Genetics of Drosophila

In 1865, Gregor Mendel published a paper on the patterns of genetic inheritance in the common garden pea. This revolutionary work provided the basis for future study of genetics. Mendel hypothesized that heredity was passed on by discrete particles, rather than by the blending of parental traits, as was believed at the time, strongly affecting the argument over Darwin's theory of evolution.

Mendel proposed two very basic laws which serve as the cornerstones of modern genetics: Mendel's Law of Segregation and Law of Independent Assortment.

Mendel's Laws of Genetic Inheritance

Mendel's Law of Segregation states that for each trait (gene), each organism carries two factors (alleles), and that each of the organism's gametes contains one and only one of these factors. In this way, the alleles segregate during meiosis, providing for genetic variability among the organism's offspring. This is apparent in monohybrid crosses—matings involving only one trait.

Mendel's Law of Independent Assortment, found in dihybrid crosses (crosses involving two traits), states that the alleles for one trait will separate independently of the alleles in another trait. This means that with two genes (A and B), each with two possible alleles (A, a and B, b), there are four possible combinations gametes can receive (AB, Ab, aB, or ab). This helps to ensure genetic variability among offspring.

Although Mendel's laws have proven to be true, they have their limitations. It must be assumed that the genes involved are on different chromosomes. If they are on the same chromosome, the assortment of their alleles will be closely linked instead of being independent. It must also be assumed that the genes in question are not located on the X chromosome; this could cause the expression of the trait to be linked to the sex of the offspring. Color blindness in humans is a good example of this type of heredity. None of the traits Mendel selected in his pea plant investigations were sex linked, or located on the same chromosome.

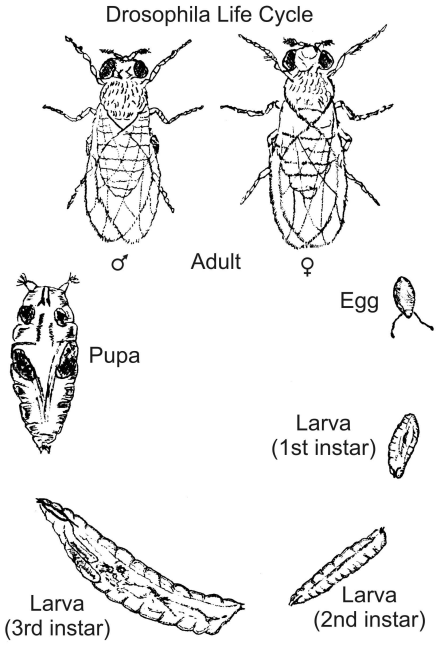
Since Mendel's time, our knowledge of genetics, as well as the tools we use, have advanced drastically. Instead of crossing varieties of pea plants and observing the results as Mendel did, we now use restriction enzymes, electrophoresis, and basepair sequencing to learn more about traits and their expression. Yet even with these advances, the basic concepts of genetic inheritance described by Mendel so many years ago still stand.

Drosophila Use in Genetic Research

Drosophila melanogaster, the common fruit fly, was first used in genetic experiments in 1907 by Thomas Hunt Morgan of Columbia University, and has been a staple of genetic research ever since. Drosophila specimens are well suited to investigations into Mendelian patterns of inheritance; they are small, produce large numbers of offspring, have many easily discernible mutations, have only four pairs of chromosomes, and complete their entire life cycle in approximately 12 days. Additionally, Drosophila are relatively easy to maintain, as they are hardy and have simple food requirements.

Since the fruit fly was selected for study nearly a hundred years ago, a great deal has been learned about its genome. In fact, the first chromosome map of any kind was constructed to

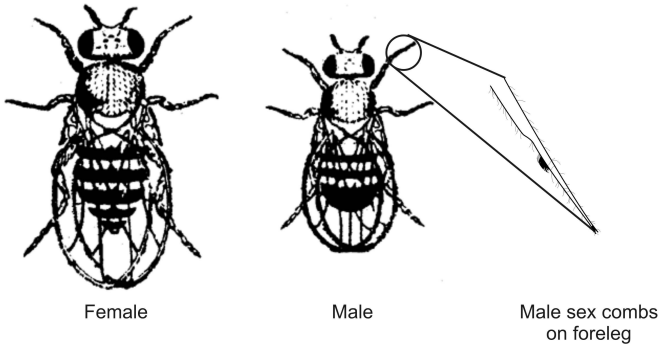
detail the fruit fly. Chromosomes 1 (the X chromosome), 2, and 3 are very large, and the Y chromosome and number 4 are extremely small. Thousands of genes reside on these four chromosomes, many of which are universal in nature, existing in most eukaryotic forms, including humans. The similarities between the Drosophila genome and those of other species is helpful in determining patterns of evolution and species divergence.

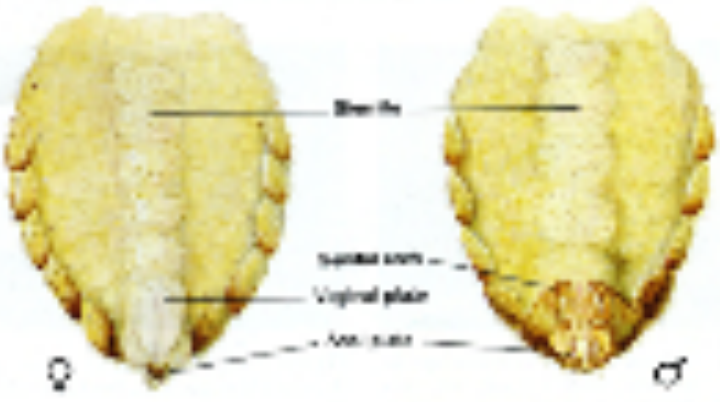


Drosophila embryos develop in the egg membrane. The egg hatches and produces a larva which feeds by burrowing through the medium. The larval period consists of three stages, or instars, the end of each stage marked by a molt. The first instar is the newly hatched larva; the third instar is the final larval stage, where the larva may attain a length of 4.5mm. Near the end of the larval period, the third instar larva will crawl up the sides of the culture vial, attach themselves to a dry surface (the jar, the filter paper, etc.) and form pupae. After a period of time the adults emerge.

It takes one or two days for Drosophila eggs to hatch into larvae, four to five days for the larvae to enter pupae, and four days for the pupa stage. The duration of these stages, however, vary with the temperature; at 20°C the average length of the egg–larval period is eight days, while at 25°C it is reduced to five days. Thus at 25°C the life cycle may be completed in about 10 days; at 20°C, 15 days are required.

Different body features characterize the male and female flies. The females are slightly larger and have a light-colored, pointed abdomen. The abdomen of the males will be dark and blunt. The male flies also have dark bristles on the upper portion of the forelegs, which are known as sex combs.





In the following experiment, parental generations (P1) of wild-type and mutant strains of Drosophila with various traits to demonstrate basic genetic principles are used. Monohybrid, dihybrid, and sex-linked crosses are performed; the offspring of the first cross, the F_1 generation, are normally allowed to breed among themselves to produce the F_2 generation. Members from the F_1 and F_2 generations are collected and their traits observed to draw conclusions about genetic inheritance.

The second filial generation, or F_2, represents the flies that result form self-fertilization or inbreeding among members of the F_1 generation.

Punnett Square

Based on the laws of segregation and independent assortment, a Punnett square is extremely important in determining the outcome of crosses in Mendelian genetics; it clearly displays the possible combinations in chart form.

The simplest Punnett square to construct is one for a monohybrid cross. A good example of this is a cross between female fruit flies with vestigial wings and male wild-type fruit flies. Determining the genotype of the flies being crossed is vital to the accuracy of the results of a Punnett square; if it is known that the trait for vestigial wings is a recessive mutation, the flies with vestigial wings must be homozygous recessive for the first trait, and therefore have a genotype of vv. Assuming that the wild-type flies are heterozygous dominant, they will have a genotype of Vv. According to the Law of Segregation, only one of the alleles for the trait can be passed on to a gamete for each parental fly. Therefore, the male wild-type fly could pass either the V or the v allele on to its offspring. Likewise, the female, vestigial fly can pass on only one of her alleles for the trait. In her case, however, they are both v. Therefore, the possible allelic combinations in the offspring are Vv and vv. This is diagrammed in a Punnett square, below.

Males

Females	V	v
v	Vv	Vv
v	Vv	vv

The two possible genotypes, Vv an vv, will exist in a 1:1 ratio, and the phenotypic ratio will also be 1:1 with as many offspring with vestigial wings as with normal wings.

Punnett squares become more complicated when diagramming a dihybrid cross. Due to the complex nature of dihybrid crosses—or even worse, crosses with three or more traits—it is even more important to diagram the crosses with a Punnett square.

For an example of a dihybrid cross, females with normal eyes and vestigial wings can be crossed with male flies that have sepia (dark brown) eyes and normal wings, assuming that the females have the genotype Ssvv, and the males have the genotype ssVV. Again, each parent can donate only one allele for each trait to the offspring, but by applying Mendel's Law of Independent Assortment, the alleles for the two traits should be distributed without regard to the distribution

Males

of the other. There are, therefore, four possible combinations of alleles each parent can donate to any one gamete. The females can donate the set of alleles Sv or sv; the males can only donate the set of alleles sV. The Punnett square for this cross is diagrammed below.

		sV	sV	sV	sV
Females	Sv	SsVv	SsVv	SsVv	SsVv
	Sv	SsVv	SsVv	SsVv	SsVv
	sv	ssVv	ssVv	ssVv	ssVv
	sv	ssVv	ssVv	ssVv	ssVv

Chi-Square Test

The chi-square test is a statistical tool that compares experiment results with an accepted set of data to determine how much the experimental values deviated from the accepted ones and whether or not that deviation can be explained solely by chance.

The square of the difference between the observed and expected values $(O - E)^2$ for each data point (phenotype category in this case) is calculated. Then, by dividing this by the expected value, the amount of deviation between the experiment data and the accepted value for that data point can be determined. Adding the statistic for each data point yields a value known as the X^2 (chi-square) statistic.

$$X^2 = \sum (O - E)^2/E$$

Because all the values for each category, or data point, are being added together, the value of X2 will rise as the number of data points used increases. For this reason, "degrees of freedom" must be included in the parameters of the analysis. The number of degrees of freedom (v) for a chi-square test is equal to the number of data points minus one. The number of degrees of freedom does not have an impact on the value of X^2 itself, but rather is used in the interpretation of the importance of the value, as shown in the chi-square table, below. The numbers get larger as you go down and the value for degrees of freedom increases.

Chi-Square Table							
DF	a						
v	P=0.99	0.95	0.80	0.50	0.20	0.05	0.01
1	0.00016	0.00393	0.06420	0.45500	1.64200	3.84100	6.63500
2	0.02010	0.10300	0.44600	1.38600	3.21900	5.99100	9.21000
3	0.11500	0.35200	1.00500	2.36600	4.64200	7.81500	11.34500
4	0.29700	0.71100	1.64900	3.35700	5.98900	9.44800	13.27700
5	0.55400	1.14500	2.34300	4.35100	7.28900	11.07000	15.08600
6	0.87200	1.63500	3.07000	5.34800	8.55800	12.59200	16.81200
7	1.23900	2.16700	3.82200	6.34600	9.80300	14.06700	18.47500
8	1.64600	2.73300	4.59400	7.34400	11.03000	15.50700	20.09000

9	2.08800	3.32500	5.38000	8.34300	12.24200	16.91900	21.66600
10	2.55800	3.94000	6.17900	9.34200	13.44200	18.30700	23.20900
15	5.22900	7.26100	10.30700	14.33900	19.31100	24.99600	30.57800
20	8.26000	10.85100	14.57800	19.33700	25.03800	31.41000	37.56600
25	11.52400	14.61100	18.94000	24.33700	30.67500	37.65200	44.31400
30	14.95300	18.49300	23.36400	29.33600	36.25000	43.77300	50.89200

First two hypotheses, H_0 and H_1, must be formulated, with H_1 stating that the variations cannot be explained solely by chance, and H_0 stating that they can be determined solely by chance. This will be determined by the value of "a", which is defined as the probability that H_1 can be accepted as true. In order to justify H_0, "a" must be quite low, depending on the specific needs of the experiment and the confidence level required in the data.

The value of "a" is determined using the chi-square chart: the value of X^2 is found in the row for the correct number of degrees of freedom (v). It is likely that it will be between values; in this case the value of "a" should be approximated. The probability that H_0 can be accepted as true can then be defined as 1 – a.

For an experiment with a relatively small sample size, a confidence level of between 50% and 80% is acceptable.

Example:

Assuming only two phenotypic categories, vestigial wings and normal wings, use the following table of results for the F_2 generation:

Phenotype	No. of Males	No. of Females
Vestigial Wings	14	12
Normal Wings	36	38

The calculation needs to be performed only twice—once for each phenotype. Adding the results together provides the value for X^2.

Total number of flies observed: 100
Flies with vestigial wings: 26
Flies with normal wings: 74
Expected ratio: 3:1
Expected number of flies with normal wings: 75
Expected number of flies with vestigial wings: 25

$$X^2 = \frac{(74-75)^2}{75} + \frac{(26-25)^2}{25} = 0.013 + 0.04 = 0.053$$

Since only two phenotypes are used, there will be only one degree of freedom, as the number of degrees of freedom is one less than the number of phenotypic categories. Since the X^2 value is

less than the X^2 value for one degree of freedom, at 0.05% (3.841), the null hypothesis can be accepted as true. Since the X^2 value (0.053) is between 0.00393 and 0.0642, the values for 80% and 95% confidence respectively, there is an 80 to 95% confidence that the deviations from the expected experiment values are due solely to chance.

OBJECTIVES

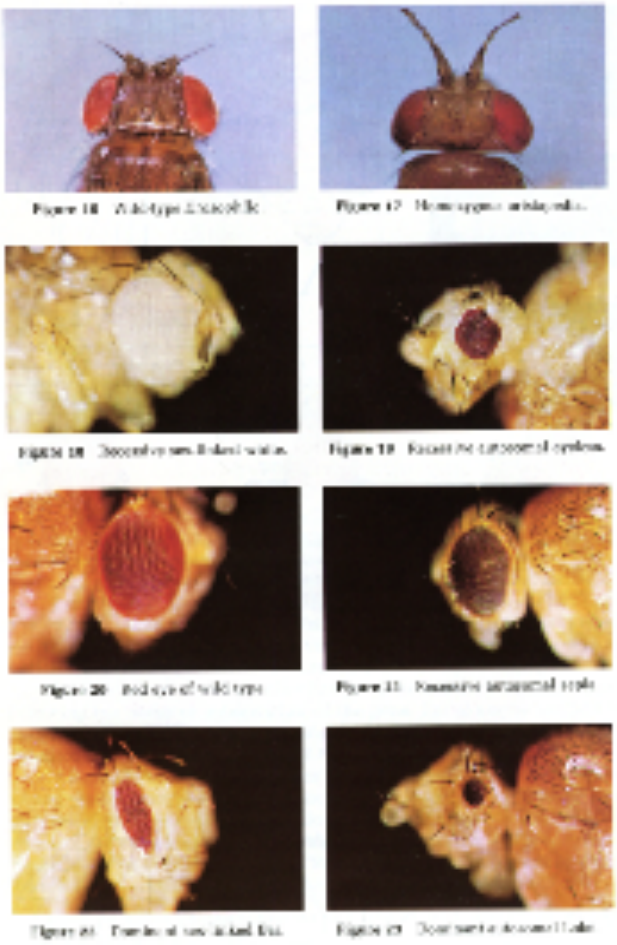
In this experiment, you will

- Learn basic handling and culture techniques for working with Drosophila.
- Apply concepts and principles of Mendelian inheritance patterns.
- Diagram sex-linked crosses.
- Gain experience sorting, sexing, and observing Drosophila phenotypes.
- Perform a chi-square statistical analysis of experimental results.

MATERIALS

culture vial of wild-type drosophila
culture vials of mutant drosophila
culture vial of white-eyed drosophila
culture vial of sex-linked cross
fly nap
paint brush
dissecting microscope
slides
drosophila vials and labels
drosophila medium, water
fly morgue
yeast

Prelab:



1) Red-eyed flies are wild type and white-eyed flies are X-linked recessive. Write out all possible crosses (red-eyed heterozygous female x red-eyed male, red-eyed homozygous female x white-eyed male, etc.) using one Punnett square per cross. There should be six different Punnett squares for each of the 6 different crosses.

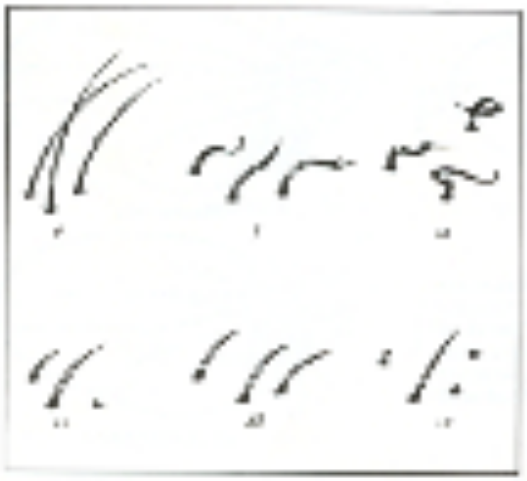
2. Calculate the percentage (ratios) of each gender and eye color phenotype that you expect to see given each cross.

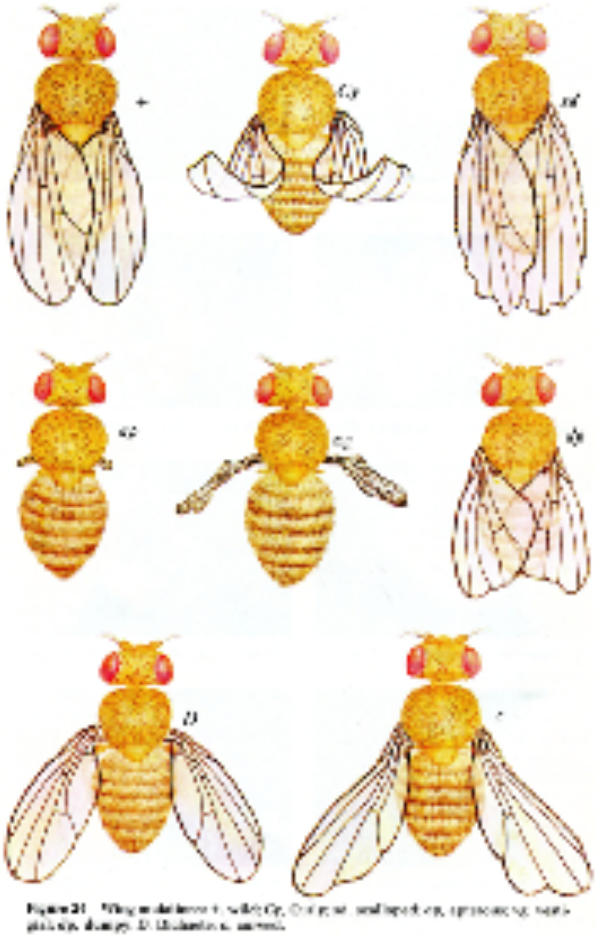
3. Calculate the ratios for each genotype in each cross.

PROCEDURE

Part A: Working with Drosophila

You will need to observe wild type Drosophila to familiarize yourself with the wild type phenotype. You will then observe different drosophila mutants. You will not be told what type of mutation the flies in your vial possess. You will have to separate the flies according to sex and phenotype and determine what trait(s) is/are different in this strain vs wild type. Please note any phenotype variations from the wild type, and name the mutations. Here are some examples of known mutations.





1. Anesthetize a vial of wild-type Drosophila. Your instructor will demonstrate the proper immobilization technique using fly nap. Knock the flies to the bottom of the vial by gently

tapping vial on a book. Quickly open the foam top and add the wand dipped in fly nap to the top of the vial and quickly replace the foam top holding the wand in place near the top of the vial. Do this quickly to prevent the flies from flying out of the vial. Make sure that you turn the vial upside down after the fly nap is added so that the flies will not fall asleep and stick to the food at the bottom of the vial.

2. Observe the flies' traits, particularly body features that distinguish males and females, eye color, wing size and shape, body color, bristles, etc. Separate the males from the females and count the total number of each gender and each phenotype. Record your observations in Table 1 in the Analysis section. If, at any time during your observations, the flies begin to become active, re-immobilize them according to your instructor. **Note:** Use the paint brush to move the flies when making observations. Replace flies in food vial when finished by putting vial on it side and gently adding the flies to the side of the vial, place the foam back on the top of the vial and turn vial upside down until the flies wake up. This is to prevent the flies from sticking the in food.

3. Anesthetize a vial of mutant Drosophila. Write down the number on the label of the vial in Table 2. Observe the flies' traits, particularly body features that distinguish males and females, eye color, wing size and shape, body color, bristles, etc. Separate the males from the females and count the total number of each gender and each phenotype. Record your observations in Table 2 in the Analysis section. Replace flies in food vial when finished using the same technique described in step 2.

Part B: Analyzing a Drosophila Cross

You will be assigned a vial with the progeny of a cross (F1 generation) without being told the genotype or phenotype of the parental strain (P). You will examine the flies in the vial and count the total number of males and females and the eye color of each gender.

3. Obtain a vial containing the progeny (F1) of a prepared Drosophila cross. These flies may exhibit a mutation.

4. Record the letter written on the vial in Table 3 in the Analysis section of the lab. This will help you to keep track of which cross you have received. This will aid in determining expected results as well as allow your instructor to identify any problems you may be having and to help correct them.

5. Immobilize flies as you have done previously and observe the flies under a stereomicroscope. If, at any time during your observations, the flies begin to become active, re-immobilize them as you have done before.

6. Separate the males from the females. Note any mutations from the wild-type phenotype, as well as whether the mutation is apparent in the male or female flies. Record your observations in Table 3.

DATA

Table 1		
Phenotypes of Wild Type Drosophila		
	Eye color	Wing size and shape
Male		
Female		

Table 2		
Phenotypes of Mutant flies		
Phenotype	No. of males	No. of females

Vial Number:____

Table 3		
Phenotypes of the F_1 Generation		
Phenotype	No. of males	No. of females

Vial Number:_____

ANALYSIS

1. Describe the parental cross you received; use genetic symbols. Example: A cross between vestigial and wild-type flies would be expressed as vv x VV. Draw a Punnett square to show the possible allelic combinations for this gene in the F_1 generation
2. Identify the genotype the F_1 flies should exhibit. Identify the phenotype. Compare your experimental results by counting the members of the F_1 generation.

3. If you allowed the F1 progeny (flies that you counted) to cross to one another to yield the F2 generation, what would you expect the genotypes and phenotypes of the males and females to be? Describe the F_1 cross you performed, and draw a Punnett square to show the allelic combinations possible in the F_2 generation.

4. Identify the genotype ratio the F_2 flies should exhibit. Identify the phenotype ratio. Explain your answer.

5. Identify the type of cross you received: monohybrid or dihybrid, autosomal or sex linked, mutations dominant or recessive.

6. Using a chi-square test, determine whether or not the variation between the observed and expected number of individuals of each phenotype can adequately be explained by chance alone. Use the following formula, and apply it to the chi-square table in the Introduction to determine the confidence that the variation is due solely to chance.

$$X^2 = \sum (O–E)^2/E$$

O = observed number of offspring for the phenotypic category

E = expected number of offspring for the phenotypic category

X^2 = _____

Confidence that variability is due entirely to chance = _____ %

QUESTIONS

1. How are the alleles for genes on different chromosomes distributed to gametes? What genetic principle does this illustrate?

2. Why was it important to have virgin females for the first cross (yielding the F1 generation), but not the second cross (yielding the F2 generation)?

3. What did the chi-square test tell you about the validity of your experimental data? What is the importance of such a test?

EXTENSION

1. Choose a new F1 generation cross (will yield the F2 generation) using two different phenotypes that you have observed or were observed in the class. Write out a Punnett square for every genotype that is associated with these two phenotypes. What ratio would you expect if the cross included a sex-linked recessive trait, a sex-linked dominant trait, a recessive autosomal trait, or a dominant autosomal trait? If you do not know whether the trait is dominant or recessive or sex-linked, look up the trait and write down the expected percentage of males with these genotypes and females with these genotypes. Now write down the percentage of males with these phenotypes and females with these phenotypes.