Introduction

In the last third of the 19th century, an Augustine monk named Gregor Mendel began an experimental study of inheritance which eventually resulted in the development of the biology discipline called genetics. The essentials of his model for inheritance has been substantiated by modern understanding for the nature of inheritance - a remarkable accomplishment since Mendel did not know about chromosomes nor the molecular basis of inheritance.

Mendel developed his model as an attempt to explain the results of breeding experiments with pea plants in the monastery garden. Working with only one variable at a time, and employing statistical procedures, he arrived at a conclusion that yielded successful predictions.

Mendel articulated his observations in the expression of his two laws of inheritance: the Law of Segregation and the Law of Independent Assortment. His Law of Segregation, states that in the formation of gametes, two members of a gene pair (alleles) segregate independently of one another. In other words, each gamete produced from a single parent will possess only one or the other of a pair of alleles for a given genetic character (e.g., seed color). Thus, given a heterozygous individual (e.g., Rr), half of his or her gametes will possess one allele (perhaps R) and the other half will possess the other allele (perhaps r). The actual gamete becoming involved in fertilization - thus contributing its single allele for every pair of alleles to the zygote - is entirely random. Note, when dealing with the inheritance of a single genetic character involving a pair of alleles, we are working with what is normally described as a monohybrid cross.

Mendel’s Law of Independent Assortment addresses the relationship, in terms of patterns of inheritance, between two or more different genetic characters (e.g., seed color and seed shape), each of which involves pairs of alleles. When dealing with two different characters, the situation is referred to as a dihybrid cross. According to the Law of Independent Assortment, whenever two or more pairs of contrasting characters are brought together in a hybrid, the alleles of different pairs segregate independently of one another during gamete formation.

This laboratory activity is designed to familiarize you with the statistical nature of Mendel’s model.

In Part I, we will attempt to understand the probabilistic aspects of monohybrid crosses (Mendel’s Law of Segregation) by “randomly” tossing special “coins” designed to simulate the genotypes of parents, focusing on a single genetic character, seed color.

In Part II, we will attempt to understand the probabilistic aspects of dihybrid crosses (Mendel’s Law of Independent Assortment) by “randomly” tossing special “dice” designed to simulate the genotypes of parents, focusing on two genetic characters, seed color and plant height.

In Part III, we will investigate various human genetic characters.

In Part IV, we will also evaluate the results of monohybrid and dihybrid crosses involving the characteristics of corn seeds.

In Part V, we will learn about the use of the Chi-Square test for statistical hypothesis testing.

Parts I-III of this lab activity will involve collection of class data. So we will proceed through these parts together as a class. But each round of data collection will involve working in pairs.

Parts IV & V will not involve collection of class data. You will proceed through these parts working in pairs.

Please read about Mendelian inheritance (Chapter 14 in your BIOL 171 textbook) before the laboratory activity. Also be sure to read any supplementary materials posted at the Laulima site for this lab activity.

A Comment on Frequencies, Percentages, Proportions and Ratios

Throughout the lab activity you will be asked to present your values as frequencies, percentages and/or proportions. It is important that you understand how these ways of presenting your results differ from each other. Frequencies are the actual counts. For example, if you tally up 150 purple corn kernels out of a total of 200 purple and yellow kernels, your frequency of purple kernels is 150. Since you counted a total of 200 kernals, the...
A percentage is a number or amount in each 100. For example, if you counted 150 purple kernels out of 200, your percentage of purple kernels was 75% \( \frac{100\% \times 150}{200} = 75\% \). The percentage of yellow kernels was therefore 25%.

A proportion is a part, share, or number considered in comparative relation to a whole where the whole equals a value of 1. For example, if you counted 150 purple kernels out of 200, your proportion of purple kernels was 0.75 \( \frac{150}{200} = 0.75 \). The proportion of yellow kernels was therefore 0.25.

For the purposes of this laboratory exercise, a ratio would present the results as a fraction of the total. For example, to express the ratio of 150 purple kernels out of 200 total kernels, you would write the result as \( \frac{150}{200} \). Note, \( \frac{150}{200} \) is essentially the same as \( \frac{75}{100} \).

Procedures and Assignments

Be sure to download and print all of the relevant downloadable documents listed with this lab activity on the Laulima site. Bring hard copies of all of these documents with you to the lab session.

I. MONOHYBRID CROSSES AND CHANCE

In this exercise, specially designed plastic disks representing the genetic make up (genotype) of parents involved in experimental cross. Each disk has a letter on each side. Some disks have an upper case “Y” on each face (representing the parental genotype YY - homozygous dominant condition). Other disks have a lower case “y” on each face (representing the parental genotype yy - homozygous recessive condition). Still others have an upper case “Y” on one side and a lower case “y” on the opposite side (representing the parental genotype Yy - heterozygous condition). The upper case “Y” represents the dominant yellow seed allele in peas, while the lower case “y” represents the recessive green seed allele in peas. By dominant, we mean that the Y allele masks the expression of the y allele in the heterozygous condition (Yy). Thus while the genotype may be Yy, its phenotype (actual physical appearance of the individual), is the yellow seed phenotype.

When considering monohybrid crosses, we are only evaluating the inheritance of alleles at a single gene locus. Bearing in mind that each chromosome of a pair of chromosomes possesses corresponding gene loci with its partner chromosome, then there will be two alleles for the genetic character determined at a particular gene locus. In the present hypothetical example, the gene locus under investigation is the gene locus involved in determining seed color. The two alleles may be the same (i.e., YY or yy) or they may be different (i.e., Yy).

When a single disk is tossed, only one face (therefore only one letter) can appear facing up. This situation is analogous to the segregation of allele pairs during meiosis in the production of gametes: only one allele from a pair of alleles can appear in each gamete. In our simulation, you are to consider the letter facing up to represent the allele found in the gamete produced by that parent. A YY parent will only produce gametes containing a single Y allele. Similarly, a yy parent will only produce gametes containing a single y allele. However, a Yy parent may produce some gametes that contain a single Y and other gametes that contain a single y.

A. Gamete Genotypes

1. Homozygous Dominant Parent (YY)

Take a single disk with upper case "Y" on each face (YY parent). Toss the disk 10 times, each time recording the letter facing up.

   a. Record your group results in terms of frequencies and percentages.

   b. What gamete genotypes are possible, from this parent?

   c. In what expected percentages?

2. Homozygous Recessive Parent (yy)

Take a single disk with lower case "y" on each face (yy parent). Toss the disk 10 times, each time recording the letter facing up.

   a. Record your group results in terms of frequencies and percentages.

   b. What gamete genotypes are possible, from this parent?

   c. In what expected percentages?

3. Heterozygous Parent (Yy)

Take a single disk with upper case "Y" on one face and lower case "y" on the opposite
face (Yy parent). Toss the disk 20 times, each
time recording the letter facing up.

a. Record your group results and the combined class results in terms of frequencies and percentages.
b. Using the combined class data, calculate the percentages of the gamete genotypes.
c. What gamete genotypes are possible from this parent?
d. In what expected percentages?
e. Were the observed percentages similar to the expected percentages?

3. One Homozygous Dominant Parent Versus One Homozygous Recessive Parent (YY x yy)

Repeat the experiment again, this time taking one YY disk and one yy disk and toss them together 10 times. Thus the two letters facing up represent the genotype of the zygote or offspring produced in a mating by two parents that exhibit different genotypes (in this case one is YY and the other is yy).

a. Record your group results in terms of frequencies and percentages of both genotypes and phenotypes.
b. What offspring genotypes are possible, from this cross?
c. In what expected percentages?
d. What offspring phenotypes are possible, from this cross?
e. In what expected percentages?

4. Both Parents Heterozygous (Yy x Yy)

a. Before coming to lab, you should carry out the Punnett Square diagram for this cross. To understand how to do this, please see Fig. 14.5 (F1 generation cross to produce F2 generation), p. 266 in Campbell Biology 9th Ed. Be sure to include this diagram in your lab summary report.
b. Repeat the experiment again, this time taking two Yy disks and toss them together 20 times. Thus the two letters facing up represent the genotype of the offspring produced in a mating by two Yy parents. This is often called the monohybrid cross because two hybrids (the heterozygous condition) are crossed.

1. Record your group results and the combined class results (frequencies of genotypes only).
2. Working only with the combined class data, calculate the observed percentages of the genotypes and phenotypes (assuming Y is dominant to y).
3. What offspring genotypes are possible from this cross?
4. In what expected percentages?
5. What offspring phenotypes are possible from this cross?
6. In what expected percentages?
7. Were the observed percentages similar to the expected percentages?

5. One Parent Heterozygous and One Parent Homozygous Recessive (Yy x yy)
   a. Before coming to lab, you should carry out the Punnett Square diagram for this cross. Be sure to include this diagram in your lab summary report.
   b. Repeat the experiment again, this time taking one Yy disk and one yy disk and toss them together 20 times. Thus the two letters facing up represent the genotype of the zygote or offspring produced in a mating by two parents that exhibit different genotypes (in this case one is Yy and the other is yy).
1. Record your group results and the combined class results (frequencies of genotypes only).
2. Working only with the combined class data, calculate the observed percentages of the genotypes and phenotypes (assuming Y is dominant to y).
3. What offspring genotypes are possible from this cross?
4. In what expected percentages?
5. What offspring phenotypes are possible from this cross?
6. In what expected percentages?
7. Were the observed percentages similar to the expected percentages?

6. Question Regarding Allele Segregation, Recombination and Chance
   a. If this experiment has been a fair simulation for inheritance, what can you say about the role of chance in determining the genotypes and phenotypes of offspring from a particular pair of parents?

II. DIHYBRID CROSSES AND CHANCE

In a monohybrid cross, we are concerned with only one genetic character at a time. For example, we may be interested in the seed color character (yellow vs. green) or the plant height character (tall vs. short). Thus, when we study seed color, we are not concerned with plant height. On the other hand, when we study plant height, we are not concerned with seed color.

Suppose, however, we wanted to know whether or not the inheritance of seed color influenced the inheritance of plant height. We would have to look at the inheritance of both characters together in the same sets of crosses.

In the following exercises, we will examine a cross between individuals that are heterozygous for both characters. In this example, seed color is determined by two alleles: yellow (Y, dominant) and green (y, recessive). Plant height is also determined by two alleles: tall (T) and short (t). The heterozygous condition for seed color would be Yy. The heterozygous condition for plant height is Tt. When considering both characters at the same time, the individual's genotype may be expressed as TtYy.

According Mendel's Law of Segregation each gamete should receive either a Y allele or a y allele and either a T allele or a t allele. According to his Law of Independent Assortment, the occurrence of Y or y in a gamete is independent of the occurrence of T or t. Thus the following combinations of alleles should occur with equal frequency among the gametes: TY, Ty, tY and ty.

A. Dihybrid Gamete Genotypes for a TtYy Parent (Independent Assortment)

You will be given two four-sided dice. Each die represents a parent whose genotype is TtYy. Each face of a single dice represents the alleles presented in a gamete. The allele combinations in the gametes are TY, Ty, tY and ty. When a single dice is tossed, the dice side facing the table is the one to read and represents the allele combination presented by the parent.

Take a single dice and toss it 20 times, each time recording the letters facing down on the table.
1. Record your group results and the combined class results (frequencies of gamete genotypes only).
2. Using only the combined class results, calculate the observed percentage for each gamete genotype.
3. What are the expected percentages for each gamete genotype?
4. Compare the observed percentages to the expected percentages. Are the observed percentages similar to the expected percentages?
B. Dihybrid Cross Punnett Square

Do the following exercise before the lab session (include your results in your lab summary):

1. Using a Punnett square, predict the offspring genotypes from a TtYy x TtYy cross assuming independent assortment.
2. Given that yellow (Y) is dominant over green (y) and tall (T) is dominant over short (t), determine the expected ratio of the phenotypes from this cross.

C. Dihybrid Cross Using Tetradice (Independent Assortment)

Take two TtYy dice and toss them together 20 times. The combination of letters facing down for both dice represents the genotype of the offspring produced in a mating by the two TtYy parents.

1. Record your group results (frequencies) and combined class results (frequencies) of the offspring genotypes.
2. Using the combined class results and, keeping in mind the dominance relationships of the alleles involved, calculate the observed frequencies (not percentages) for each offspring phenotype.
3. Based upon the total number of offspring yielded in the combined class results and the expected phenotypic ratios (see II.B.2. above), calculate the expected phenotype frequencies and compare them to the observed phenotype frequencies from above.
4. Comment on how these result fit in with Mendel's Law of Independent Assortment.

III. HUMAN GENETIC TRAITS

You should review the information (downloadable from the Laulima site) that describes and explains a variety of human genetic traits before the lab activity. Be sure you understand how to determine which phenotypes you exhibit. Also be sure you understand which traits are dominant and which are recessive.

A. Your Own Phenotypes
1. Compare your own traits (phenotypes) to the ones depicted in the downloadable information sheets on human genetics.
2. Fill out the sheet as instructed, circling the gene symbols that apply to you.

B. Class Phenotypic Percentages

Class data will be pooled so that you may compare yourself to the class average genetic makeup.

1. For each trait determine the percentage of the class represented by each phenotype. For example, if out of 20 students, 12 can roll their tongues, while 8 cannot, the percentages for tongue rolling and inability for tongue rolling are 60% and 40% respectively.
2. What can you say about the relative abundance of recessive, versus dominant, phenotypes in our small population?

IV. CORN GENETICS

A. Monohybrid Cross

You will be given an ear of corn whose kernels exhibit different characteristics. Some cobs will have yellow (r allele) and purple (R allele) kernels (note purple is dominant). Other cobs may have smooth (referred to as "starchy"; S allele) and wrinkled (referred to as "sweet"; s allele) kernels (note starchy is dominant). Consider the kernels to represent individual offspring from a cross between two parents whose genotypes are unknown to you. Your task will be to determine the most likely genotypes for these parents.

1. Avoiding any kind of systematic bias, observe and record the phenotypes (observed frequencies) of at least 100 kernels on your corn cob.
2. From these observed frequencies, hypothesize the two parental genotypes responsible.
3. Based upon your hypothesized cross, calculate the expected phenotypic frequencies for the 100 kernels (use a Punnett square diagram to determine the expected phenotypic ratio).
4. Make a table that presents your observed and expected frequencies for each phenotype.
5. Were your observed results reasonably close to your expected values?
B. Dihybrid Cross

You will be given an ear of corn whose kernels exhibit two different genetic characters at the same time. Some kernels will be yellow (r allele), while others will be purple (R allele; note purple is dominant). Some kernels will also be smooth (referred to as "starchy"; S allele; dominant), while others are wrinkled (referred to as "sweet"; s allele; recessive). Because we are considering these characters together the possible phenotypes will be: purple-starchy (R_S_), purple-sweet (R_ss), yellow-starchy (yyS_) and yellow-sweet (yyss). Consider the kernels to represent individual offspring from a cross between two parents whose genotypes are unknown to you. Your task will be to determine the most likely genotypes for these parents.

1. Avoiding any kind of systematic bias, observe and record the phenotypes (observed frequencies) of at least 120 kernels on your corn cob.
2. From these observed values, hypothesize the two parental genotypes responsible.
3. Based upon your hypothesized cross, calculate the expected phenotypic frequencies for the 120 kernels (use the Punnett square diagram to determine the expected phenotypic ratio).
4. Make a table that presents your observed and expected frequencies for each phenotype.
5. Were your observed results reasonably close to your expected values?

V. HYPOTHESIS TESTING: THE CHI-SQUARE TEST

Rarely do the observed results of a cross reflect the expected values based upon the hypothesized model. Because the allocation of alleles to gametes and the fertilization of gametes are random processes, there is a real, finite probability of getting results that deviate from the expected values. However, getting a result that deviates substantially from the expected values is not likely. Therefore, if your observations deviate significantly from your expected values, you may conclude that your model must be incorrect. Several possible explanations may be offered: your hypothesized cross is incorrect (try hypothesizing another); some alleles may influence the success of gametes; or, in the case of the dihybrid situation, genetic characters may be linked.

How large a deviation between observed and expected results would be enough to convince you that your model is incorrect? The chi-square test offers a non-biased approach to answering this question. The chi-square test procedure will be explained to you in class. The calculations are illustrated on a downloadable document on the Laulima site.

A. Chi-Square Test for Corn Monohybrid Cross

In the lab activity above dealing with monohybrid crosses in corn (Part IV.A.), you hypothesized the parental genotypes yielding the observed corn kernel results.

1. Apply the chi-square test to evaluate the robustness of your hypothesis. Be sure to show your calculations. These calculations will be comparing the observed phenotypic frequencies to the expected phenotypic frequencies.
2. What was your conclusion regarding your hypothesis? If your hypothesis failed the test, suggest another.

B. Chi-Square Test for Corn Dihybrid Cross

In addition, test your hypothesis for the dihybrid corn example above (Part IV.B.). Present your conclusions regarding your hypothesized cross. Again, if the hypothesis failed the test, suggest another.

VOCABULARY

- genetics
- monohybrid
- genotype
- homozygous
- heterozygous
- dominant
- recessive
- allele
- phenotype
- gene locus
- segregation
- independent study
- antibody
- antigen
- Rh factor
- expected frequency
- observed frequency
- chi-square test
- degrees of freedom
Lab Summary

Please include the following in your lab summary.

1. Descriptive title.
2. Introduction describing purpose and objectives of this lab activity.
3. The Results and Discussion section of your laboratory summary should consist of the specific answers requested in the “Procedures and Assignments” section described above. Please organize your presentation of answers by referring to the parts listed above (e.g., Part III.A.1).
4. A Conclusion paragraph summarizing what was learned and could be concluded from the data.