MENDELIAN GENETICS

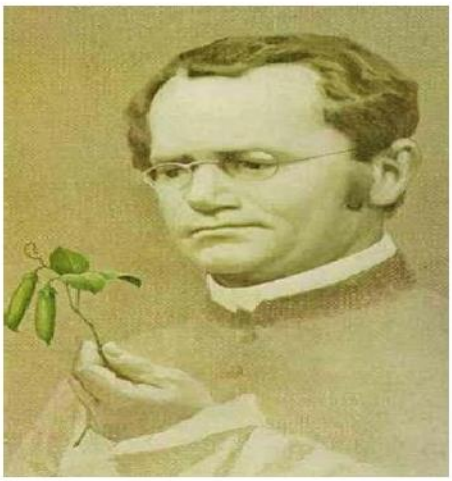
Objectives

Upon completion of this lab, students should:

1. Understand the principles and terms used in Mendelian genetics.
2. Know how to complete a Punnett square to estimate phenotypic and genotypic ratios in offspring.
3. Be able to solve inheritance problems for plants.
4. Know how the results from mono and dihybrid corn crosses support Mendel's first and second laws.

Introduction

In the mid-1800's, an Austrian monk named Gregor Mendel conducted experimental crosses with garden peas in an attempt to understand the basic patterns of inheritance. Mendel's meticulous work provided the basis for modern genetics. Although Mendel used garden peas for his experimental crosses, we will use corn since it is easier to observe results. Today, you will examine two kinds of crosses performed by Mendel and see how he used the results from these crosses to formulate his 1st and 2nd laws. Then you will examine and solve some problems of Mendelian inheritence in plants.



Mendel's Laws Of Inheritance

- **Mendel's First Law** (The Principle of Segregation) - The two alleles of a homologous pair of chromosomes separate (segregate) during gamete formation (Meiosis) such that each gamete receives only one allele randomly.

• **Mendel's Second Law** (The Principle of Independent Assortment) - Alleles of a gene pair assort independently of other gene pairs on non-homologous chromosomes.

*** Please read and make sure you understand the following terms before you go on:**

A gene is a unit of heredity on a chromosome and has alternate forms called alleles. In sexually reproducing organisms each parent contributes one allele to their offspring that may or may not be like the other parent's allele.

Alleles for a particular gene occur in pairs. Alleles that mask expression of other alleles of a particular gene, but are themselves expressed are dominant, and are usually designated by a capital letter (for example, "T"). Alleles whose expression is masked by dominant alleles are recessive, and are designated by a lower case letter (for example, "t").

A cross between two individuals who are both hybrid for a single trait of interest is known as a **monohybrid cross**. A cross between two individuals who are both hybrid for two traits of interest is known as a **dihybrid cross**. The prefix of a hybrid cross depends on the number of traits being examined (there are trihybrid, tetrahybrid, and even dodecahybrid crosses!), but remember that in such a cross BOTH PARENTS MUST BE HYBRID FOR ALL TRAITS UNDER CONSIDERATION.

A test cross is performed when the breeder wishes to know the genotype of an individual expressing the dominant allele. Since this individual could be either homozygous dominant or heterozygous, breeding it with an individual who is homozygous recessive and counting the offspring phenotypes will reveal the genotype of the dominant-expressing individual.

The genotype of an organism includes all the alleles present in the cell, whether they are dominant or recessive. The physical appearance of the trait is **the phenotype**. Tallness (T) is dominant to dwarfism (t), a plant with a tall phenotype can have a genotype of (TT) or (Tt). A plant with a dwarf phenotype can only have genotype (tt). When the paired alleles are identical (TT or tt), the genotype is homozygous (homo =same). Heterozygous (hetero = different) refers to a pair of alleles that are different (Tt).

YOUR LABORATORY INSTRUCTOR WILL TELL YOU WHAT YOU ARE TO TURN IN FOR THIS LAB EXERCISE.

Data collection

Each group will be provided with samples from different parental crosses. Given what you know about Mendelian genetics, construct the null hypothesis concerning phenotypic and genotypic ratios for each of the samples. In order to visualize a cross between these two parents you should

to construct a Punnett square. Make a Punnett square on a sheet of paper to help you calculate the genotypes of the F1 individuals resulting from this cross. To construct a Punnett square, alleles present in the gametes (N) of one parent are written along the top and those from the other parent are listed down the side. The combinations produced by filling in each box of the Punnett square show the possible genotypes of the offspring. Tabulate your findings below.

As you know all too well by now, a valid test of your hypotheses, requires more than a mere visual inspection of the data. The investigator can make no claims about significance without first performing a statistical test on his/her data. When you have finished counting and tabulating your results, you will perform a simple, non-parametric test that will allow you to determine whether your observed results exhibit any significant deviation from the expected ratios. If they do, it is up to you to hypothesize WHY?

Data analysis

Remember: a scientifically observed result is said to be statistically significant if there is less than or equal to a 5% ($P \leq 0.05$) probability that the phenomenon is due simply to random chance. Conversely, this means that there is a 95% chance that the phenomenon is due to some factor other than chance.

To determine whether the variation in phenotype frequency you counted is significantly different from the frequencies expected from each of the parental genotypes, you will use the Chi square test.

The Chi square test

Before we count the offspring of each sample, we must ask whether the ratio of the phenotypes we actually count is likely to occur in a random sample produced by a monohybrid cross.

If our count is not significantly different from the expected, we have no reason to reject our null hypothesis. However, if our number is significantly different from the expected, we conclude that there is very little probability of obtaining such a ratio by random chance. Some other factor must be at work here--one we must try to explain in terms of other genetic events such as gene linkage, lethal genes, etc.(from lecture).

We will use a simple, non-parametric test called the Chi square (X^2) to determine whether our observed (counted) ratios of sample phenotypes are significantly different from the expected (from the known parental genotypes) ratios. The formula for this test is as follows:

$$X^2 = \sum \frac{(\text{Observed frequencies} - \text{Expected frequencies})^2}{\text{Expected frequencies}}$$

$$= \sum \frac{(Fo - Fe)^2}{Fe}$$

in which

- Fo = the observed (counted) number of grains of a particular phenotype
- Fe = the expected number of grains of a particular phenotype.
- Σ represents the summation of X^2 values over every phenotypic category (1 through n)

Run the X^2 test on each samples to determine whether their phenotypic ratios deviate significantly from the expected. Test all three of your hypotheses, accept or reject each one, and explain your results.

*** Please read and make sure you understand the following term before you go on:**

The hypothesis is termed the **null hypothesis** which states that there is no substantial statistical deviation between observed and expected data.

EXAMPLE

If you knew the parents of 100 kernels of corn on a cob were a monohybrid cross for the purple vs. yellow trait, your knowledge of Mendelian genetics would lead you to expect 75 purple kernels and 25 yellow kernels. Your null hypothesis would be that the ratio of purple to yellow corn kernels should not differ from a 3:1 ratio. Your observed, ratio, however, is 70 purple: 30 yellow. Is this deviation significant, or simply due to random chance?

If we calculate our X^2 value for the above example, we obtain:

For the purple kernels: For the yellow kernels:

$X^2 = (70 - 75)^2/75 = 0.333$ $X^2 = (30 - 25)^2/25 = 1.0$

Summing (Σ) the two X^2 values, we obtain a X^2 statistic of 1.333.

How to use the table:

1. To accept or reject our null hypothesis, we must now determine the degrees of freedom. For the Chi Square test, df = n (# of phenotypes)- 1, where n = the number of phenotypic catgories possible. In our case, n = 2 (purple and yellow). Calculate the dF for our example problem.
2. In the far left vertical column of the table of Chi Square Critical Values locate the dF you obtained for the sample problem.

3. Now go across the appropriate dF row until you find a X^2 value closest to the value we obtained in our sample problem. Our X^2 statistic was 1.333. If you look across the dF = 1 row, you will find that this X^2 value lies between 1.323 and 2.706 on the Chi Square table.
4. Next, look vertically above the two X^2 values closest to our example's X^2 value to locate the P values associated with the X^2 at one degree of freedom. The P value of our sample statistic lies between 0.25 and 0.1, or 0.25 > P > 0.1.
5. Literally, this means that the probability that our observed corn phenotype ratio's deviation from the expected corn phenotype ratio is due simply to chance is between 10 and 25%. That's a fairly big probability that chance is the culprit for our deviation.
6. Recall that for P to be significant, it must be less than or equal to 0.05 (5%).

As you can see, our example does not support rejection of Hypothesis. A ratio of 70 : 30 purple to yellow corn grains is not a significant deviation from the expected 75 : 25 ratio, and is probably due to chance and random sampling error.

Table . A partial table of critical values of the Chi-square distribution.

	P								
df	**.995**	**.975**	**.9**	**.5**	**.1**	**.05**	**.025**	**.01**	**.005**
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801

P, probability; df, degrees of freedom.

WORK SHEET

1) Predict the ratio between the phenotypes resulted from crossing of a homozygous green seeded pea plant and a homozygous yellow seeded one, knowing that yellow color is the dominant phenotype. What is the ratios expected from back crossing of offspring with each parent?

2) What is the ratio between phenotypes resulted from self-pollination of heterozygous yellow seeded pea plant?

3) You are provided with the results observed following crossing of pea plants. Find the possible phenotypes of offspring and their parents.

a) Green X Yellow ⟶ 100% yellow

b) Green X Yellow ⟶ 50% yellow and 50% Green.

c) Yellow X Yellow ⟶ 100% Yellow

d) Yellow X Yellow ⟶ 25% Green and 75% yellow.

e) Green X Green ⟶ 100% Green.

4) Predict the ratio between the phenotypes resulted from crossing of a homozygous green wrinkled seeded pea plant and a homozygous yellow rounded seeded one, knowing that yellow color and rounded shape are the dominant phenotypes. What is the ratios expected from back crossing of offspring with each parent?

5) What is the ratio between phenotypes resulted from self-pollination of heterozygous yellow rounded seeded pea plant?

6) You are provided with the results observed following crossing of pea plants. Find the possible genotypes of offspring and their parents.

a) Green wrinkled X Yellow rounded ⟶ 100% yellow rounded.

b) Green wrinkled X Yellow rounded ⟶ 50% yellow rounded and 50% Green wrinkled.

c) Yellow rounded X Yellow wrinkled ⟶ 50% Yellow rounded and 50% Yellow wrinkled.

d) Yellow rounded X Yellow wrinkled ⟶ 100% Yellow rounded.

e) Yellow rounded X Yellow wrinkled ⟶ 75% yellow rounded and 25% Green rounded.

f) Yellow rounded X Yellow rounded ⟶

9 Yellow rounded, 3 Yellow wrinkled, 3 Green rounded and 1 Green wrinkled.

7) A scientist performed a series of crossing experiments to study the inheritance of flower color in *Bougainvillea glabra*. Two color phenotypes were observed, purple and yellow. He hypothesized that color was controlled by a single gene where the allele for purple was dominant (P) and the allele for yellow was recessive (p). He crossed the two heterozygous plants (Pp X Pp).

a) What is the expected genotypic ratio in the offspring?

b) From the provided flowers, use the Chi square (X^2) to determine whether the observed (counted) ratios of phenotypes are significantly different from the expected (from the known parental genotypes) ratios for Mendel's law of segregation.

Phenotype	**Genotype**	**Observed Number**	**Expected Ratio**	**Expected Number**	**(Fo-Fe)**	**$(Fo-Fe)^2$**	**$(Fo-Fe)^2/Fe$**
Purple							
yellow							
Total							

Chi-square =

Number of degrees of freedom=

Probability of a match=

Comment on your results.