Genetics is the “study of heredity”. A more modern definition is the study of composition and functioning of the genetic material. Genetics as a set of principles and analytical procedures did not begin until the 1860s, when an Augustinian monk named Gregor Mendel performed a set of experiments that pointed to the existence of biological elements that we now call genes. Thus genetics can be defined as the study of genes.

Mendel studied the garden pea (*Pisum sativum*), which he chose as his object of study. The choice was a good for the following reasons:

- Can be grown in a small area.
- Produce lots of offspring.
• Available in many varieties with distinct heritable features with different variations: flower color, seed color, seed shape, etc.
• Self and cross pollinations can occur.

Generally, the choice of an organism for studying genetics should consider the presence of the following:
Short life cycle
Available easy system for growth and maintenance.
Sexually reproduction
Controlled matting
Large number of offsprings
Features with different variations.

Mendel’s experimental design

Mendel was careful to focus on only a few specific differences between the plants he was using and to ignore the countless other differences he must have seen. Consequently, he studied seven characters. He usually conducted his experiments in three stages:

1. Mendel allowed plants of a given variety to self-cross for multiple generations to assure himself that the traits he was studying were indeed true-breeding, that is, transmitted unchanged from generation to generation.

2. Mendel then performed crosses between true-breeding varieties exhibiting alternative forms of characters. He also performed reciprocal crosses.

3. Finally, Mendel permitted the hybrid offspring produced by these crosses to self-fertilize for several generations, allowing him to observe the inheritance of alternative forms of a character. Most important, he counted the numbers of offspring exhibiting each trait in each succeeding generation. This quantification of results is what distinguished Mendel’s research from that of earlier qualitative investigations. Mendel’s mathematical analysis of experimental results led to the inheritance model that we still use today.
The Principle of Segregation

A monohybrid cross is a cross that follows only variations on a single character, such as yellow- and green-colored seeds. A monohybrid cross is made by mating true-breeding individuals from two parent strains, each exhibiting one of the two contrasting forms of the character under study. This deceptively simple kind of cross can lead to important conclusions about the nature of inheritance. The seven characteristics, or characters, Mendel studied in his experiments possessed two variants that differed from one another.
in ways that were easy to recognize and score. We examine in detail Mendel’s crosses with seed color. His experiments with other characters were similar, and produced similar results.

When Mendel crossed green-seeded and yellow-seeded plants, the hybrid offspring (customarily referred to as the first filial generation, or F1) all had yellow seeds. Mendel referred to the form of each trait expressed in the F1 plants as dominant, and to the alternative form that was not expressed in the F1 plants as recessive. For each of the seven pairs of contrasting traits that Mendel examined, one of the pair proved to be dominant and the other recessive.

After allowing individual F1 plants to mature and self-fertilize, Mendel collected and planted the seeds from each plant to see what the offspring in the second filial generation, or F2, would look like. He found that although most F2 plants had yellow seeds, some exhibited green seeds, the recessive trait. Although hidden in the F1 generation, the recessive trait had reappeared among some F2 individuals. Believing the proportions of the F2 types would provide some clue about the mechanism of heredity, Mendel counted the numbers of each type among the F2 progeny. In the cross between the yellow-seeded F1 plants, he obtained a total of 929 F2 individuals. Of these, 705 (75.9%) had yellow seeds, and 224 (24.1%) had green seeds.

Mendel went on to examine how the F2 plants passed traits to subsequent generations. He found that plants exhibiting the recessive trait were always true-breeding. For example, the green seeded F2 individuals reliably produced green seeded offspring when they were allowed to self-fertilize. By contrast, only ⅓ of the dominant, yellow-seeded F2 individuals (of all F2 offspring) proved true-breeding, but ⅔ were not. This last class of plants produced dominant and recessive individuals in the third filial generation (F3) in a 3:1 ratio. This result suggested that, for the entire sample, the 3:1 ratio that Mendel observed in the F2 generation was really a disguised 1:2:1 ratio: true-breeding dominant
A summary for Mendel’s observations starting with crossing of 2 True breeding individuals (yellow and green seeded) and subsequent self crossing of the offspring for next 2 generations.
individuals, not-true-breeding dominant individuals, and true-breeding recessive individuals.

**From This experiments** we can extract the following conclusions upon which Mendel proposed a simple model that has become one of the most famous in the history of science:

1. Variations (traits) of a character are determined by discrete information for each trait. Mendel called these information “factors” We now call these factors alleles.

2. Crossing of true breeding plants (parents) carrying opposite traits of a character results in offspring (F1) all carrying one of these traits while the other disappear. F1 plants did not have an intermediate appearance, as a hypothesis of blending inheritance would have predicted. Instead, different plants inherited each trait intact, as a discrete feature.

3. Upon self-crossing of the offspring (F1), the disappeared trait appears only in ¼ of the resultant plants (F2). The trait that “disappeared” must therefore be latent (present but not expressed) in the F1 individuals. Also, the factors (alleles) remain discrete—they neither blend with nor alter each other. This characteristic 3:1 ratio is referred to as the **Mendelian ratio** for a monohybrid cross.

4. Factors (alleles) for any character are not always the same. In such case only factors (called dominant factors) are expressed while the others (called the recessive factors) are not expressed. Dominant factors here are responsible for yellow seeds while recessive ones are responsible for green seeds. Now we call plants carrying the same alleles for a character homozygous (true breeding) for this character while those carrying different factors are called heterozygous (not true breeding).

5. Self-crossing of F2 plants showed that green seeded plants are true breeding (homozygous); carrying only factors for green seeds while only 1/3 of the F2 yellow seeded plants are true breeding (homozygous); carrying only factors for yellow seeds and the other 2/3 are not true breeding (heterozygous); carrying factors for both of yellow and green seeds. Thus, F2 plants are divided into homozygous for the
dominant trait, heterozygous and homozygous for the recessive trait in the ratio 1: 2: 1.

6. Based on 5, we have 4 equal (1+2+1) probabilities for offspring of monohybrid self-cross. This indicates that heterozygous plant has two different factors for seed color. Factors segregate and rejoin randomly upon crossing. Each individual receives a factor (allele) from each parent for each character.

Back crossing (crossing offspring with parents) was used to confirm this assumption. Back crossing of F1 with the parent carrying dominant trait results in plants all carrying dominant phenotype. Back crossing with the parent carrying recessive trait (it is also called test cross) results in 50% plants carrying dominant phenotype and 50% carrying the recessive trait. These results are in agreement with Mendel’s assumption and generalized it to all possible crossings.

Geneticists now refer to the set of alleles that an individual contains as the individual’s genotype. The physical appearance or other observable characteristics of that individual, which result from an allele’s expression, is termed the individual’s phenotype. In other words, the genotype is the blueprint, and the phenotype is the visible outcome in an individual. This also allows us to present Mendel’s ratios in more modern terms. The 3:1 ratio of dominant to recessive is the monohybrid cross phenotypic ratio. The 1:2:1 ratio of homozygous dominant to heterozygous to homozygous recessive is the monohybrid cross genotypic ratio. The genotypic ratio is modified into the phenotypic ratio due to the action of the dominant allele making the heterozygote appear the same as homozygous dominant.

Mendel’s model accounts for the ratios he observed in a neat and satisfying way. His main conclusion—that alternative alleles for a character segregate from each other during gamete formation and remain distinct—has since been verified in many other organisms. It is commonly referred to as Mendel’s first law of heredity, or the Principle of Segregation. It can be simply stated as: The two alleles for a gene segregate during gamete formation and are rejoined at random, one from each parent, during fertilization. The physical basis for allele segregation is the behavior of chromosomes.
during meiosis. Homologues for each chromosome disjoin during anaphase I of meiosis. The second meiotic division then produces gametes that contain only one homologue for each chromosome. Thus, Mendel through his analysis arrived at the correct scheme, even though he had no knowledge of the cellular mechanisms of inheritance; neither chromosomes nor meiosis had yet been described.

To test his model, Mendel first expressed it in terms of a simple set of symbols (letters: A, B...etc). He then used the symbols to interpret his results. Geneticists have several different systems for using symbols to represent genes. We will review a number of these conventions during the course, but for now, we will adopt one to use consistently throughout this part. According to this convention, the first letter of the dominant trait symbolizes the character in question; in lowercase italic, it designates the allele for the recessive trait, and in uppercase italic, it designates the allele for the dominant trait.

Consider again Mendel’s cross of yellow-seeded with green-seeded plants. By convention, we assign the symbol Y to the dominant allele, associated with the production of yellow seeds, and the symbol y to the recessive allele, associated with the production of green seeds. In this system, the genotype of an individual that is true breeding for the recessive green-seeded trait would be designated yy. Similarly, the genotype of a true-breeding yellow-seeded individual would be designated YY. In contrast, a heterozygote would be designated Yy (dominant allele first). Using these conventions and denoting a cross between two strains with “X” we can symbolize Mendel’s original yellow × green cross as YY × yy. Because a green-seeded parent (yy) can produce only y gametes, and a true-breeding yellow-seeded parent (YY, homozygous dominant) can produce only Y gametes, the union of these gametes can produce only heterozygous Yy offspring in the F1 generation. Because the Y allele is dominant, all of these F1 individuals are expected to have yellow seeds. When F1 individuals are allowed to self-fertilize, the Y and y alleles segregate during gamete formation to produce both Y gametes and y gametes. Their subsequent union at fertilization to form F2 individuals is random.

The F2 possibilities may be visualized in a simple diagram called a Punnett square, named after its originator, the English geneticist R. C. Punnett. Mendel’s model,
analyzed in terms of a Punnett square, clearly predicts that the F2 generation should consist of \( \frac{3}{4} \) yellow-seeded plants and \( \frac{1}{4} \) green-seeded plants, a phenotypic ratio of 3:1.

**The Testcross: Revealing Unknown Genotypes**

In a testcross, an individual with unknown genotype is crossed with the homozygous recessive genotype—that is, the recessive parental variety.

Consider a yellow-seeded pea plant. It is impossible to tell whether such a plant is homozygous or heterozygous simply by looking at it. To learn its genotype, you can perform a testcross to a green-seeded plant. In this cross, the two possible test plant genotypes will give different results:

*Alternative 1:* Unknown individual is homozygous dominant \((YY)\) \(YY \times yy\). All offspring have yellow seeds \((Yy)\).

*Alternative 2:* Unknown individual is heterozygous \((Yy)\) \(Yy \times yy\). \(\frac{1}{2}\) of offspring have yellow seeds \((Yy)\), and \(\frac{1}{2}\) have green seeds \((yy)\). Put simply, the appearance of the recessive phenotype in the offspring of a testcross indicates that the test individual’s genotype is heterozygous.
Punnett square showing results of crossing of 2 True breeding individuals (yellow and green seeded) and subsequent self crossing of the offsprings.
Punnett square showing results of back cross between F1 plant and its parents.

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50% yellow seeded
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100% yellow seeded

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50% yellow seeded
50% green seeded

Punnett square showing results of test crosses between the possible genotypes of yellow seeded plants and green seeded plant.
Important Definitions

**Gene**
A part of DNA carries information about a specific character. Genes are found on chromosomes and each gene has a designated place on every chromosome, called a locus.

**Alleles**
The different versions of the same gene. Each allele is responsible for a trait of the character the gene controls. They are referred to by letters.

**Character**
A heritable feature that varies among individuals. An example would be flower color.

**Trait**
A variant for character, such as white or purple colors for flowers.

**Homozygous**
The individual is called homozygous for certain character if the two alleles controlling such character are similar.

**Heterozygous**
The individual is called heterozygous for certain character if the two alleles controlling such character are different.

**Dominant and Recessive**
In heterozygous individual one allele is expressed and the other allele is not expressed or masked.

- The expressed allele is dominant. The allele is referred to by capital letter.
- The allele not expressed or masked is recessive. The allele is referred to by small letter.

**Genotype**
It refers to alleles the individual carries eg: YY, Yy and yy.

**Phenotype** (appearance)
The way an organism looks and behaves.
Dihybrid Crosses: The Principle of Independent Assortment

The Principle of Segregation explains the behavior of alternative forms of a single trait in a monohybrid cross. The next step is to extend this to follow the behavior of two different traits in a single cross: a dihybrid cross. With an understanding of the behavior of single traits, Mendel went on to ask if different traits behaved independently in hybrids. He first established a series of true-breeding lines of peas that differed in two of the seven characters he had studied. He then crossed contrasting pairs of the true-breeding lines to create heterozygotes. These heterozygotes are now doubly heterozygous, or dihybrid. Finally, he self-crossed the dihybrid F1 plants to produce an F2 generation, and counted all progeny types.

Traits in a dihybrid cross behave independently

Consider a cross involving different seed shape alleles (round, R, and wrinkled, r) and different seed color alleles (yellow, Y, and green, y). Crossing round yellow (RR YY) with wrinkled green (rr yy), produces heterozygous F1 individuals having the same phenotype (namely round and yellow) and the same genotype (Rr Yy). Allowing these dihybrid F1 individuals to self-fertilize produces an F2 generation.

The F2 generation exhibits four types of progeny in a 9:3:3:1 ratio

If the traits behave independently, we expect to see all possible combinations between traits of both characters: round yellow, round green, wrinkled yellow and wrinkled green seeds. Again, we expect the two types of gametes found in the parents: RY and ry and new combinations of alleles i.e. Ry and rY.

We can then construct a Punnett square with these gametes to generate all possible progeny. This is a 4 × 4 square with 16 possible outcomes. Filling in the Punnett square produces all possible offspring. From this we can see that there are 9 round yellow, 3 wrinkled yellow, 3 round green, and 1 wrinkled green. This predicts a phenotypic ratio of 9:3:3:1 for traits that behave independently.
Punnett square showing results of crossing of 2 True breeding individuals (yellow rounded and green wrinkled seeded) and subsequent self crossing of the offsprings.
What did Mendel actually observe?

From a total of 556 seeds from self-fertilized dihybrid plants, he observed the following results:

- 315 round yellow (signified $R_\_ Y_\_$, where the underscore indicates the presence of either allele),
- 108 round green ($R_\_ yy$),
- 101 wrinkled yellow ($rr Y_\_$), and
- 32 wrinkled green ($rr yy$).

These results are very close to a 9:3:3:1 ratio. (The expected 9:3:3:1 ratio from this many offspring would be 313:104:104:35.) The alleles of two genes appeared to behave independently of each other. Mendel referred to this phenomenon as the traits assorting independently. Note that this independent assortment of different alleles in no way alters the segregation of individual pairs of alleles for each gene. Round versus wrinkled seeds occur in a ratio of approximately 3:1 (423:133); so do yellow versus green seeds (416:140).
Mendel obtained similar results for other pairs of traits. We call this Mendel’s second law of heredity, or the Principle of Independent Assortment. This can also be stated simply: *In a dihybrid cross, the alleles of each gene assort independently.*

A more precise statement would be: **the segregation of different allele pairs is independent.** This statement more closely ties independent assortment to the behavior of chromosomes during meiosis. The independent alignment of different homologous chromosome pairs during metaphase I leads to the independent segregation of the different allele pairs.

**Q: What are the possible results for test crossing of yellow rounded, yellow wrinkled and green rounded seeded pea plants?**

**Note:**
Yellow rounded seeded plants may carry *YYRR, YYRr, YyRR* or *YyRr* genotype.
Yellow wrinkled seeded plants may carry *YYrr* or *Yyrr* genotype.
Green rounded seeded plants may carry *yyWW* or *yyWw* genotype.

**Probability: Predicting the Results of Crosses**

Probability allows us to predict the likelihood of the outcome of random events. Because the behavior of different chromosomes (carrying alleles) during meiosis is independent, we can use probability to predict the outcome of crosses. The probability of an event that is certain to happen is equal to 1. In contrast, an event that can never happen has a probability of 0. Therefore, probabilities for all other events have fractional values, between 0 and 1. For instance, when you flip a coin, two outcomes are possible; there is only one way to get the event “heads” so the probability of heads is one divided by two, or ½ . Another example is rolling a die; only one outcome (of six) is possible. Thus, the probability any outcome is one divided by six, or 1/6.

In the case of genetics, consider a pea plant heterozygous for the seed color alleles *Y* and *y*. This individual can produce two types of gametes in equal numbers, again due to the behavior of chromosomes during meiosis. So the probability of any particular gamete carrying a *Y* allele is 1 divided by 2 or, just like the coin toss.
We can use probability to make predictions about the outcome of genetic crosses using only two simple rules. Before we describe these rules and their uses, we need another definition. We say that two events are *mutually exclusive* if both cannot happen at the same time. The heads and tails of a coin flip are examples of mutually exclusive events. Events occurring of any of them does not affect the chance for occurring the others. Ex: Two consecutive coin flips where you can get two heads or two tails.

**The rule of addition**

Consider a six-sided die instead of a coin: for any roll of the die, only one outcome is possible, and each of the possible outcomes are mutually exclusive. The probability of any particular number coming up is 1/6. The probability of either of two different numbers is the sum of the individual probabilities, or restated as the **rule of addition**: For two mutually exclusive events, the probability of either event occurring is the sum of the individual probabilities. Probability of rolling either a 2 or a 6 is:

\[ \frac{1}{6} + \frac{1}{6} = \frac{2}{6} = \frac{1}{3} \]

To apply this to our cross of heterozygous yellow F1, four mutually exclusive outcomes are possible: YY, Yy, Yy, and yy. The probability of being heterozygous is the same as the probability of being either Yy or Yy, or \( \frac{1}{4} \) plus \( \frac{1}{4} \), or \( \frac{1}{2} \). Similarly, the probability of being yellow is \( \frac{3}{4} \) and self crossing of heterozygous yellow rounded seeded plants results in a probability of (3/16) for yellow wrinkled (explain).

**The rule of multiplication**

The second rule, and by far the most useful for genetics, deals with the outcome of independent events (Events occurring of any of them does not affect the chance for occurring the others). This is called the **product rule**, or **rule of multiplication**, and it states that the probability of two independent events both occurring is the *product* of their individual probabilities. We can apply this to a monohybrid cross in which offspring are formed by gametes from each of two parents. For any particular outcome then, this is due to two independent events: the formation of two different gametes. Consider the yellow F1 parents from earlier. They are all Yy (heterozygotes), so the probability that a particular F2 individual will be yy (homozygous recessive) is the
probability of receiving a y gamete from the male (½) times the probability of receiving a y gamete from the female (½), or ¼. This is actually the basis for the Punnett square that we used before. Each cell in the square was the product of the probabilities of the gametes that contribute to the cell. Similarly, self crossing of heterozygous yellow rounded seeded plants results in a probability of (1/16) for green wrinkled (explain).

**Statistical Tests for Mendelian Inheritance**

Dealing with biological issues usually faces some differences between expected and observed results. To judge whether these differences are significant or not statistical tools should be used.

**Chi Square (χ²) is “Goodness of Fit Test”**

\[ \chi^2 = \sum \frac{(\text{Observed results} - \text{Expected results})^2}{\text{Expected results}} \]

- The test statistic is compared to a theoretical probability distribution
- In order to use this distribution properly you need to determine the degrees of freedom = (phenotypic possibilities in the cross – 1).

If the calculated χ² is smaller than its value in table for critical values of χ² distribution at 0.05 at the same degree of freedom then the hypothesis is accepted and the data is useful.

If the calculated χ² is greater than its value in table for critical values of χ² distribution at 0.05 at the same degree of freedom then the hypothesis is rejected and the data is not useful.

Example:
The results of test crossing of a pea plant producing yellow rounded seeds were 160 plant of which:
35 Plants with yellow rounded seeds
42 Plants with yellow wrinkled seeds
47 Plants with green rounded seeds
36 Plants with green wrinkled seeds

Examine the agreement of these results with the principle of independent assortment.

**Answer**

The appearance of recessive traits of both characters reveals that the yellow rounded parent is heterozygous for both characters i.e. carrying YyRr genotype.

According to the principle of independent assortment, the predicted results of this test cross will be:

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Number of plants in each class = probability of its occurrence X total number of plants.

Plants with yellow rounded seeds = 160 X ¼ = 40 Plant
Plants with yellow wrinkled seeds = 160 X ¼ = 40 Plant
Plants with green rounded seeds = 160 X ¼ = 40 Plant
Plants with green wrinkled seeds = 160 X ¼ = 40 Plant

\[ \chi^2 = \sum \frac{(\text{Observed results} - \text{Expected results})^2}{\text{Expected results}} \]

\[ \chi^2 = \frac{(35 - 40)^2}{40} + \frac{(42 - 40)^2}{40} + \frac{(47 - 40)^2}{40} + \frac{(36 - 40)^2}{40} \]

\[ \chi^2 = 2.35 \]

We have 4 phenotypic possibilities:
  • Yellow Rounded
  • Yellow wrinkled
  • green Rounded
  • green wrinkled

Degree of freedom = (phenotypic possibilities in the cross – 1) = 4 -1 = 3.

In table for critical values of \( \chi^2 \) distribution at 0.05 at 3 degrees of freedom

\[ \chi^2 = 7.815 \]

Thus calculated \( \chi^2 \) is smaller than that of table i.e. these data agree with the principle of independent assortment.
## Critical values of the $\chi^2$ distribution

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*P*, probability; *df*, degrees of freedom.