# Independent Assortment: Dihybrid cross

- Independent assortment can be observed when studying more than one character at a time.
- Dihybrid cross is a mating between parents that are heterozygous for two characters (dihybrids).
  - For example, Mendel began his experiments by crossing truebreeding parent plants that differed in two characters such as seed color (yellow or green) and seed shape (round or wrinkled).
  - The allele for yellow seeds (Y) is dominant to the allele for green (y), and round (R) is dominant to wrinkled (r).
  - If plants homozygous for round yellow seeds (*RRYY*) are crossed with plants homozygous for wrinkled green seeds (*rryy*), the resulting F<sub>1</sub> dihybrid progeny would be heterozygous for both traits (*RrYy*) and have round yellow seeds, the dominant phenotypes.



- The F<sub>1</sub> progeny self-pollinate. Two alternate hypotheses predict different outcomes:
  - If the two characters segregate *independently*, the F<sub>1</sub> hybrids will produce four classes of gametes (*RY*, *Ry*, *rY*, *ry*), and the F<sub>2</sub> progeny will show a 9:3:3:1 ratio.
  - **Experiment:** Mendel performed a dihybrid cross by allowing self-pollination of the F<sub>1</sub> plants (*RrYy* X *RrYy*).
  - Results: Mendel categorized the F<sub>2</sub> progeny and determined a ratio of 315:108:101:32, which approximates 9:3:3:1. These results were repeatable. Mendel performed similar dihybrid crosses with all seven characters in various combinations and found the same 9:3:3:1 ratio in each case.



- **Conclusion:** The experimental results supported the hypothesis that each allele pair segregates independently during gamete formation.
- Mendel's law of independent assortment states that each allele pair segregates independently of other gene pairs during gamete formation.
- Mendel observed that different genes assort independently of each other. It was as if each gene had its own chromosome. (Chromosomes had not yet been discovered when Mendel was alive. The link between chromosomes and genes had not yet been made.)

# Problem:

In pumpkin fruits the white W color is dominant to the yellow w and the discoid shape D is dominant to the round d. We fertilized a pure plant that have yellow discoid fruit with another pure plant that have white round:

What is the phenotype and genotype of F1 and F2?

What we will have if we fertilized the resulting Individuals with the both parents?

## **Testing a dihybrid Cross**

- 1. Count the number of purple starchy, purple sweet, yellow starchy and yellow sweet kernels in five of the rows on your ear of corn and record the number on the chart. Be sure to use the same five rows for each calculation.
- 2. Make a table and but your data on.
- 3. Calculate the percentage between the individual traits.



#### Testing a dihybrid test cross

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# THE CHI-SQUARE TEST

Genetics is the study of inheritance, but it is also a study of probability. Most eukaryotic organisms are diploid, meaning that each cell contains two copies of every chromosome, so there are two copies of each gene that controls a trait (alleles). In sexual reproduction, these two copies of each chromosome separate, and are randomly sorted into the gametes.

When gametes from two different parents combine in fertilization, new combinations of alleles are created. Thus chance plays a major role in determining which alleles, and therefore which combinations of traits end up in each new individual.

**The chi-square test:** It is a statistical test that can be used to determine whether observed frequencies are significantly different from expected frequencies or not.

#### **Chi-square Analysis**

Genetics uses the Chi-square to evaluate data from experimental crosses to determine if the assumed genetic explanation is supported by the data. The Chi-square test helps you to decide if the difference between your observed results and your expected results is probably due to random chance alone, or if there is some other factor influencing the results.

### **Determining the Chi-square Value**

Chi-square is calculated based on the formula below: but we must begin by stating a **null hypothesis** 

H0: there is no significant difference between observed and expected frequencies.

H1: there is a significant difference.

Based on the outcome of the chi-square test we will either reject or fail to reject the null hypothesis.

When you examine the results of a genetic cross you may ask if the numbers you observe are in agreement with the hypothetical outcome of the cross.

If the difference between your observation and the expected result small enough that it could have been produced by chance alone. This is the null hypothesis that there is no real difference between the observed data and the predicted data.

**Importance:** Chi-square tests enable us to compare observed and expected frequencies objectively, since it is not always possible to tell just by looking at them whether they are "different enough" to be considered statistically significant. Statistical significance in this case implies that the differences are not due to chance alone, but instead may be indicative of other processes at work.

Then calculate the chi-square statistic using this formula.

You need to memorize the formula!

$$X^{2} = \sum \frac{(o-e)^{2}}{e} = \sum \frac{(d)^{2}}{e}$$

χ2	the chi-square test statistic
0	observed count or frequency
е	expected count or frequency
d	The difference between the observations and the expected numbers

Note that you must use the number of individuals, the counts, and NOT proportions, ratios, or frequencies.

#### You can use this table:

Phenotypes	Observed	Expected	d = o -	d2	d2/e
	(0)	(e)	е		
Total					

#### **Degrees of Freedom**

A critical factor used in the chi-square test is the "degrees of freedom", which is essentially the number of independent random variables involved.

Degree of freedom is simply the number of classes of offspring minus 1.

For our example, there are 2 classes of offspring: purple and white. Thus, degrees of freedom (d.f.) = 2 - 1 = 1.

The rows in the Chi-square Distribution table refer to degrees of freedom.

After the cross you would then compare your observed results against your expected results and complete a Chi-square analysis by given your conclusion.

# **Conclusion:**

If the observed chi-square is greater than the critical value (p value) then you would accept your null hypothesis (differences are due to random chance alone) and your genetic explanation for this trait is supported.

If the p value is determined to be less then you would reject your null hypothesis your genetic explanation for this trait is unsupported. You therefore have to consider alternative factors influencing the inheritance of the mutant trait.

## EXAMPLE

Suppose you counted 79 R\_ and 33 rr. The total number of individuals you counted, N, is 112. You expect 3/4 to be R\_ (84) and 1/4 to be rr (28). Are your results close enough to these ratios for you to accept the null hypothesis—that there is no real difference? The Chi-square test is one tool for making this decision.

Phenotypes	Observed (o)	Expected (e)	d = o - e	d2	d2/e
R_	79	3∕4 x 112 = 84	-5	25	.30
rr	33	1⁄4 x 112 = 28	5	25	.89
Total	112	112	0		1.19

X2 =  $\sum$  (observed-expected)2 / (expected). This means add up the values in the last column.

You can compare the chi-square sum, 1.19, with the numbers in a table of critical values to decide whether to accept the null hypothesis—that the observed results are so close to expected results that there is no difference, and our original hypothesis is accepted.

Degrees of freedom: In a two-phenotype system, when you know the number for one phenotype, the result for the other (the rest of the population) is automatically determined. In this kind of genetic data, the **number of degrees of freedom is one less than the number of different phenotypes observed**.

As a rule, if the probability of obtaining a particular **X2 value is greater than 5 in 100 (P > 0.05)**, then the difference between expected and observed is not considered statistically significant, and the **null hypothesis is accepted**.

### In general the steps to solve a chi square test are:

- 1. Calculate the degree of freedom = number of phenotypes -1.
- 2. Calculate the expectation = number for individuals  $\times$  expected percentage.
- 3. Extract the tabled  $x^2$  by comparing the degree of freedom column with the moral level row.(if it's not given it is usually .05).
- 4. Put the data in the  $x^2$  table.
- 5. Write your conclusion by comparing the tabled  $x^2$  with the calculated  $x^2$ .

# **EXPERIMENTAL DATA**

Fill in the tables below with your observed data, calculate the expected result according to your hypothesis. Determine the X2 value for each experiment, and use the table of probabilities to accept or reject the null hypotheses.

#### Monohybrid cross

Phenotypes	Observed	Expected	d = o -	d2	d2/e
	(o)	(e)	е		
Total			0		

X2 = \_\_\_\_\_ degrees of freedom: \_\_\_\_\_

Concusion:

# **Dihybrid cross**

Phenotypes	Observed (o)	Expected (e)	d = o - e	d2	d2/e
Total					

X2 = \_\_\_\_\_ degrees of freedom: \_\_\_\_\_

Concusion:

# **Genetic Variation in Humans**

Variation in humans traits fall into two categories: continuous and discontinuous. A trait shows continuous variation if it varies in a continuous fashion, from one extreme to another. Weight, hair color, and height are three traits that fall into this category. Traits that show discontinuous variation can be divided into two or more distinct categories with little intergrading between them. They are usually the expression of a single pair of genes. Several variations of this type will be illustrated in this lab exercise.

Determine your phenotype (and genotype, if possible) for as many of the human traits listed below. Record on the board your results for the following traits:

**Tongue rolling**: stick your tongue out and see if you can roll the edges into a loop. This trait is \*thought to be\* dominant, meaning you only need one "working" copy of the gene to have it - Tt, or TT. I say "thought to be dominant" as there have been studies suggesting that the trait can be learned. Additionally, identical twins do not always share the tongue rolling trait - a piece of information which indicates that it may not be genetically controlled.

**Hitchiker's thumb:** Stick your thumb up, as though "thumbing" a ride. You have the trait if the first part of the thumb bends back more than 45 degrees. This trait is recessive meaning you need two copies of the gene to have it (hh).

**Ear lobe attachment:** See whether the ear lobes are attached to the side of the head, or whether they hang freely. Unattached earlobes are dominant to attached earlobes.

**Widow's peak:** People whose hair comes to a point over the middle of their forehead have a widows peak (a dominant trait).



# Make a survey among your family and put your data in this table:

Traits	Tongue	Hitchiker's	Ear lobes	Widow's	Cleft chin	Bent finger
Me	loning			peak		
Mom						
Dad						
Brothers						
Sisters						
Grandparents						
Aunts						
Uncles						
Cousins						