phenotype). You will examine two different mutants in wing shape, Curly (in which the wings grow curled upwards) and Diachete (in which the wings grow outwards at a slight angle instead of straight back), which are due to alleles of different genes. In the case of eye color, the wild type phenotype is brick-red and you will study the inheritance of an allele that gives rise to purple eyes.

The cross that you will perform is as follows:

Male with Curly wings and Purple eyes x Female with Diachete wings and red eyes

What you are looking for in the progeny is the result of the inheritance of the alleles that give rise to each particular trait variation. First we will consider wing shape. Curly wings are caused by a dominant allele of a gene on chromosome number 2 in Drosophila while Diachete wings are caused by a dominant allele of a different gene, which is on chromosome 3. Therefore, the two genes will segregate independently and be inherited separately. The male fly with Curly wings is heterozygous, so it has one Curly allele and one wild type allele (but has Curly wings since Curly is dominant). The female fly is also heterozygous, having one Diachete allele and one wild type allele. During segregation, the Curly allele (Cy) and the wild type allele (Cy+) will segregate to different gametes so that half of all gametes that the male fly produces will carry Cy and the other half will carry Cy+. Similarly, for the female, half of the gametes will carry the Diachete allele (D) while the other half will carry the wild type allele for this gene (D+). What is important to keep in mind is that the male fly has no Diachete alleles, only D+ (so we refer to its genotype as D+D+) while the female fly has no Curly alleles, only Cy+ (so she is Cy+Cy+).

When the various sperm and egg cells fuse to generate the progeny that you will count we expect the following:

  i. All sperm will carry the D+ allele. Half will carry Cy+ and half will carry Cy
  ii. All eggs will carry the Cy+ allele. Half will carry D+ and half will carry D.

When a zygote is formed the alleles it will inherit will depend upon which alleles are carried by the specific sperm and egg involved. Based on (i) and (ii) however, we can easily see that we expect the following:

¼ of zygotes are expected to carry D+D+ and Cy+Cy+. Since neither Cy nor D alleles are inherited these will develop into flies with wild type wings.

¼ of zygotes are expected to carry D+D and Cy+Cy+. The D allele will cause these to develop into flies with Diachete wings.

¼ of zygotes are expected to carry D+D+ and Cy+Cy. The Cy allele will cause these to develop into flies with Curly wings.

¼ of zygotes are expected to carry D+D and Cy+Cy. The inheritance of both D and Cy means that these flies will develop into flies with Curly wings that are also Diachete.

Overall, therefore, we expect that when you count the progeny you expect to observe the four phenotypes of (i) wildtpye (ii) Curly (iii) Diachete and (iv) Curly *and* Diachete and each of them should be roughly ¼ of the total progeny.

Now we can consider eye color, the second trait you will study. The male flies you will use in the cross have Purple eyes, a phenotype that is caused by a dominant allele (P) on chromosome 2. These males are heterozygous meaning that they have one copy of P and one copy of the wild type allele (P+). The females do not have Purple eyes. Instead they carry two copies of P+ and have the wild type eye color, which is red. As a result of segregation in the male, half of the gametes produce will carry P and half will carry P+. Therefore, in the zygotes we expect:

½ of the zygotes should carry PP+. These will develop into flies with Purple eyes.

½ of the zygotes will inherit the P+ from the sperm and have P+P+. These will develop into flies with wild type eye color since there is no P allele.

Overall, therefore, we expect that when you count the progeny you will find that ½ have Purple eyes and ½ have red eyes.

Combining the traits: What we have discussed above is what you expect to observe in the progeny of the cross as a result of the alleles involved. Obviously, we considered wing shape and eye color separately and discussed the expectation for each trait separately, but each progeny fly that you will examine has both wings and eyes so we must consider what we expect in terms of both traits together.

Normally, the two genes involved in wings shape (Cy and D) and the gene involved in eye color (P; but remember, these are only the genes you happen to be studying, many other genes are involved but don't contribute to the variation in our particular cross) would be inherited independently, and in fact we discussed inheritance of Cy and D independently (i.e. segregation of Cy does not affect segregation of D). However, in this particular study, both Cy and P are on the same chromosome, very close together, and violate Mendel's second law. If you paid attention above you noticed that both Cy and P are on chromosome 2, even though they are alleles of distinct genes. In addition, if you remember from above, the male flies you use in the cross are both Cy+Cy and P+P. However, it is important to note that they have been bred so that the Cy allele is on one copy of chromosome 2 and P is on the other copy of chromosome 2. Another way to think of this is that Cy and P+ are on one copy while Cy+ and P are on the other copy. Segregation in genetics occurs because the two copies of a chromosome migrate to opposite poles during meiosis. When this occurs here, Cy and P *must* segregate to opposite poles, since they are on different copies of chromosome 2, and therefore, *must* end up in different gametes. The result is that a fly that inherits Cy (meaning that it will have Curly wings) cannot also inherit P, meaning that it will have red eyes.

Therefore, when we combine wing shape and eye color, the ratios we discussed above still hold. However, progeny with Curly wings should have red eyes while progeny with flat wings (regardless of Diachete) will have Purple eyes. Therefore we expect:

¼ of progeny should have Curly, straight wings with red eyes.

¼ of progeny should have Curly, Diachete wings with red eyes.

¼ of progeny should have flat, Diachete wings with Purple eyes.

¼ of progeny should have wild type wings (flat and straight) and Purple eyes.

In the last week of the lab you will look at wing shape and eye color of all progeny and compare your results with these expectations using a statistical test.
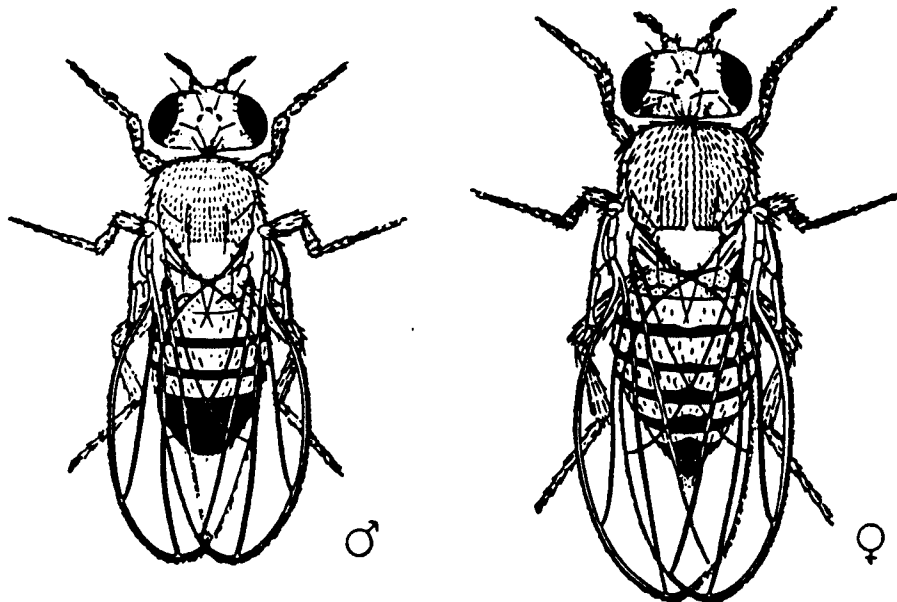
Procedure

Week 1. Setting up your cross:

In the first lab you will familiarize yourself with Drosophila. The purpose is to make sure that you can distinguish the traits that you will be studying and to be able to differentiate males and females so that you can set up crosses. You will be provided with flies with the following phenotypes:

     i.   All wild type (flat wings, no diachete and red eyes).
     ii.   Curly wings
     iii.   Diachete wings
     iv.   Curly *and* Diachete wings
     v.   Purple eyes

1. Look at the flies with the wild type phenotype. Compare these to flies with phenotypes (ii) through (v) listed above.

2. You will be provided with a mixture of flies of types (i) through (v). Separate them into five groups based on phenotypes and have your instructor check that you have done this correctly.

3. You will be provided with some more wild type flies. Find 5 males and 5 females and separate them and have your instructor check that you have separated them correctly.

4. Take the virgin flies that are provided. These are flies that have been secluded since they eclosed so that we know that they have not mated. This is very important, particularly for the females, since we must be certain about what gametes gave rise to the progeny you count. Find a male and a female and place them in a vial together. Label the vial with your group number and section and leave it with your instructor. The flies will mate and produce mature progeny within 2 to 3 weeks.

Week 2. Analysis:

1. Count all of the flies with the following phenotypes:

     Curly, straight wings and red eyes.
     Curly, Diachete wings and red eyes.
     Flat, Diachete wings and Purple eyes.
     Flat, straight wings and Purple eyes.

Also, record any flies that have phenotypes other than the 4 expected.

Calculations

Genetics is a science that involves some degree of random, or chance, variation. When we discussed the expected results for the cross we gave the proportion of progeny expected to display a particular phenotype based on what alleles would be inherited. However, in real life, you have a finite number of progeny so you may not observe *exactly* the proportion expected. This is the same as a situation in which a coin is tossed 10 times. You expect to observe 5 heads and 5 tails but also know that you will not always observe these exact numbers due to chance variation.

The existence of this chance variation gives rise to the difficulty in Genetic of determining with confidence whether or not what we observe in our progeny matches what we expected. Therefore, a statistical test called the Chi Square test is often used to determine whether there are significant differences between observed and expected results. The simple idea behind the test is to compare the difference between what we observed and what we expected to the difference that should arise due to chance (or random) variation, allowing us to assess whether or not any difference we see is likely to have arisen from such random variation.

Chi Square Test

In cases where the expected outcomes of an experiment are known the experiment yields data that can be broken down into classes, a statistical test known as Chi Square (χ^2) test can be used to determine if the observed results fit the expected results. For example, the expected results of genetic crosses should conform to the Mendelian ratios. In a dihybrid cross involving dominant and recessive traits, the expected results of a cross between heterozygous individuals would yield a phenotypic ratio of 9:3:3:1.

Suppose this experiment were actually performed with colored kernels of corn and 300 kernels were collected with the following phenotypic frequencies:

> 150 phenotypes dominant for both traits
> 60 phenotypes dominant for the first trait and recessive for the other
> 67 phenotypes recessive for the first trait and dominant for the other
> 23 phenotypes recessive for both traits

How would you determine whether these results were a variation of a 9:3:3:1 ratio? The actual ratio in this case is close to the 6:3:3:1 . Has the experiment failed to show Mendelian inheritance or are the results simply the consequences of variability? How do you decide whether to accept or reject the hypothesis that Mendelian inheritance does not apply to this genetic experiment?

The Chi Square test can be applied to provide an objective comparison of the observed results and the expected results. In the test, a number that represents the level of variability in the data is calculated and compared to a number that represents "acceptable" variability. This number is called the Chi Square Value (χ^2). To perform this test, a frequency table must be created (see **Example table**). The expected results are based on a 9:3:3:1 ratio for the 300 kernels.

Example table for this experiment (showing observed and expected results):

	Dominant	Dom+Rec	Rec+Dom	Recessive	Total
Observed	150	60	67	23	300
Expected	169	56	56	19	300

The Chi Square Value is calculated using the following formula:

$$?^2 = ? (O-E)^2 / E$$

> ? is the Greek letter sigma, indicating "sum of"
> O is the observed frequency
> E is the expected frequency

It should be clear from inspecting this formula that the value of $?^2$ will be zero when there is perfect agreement between the observed and expected results, while its value will be large when the difference between observed and expected results are great.

To calculate $?^2$ for the data in the example requires the following steps:

$$?^2 = (150-169)^2/169+ (60-56)^2/56+ (67-56)^2/56+ (23-19)^2/19$$
$$= (-19)^2/169 + (4)^2/56 + (11)^2/56 + (4)^2/19$$
$$= 2.14 + 0.29 + 2.16 + 0.84$$
$$= 5.43$$

To determine if a value of 5.43 for $?^2$ is large and indicates poor experimental agreement, the calculated $?^2$ value must be compared to a theoretical $?^2$ value for similar experimental designs. A theoretical table is given at the end of the lab (Chi-Square Distribution table).

To use this table it is necessary to understand that $?^2$ represents a value describing variation in results. These results should occur in proscribed classes. The number of classes in our example is four. To use the table you must first also have a parameter called the degrees of freedom (n) that is numerically equal to one less than the number of classes.

n = (no. of classes - 1)

In our sample,
    n = (4 - 1) = 3 degrees of freedom

For any data set consisting of classes of results it is possible to calculate the $?^2$ value. If the difference between the observed and the expected results varies randomly, then the $?^2$

values should vary from zero to some very large number. If the χ^2 value is large, then there is a marked deviation from the expected ratio. How does one determine if the observed deviations are within the limits expected by chance? Generally, statisticians have agreed on the arbitrary limits of odds of 1 chance in 20 (probability or P=0.05) for drawing the line between acceptance and rejection of the data as a satisfactory fit to the expected ratio.

The Chi Square table lists a statistical table of Chi square values. Across the top of the table are the probability (P) values while the degrees of freedom (n) are on the side of the table. For our example, using three degrees of freedom (n=3) and P = 0.05, the χ^2 value is 7.8, the maximum value of Chi Square that we are willing to accept and still attribute the deviations to chance alone.

For our example, in the n=3 row, 5.43 falls between 0.10 and 0.20 probability columns. This means the probability that the deviations we observed may be attributed to chance alone is 10% to 20%. Our χ^2 value is within the acceptable limit (i.e., it "fits"!). The deviations can be attributed to chance alone and we can accept the data as fitting the 9:3:3:1 ratio.

Chi-Square Distribution

Degrees of Freedom	Probability						
	.90	.70	.50	.30	.10	.05	.01
1	.02	.15	.46	1.07	2.71	3.84	6.64
2	.21	.71	1.39	2.41	4.60	5.99	9.21
3	.58	1.42	2.37	3.66	6.25	7.82	11.34
4	1.06	2.20	3.36	4.88	7.78	9.49	13.28
5	1.61	3.00	4.35	6.06	9.24	11.07	15.09
6	2.20	3.83	5.35	7.23	10.64	12.59	16.81
7	2.83	4.67	6.35	8.38	12.02	14.07	18.48
8	3.49	5.53	7.34	9.52	13.36	15.51	20.09
9	4.17	6.39	8.34	10.66	14.68	16.92	21.67
10	4.86	7.27	9.34	11.78	15.99	18.31	23.21
	Non - significant					Significant	

Based on the example above, calculate the χ^2 value for your results and determine whether or not your data fall within the limits of chance error based on your expectation.

Lab Report

There is a lab report due for this experiment. It should include the χ^2 analysis and an interpretation of the results. In addition, make sure that you provide a possible explanation for any significant deviation you might find using the χ^2 test as well as an explanation of any unexpected phenotypes you may have observed in the progeny.