

INDEX

BIOSCIENCE – VOLUME III

CHAPTER NO.	CHAPTER NAME	PAGE NO.
5	GENETICS	1
	• Mendelian Genetics	1
	• Extensions of Mendelian Principles	16
	• Gene Interactions	25
	• Extra Mendelian Inheritance	34
	• Gene Mapping	43
	• Molecular Markers	52
	• Cytoplasmic Inheritance	55
	• Human Genetics	68
	• Quantitative Genetics	83
	• Microbial Genetics	89
	• Mutation	99
	• Structural and Numerical Changes of Chromosomes	114
	• Recombination	131
6	MICROBIOLOGY	134
	• Introduction	134
	• History of Microorganism	135
	• The Evolutionary Tree of Microorganism Life	142
	• General Characters of Bacteria	147
	• Isolation of Microorganism	171
	• Bacterial Diseases and Causative Organisms	174
	• Virus	181
	• Sterilization and control of Microorganism	191
	• Applied and Industrial Microbiology	201
	• Environmental Microbiology	215
7	TECHNIQUES	227
	• Isolation and Purification of RNA and DNA	227
	• Gel Electrophoresis	232
	• Protein Sequencing	244
	• The Methodology for DNA Sequencing	247
	• Strategies of Genome Sequencing	251
	• Hybridization Techniques	257
	• Method of Analysis of Gene Expression	258
	• Co-immunoprecipitation	274

• Mtt Assay	275
• Polymerase Chain Reaction	279
• Molecular Analysis Using UV/Visible Spectroscopy	281
• Fluorescence Spectroscopy	284
• Optical Rotatory Dispersion (ord) / Circular Dichroism (cd) Spectroscopy	286
• Nuclear Magnetic Resonance Spectroscopy (nmr)	288
• Electron Spin Reversal Spectroscopy (esr)	290
• Microscopic Techniques	295
• The Electron Microscope	303
• Stereo Electron Microscopy and 3-d Electron Tomography	309
• Centrifugation	310
• Statistical Methods	313
• D2. Samples and Populations	315
• Measures of Central Tendency (average, mean, median, mode)	317
• D5. Tests of Statistical Significance; Standard Error; Confidence Limits	322
• The chi-square Test	325
• D7. Probability Distributions (normal, biomial and poisson)	326
• D8. Correlation	329
• D9. Regression Analysis	330
• D10. Analysis of Variance (anova)	330
• Radiolabeling Techniques	332
• E2. Radioactive Decay and Half-life	334
• E3. Applications of Radioisotopes in Biology	335
• E4. Quantification of Radioactivity	339
• Modern Dating Techniques	341
• Effects of Radiations on Biological Systems and Safety Concerns	343
• Transgenesis	345
• Plant Tissue Culture	349
• Chromatography	351

Chapter-5 Genetics

1. Mendelian Genetics

Since prehistoric times, the observation that living things inherit traits from their parents has been used to improve crop plants and animals through selective breeding. But how characters are passed from one generation to next? What are the fundamental principles involved? We can now answer these questions thanks to the excellent work by “**Gregor Johann Mendel**”.

Genetics is the scientific study of inheritance and variation—of how certain qualities or traits are passed from parents to offspring as a result of changes in DNA sequence. **William Bateson** in 1905 used the word ‘genetics’ to designate ‘the science of heredity and variation’

Mendel’s Work

Mendel was born into a German-speaking Czech family in a village in Northern Moravia (now a part of the **Czech Republic**) on 22 July 1822. During his childhood, Mendel worked as a gardener and studied beekeeping. From 1840 to 1843, he studied practical and theoretical philosophy and physics at the Philosophical Institute of the University of Olomouc. His family valued education but had little resources to send him to school, so he struggled to pay for his education.

His professor recommended Mendel to the Augustinian monks in Brunn (now Brno), who valued science, research, and education. He became a monk in part as it enabled him to obtain an education without having to pay for it himself.

After entering the priesthood he was sent to the University of **Vienna**, where he studied biology, physics, and mathematics. He returned to the Brno monastery in 1853 to teach. With teaching, he also started conducting experiments on plants in a small plot (2 hectares) of land which was available in the monastery. Over seven years (1856-1863), Mendel cultivated and tested some 28,000 plants, the majority of which were common pea plants (*Pisum sativum*).

Mendel selected *Pisumsativum* for his investigations to establish fundamental principles of inheritance. He developed a technique of emasculation and **bagging** for hybridization in plants. Flowers of the pea plant are bisexual. For cross pollination one plant is considered as male and another as female. To designate the female plant, the stamens of the plant were removed at juvenile stage, this is called **emasculation**. Emasculation was done to prevent self-pollination. Emasculated flowers covered by bags, which is called **bagging**. Bagging is used to prevent undesirable cross pollination. For cross pollination, mature pollen grains are collected from male plants and spread over emasculated flowers. Hybrid seeds are formed in the female flower after pollination. The plants that are obtained from these seeds are called **first filial generation** or F1 generation.

His experiments led him to make three fundamental principles of inheritance – **concept of dominance**, **law of segregation** and **law of independent assortment**. Experiments performed by Mendel on genetics and description of mechanisms of hereditary processes and formulation of principles are known as **Mendelism**.

On February 8, 1865, Mendel presented his work to the Brunn Society for Natural Science. His paper, “**Experiments on Plant Hybridization**,” was published the next year



Figure 1: Gregor Johann Mendel

Initial reaction to Mendel's work

Mendel's work was appreciated for its thoroughness, no one seemed to grasp its importance. His work received a few favorable press reports in local papers in 1866 but was largely ignored by the scientific community. When Mendel's paper was published, it was seen as primarily about hybridization rather than inheritance, had little impact, and was cited only about three times over the next thirty-five years. The work was simply too ahead of its time, too contrary to popular beliefs about heredity. He died in 1884 without getting any credit for his work due to kidney disease (Bright disease).

Mendel worked with traits that were inherited in distinct classes (specifically, tall versus dwarf plant height). Such variations which show two or more distinct phenotypes (limited number) for a particular character in a population are referred to as **discontinuous variation**. Mendel believed that heredity is the result of discrete units of inheritance (**particulate inheritance**), and every single **unit** or **factor** was independent in its actions in an individual's genome. Mendel used higher statistical calculation in his experiments so the results were complicated to understand.

During Mendel's lifetime, most biologists including Charles Darwin held the idea that all characteristics were passed to the next generation through **blending inheritance**, in which the traits from each parent are averaged, which is incorrect.

Although this blending hypothesis appeared to be correct in the majority of cases because of what we know now as **continuous variation**. Continuous variation shows phenotypic variation from one extreme to another and results from the action of many genes to determine a characteristic like human height. Offspring appear to be a "**blend**" of their parents' traits when we look at characteristics that exhibit **continuous variation**.

Darwin tried unsuccessfully to explain inheritance through a **theory of pangenesis**. According to this theory every cell, tissue and organ of the animal body produces many minute particles known as pangenes or gemmules. These gemmules are discharged in the bloodstream and are deposited in the reproductive organs. These reproductive cells contain these pangenes and a child develops as a

result of blending of the pangenesis from two parents. Thus the individual would represent the mixture of both of the parents. Notably, Charles Darwin was not aware of Mendel's paper, and it is believed that if he was aware of Mendel's work the genetics as we know it today would have emerged much sooner.

Rediscovery of Mendel's work:

Mendel presented his theories in two public lectures in **1865** and a detailed written publication in **1866**, but his work was unfortunately paid no attention and ignored for 34 years. This is perhaps due to the controversies arising from the publication of Darwin's book on '**origin of species**'.

By 1900, the discovery of chromosomes had suggested to biologists that genes might be carried on chromosomes. When they read Mendel's work on **particulate inheritance**, the biologists connected the links between genes and chromosomes. During this time research aimed at finding a successful theory of discontinuous inheritance rather than blending inheritance led to independent replication of Darwin work by **Hugo de Vries (Holland)** and **Carl Correns (Germany)**, and **Erich von Tschermak (Austria)** and rediscovery of Mendel's writings. Correns framed the Mendels working in the form of two laws of Mendelism. Though was originally also credited with rediscovery, this is no longer accepted because he did not understand Mendel's work. All three of these researchers, each from a different country, published their rediscovery of Mendel's work within a two-month span in the spring of 1900.

After the rediscovery, Mendel's laws were widely accepted and called Mendel as the '**Father of Classical Genetics**'. Mendel's principles of inheritance, originally given for pea plants, were equally applicable for most other organisms including humans. This helped clinicians to understand the genetic basis of human disease for example, within just a couple of years of the rediscovery of Mendel's work, **Garrod** applied Mendel's principles to decipher genetic basis of inborn error of metabolism like **alkaptonuria** and **phenylketonuria**.

Reason for Mendel's Success

Prior to Mendel, other researchers worked on several varieties of plants that differed from one another in many complex characteristics and produced fertile hybrids

through artificial pollination, but they were unable to explain the mechanism of heredity.

The two major reasons behind their failure were:

1. For hybridization, they selected parents showing variations in multiple characters. This made them confused. Due to the confusion, they were not able to trace individual characters through successive generations, nor could they keep complete numerical records of results.
2. They believed in blending inheritance that the hereditary characters of two parents (mother and father) become thoroughly mixed in the offspring.

Mendel was aware of the reasons that contributed to failure of earlier workers. In order to overcome these obstacles, he selected pea plants, carefully planned his experiments and maintained a detailed record of observations after each cross. The important reason for Mendel's success were

1. He chose the pea plant (*Pisum sativum*) as the experimental subject due to its numerous advantages for genetic research. It is easily grown in small plots, and Mendel had access to the monastery garden and greenhouse. These plants grow rapidly, producing an entire generation within a single growing season. Pea plants also produce a large number of seeds. As the sample size (progeny) increases, the chance factor declines, and thus some statistically significant result can be drawn after experimentation.
2. Mendel had access to a large number of pea varieties with distinct variations. Due to natural self-pollination, these varieties differed in various traits and were genetically pure. As a result, cross pollination can be performed in it artificially so hybridization can be made possible.
3. His choice of seven pea plant characteristics to study was also crucial to his success (**Figure 1**). He chose those characteristics that have two easily distinguishable traits, such as tall versus dwarf height, round versus wrinkled seeds, and purple versus white flower, over those that have a lot of variation. The flowers of pea plants are bisexual. Flower is closed typed such that pollination is limited to self. Cross pollination can be easily done by removing stamens

before pistils of flower mature by the process called **emasculation** and cross with stigma of desired pea plant. The hybrids of pea plant produced by cross pollination are perfectly fertile

Character	Contrasting trait		F ₁ Results
Seed shape	round/wrinkled		all round
Seed color	yellow/green		all yellow
Pod shape	full/constricted		all full
Pod color	green/yellow		all green
Flower color	violet/white		all violet
Flower position	axial/terminal		all axial
Stem height	tall/dwarf		all tall

Figure 1: Seven characters in pea plant with contrasting traits selected by Mendel for his investigations.

4. Mendel selected one character at one time in his experiments of hybridization. So it was easy. Other scientists also performed cross-hybridization for many characters, this made the experiments complex and they could not accurately explain the results.
5. To test his hypothesis, Mendel used scientific methods. Prior to Mendel, researchers simply described the results of crosses, whereas Mendel developed hypotheses based on his primary observations and then tested his hypotheses with additional crosses. He kept detailed records of the number of progeny with each type of trait and calculated ratios between them. He paid great attention to detail, was expert at seeing patterns in detail, and was patient and thorough, conducting his experiments for more than seven years before trying to write his research results.
6. The characters selected by Mendel in pea plant were present on different chromosomes or if were on same chromosome was separated by long distance, Further, all the seven pairs of contrasting traits selected by him showed complete dominance and single gene was responsible for each character. Thus, Mendel was very

lucky that the plant did not show linkage, incomplete dominance or epistasis for the characters selected by him.

Common Genetic Terminology

Character or characteristics: An attribute or feature that has been chosen for investigation. For e.g. Plant height or human skin color are characters.

Chromosome: A chromosome is a thread-like structure with an organized package of DNA, histone and other proteins found in the nucleus of the cell. Different organisms have different numbers of chromosomes. Each chromosome has single double stranded DNA which carries multiple genes.

Homologous chromosomes: They are composed of chromosome pairs that have the same length, centromere position, and staining pattern for genes with the same corresponding loci. The organism inherits one homologous chromosome from its mother and the other from its father.

Chromatids: At meiosis, each of the homologous chromosomes is composed of two identical chromatids joined together by a common kinetochore (centromere). The identical chromatids are specifically called **sister chromatids** to distinguish them from the so-called non-sister chromatids.

Gene (Mendel's factor): Danish geneticist Wilhelm Johannsen first used it. The concept of a gene (the word coined by Bateson) is due to Mendel, who used the German word "Factor". Gene is a segment of a DNA molecule that codes for the synthesis of a single polypeptide; it is an inherited factor that determines a characteristic.

Allele: One of two or more alternate forms of a gene or marker at a particular locus on a chromosome. e.g., *R* and *r* are alleles for seed shape (**Figure 2**). A locus is the location on the genome of a gene. Thus, there is a specific place—a **locus**—on a chromosome in pea plants where the shape of seeds is determined. This locus might be occupied by an allele for round seeds (*R*) or one for wrinkled seeds (*r*). In a natural population the most common allelic form is usually called the **wild-type allele**. However, in many populations there can be multiple variants at the DNA sequence level that are visibly indistinguishable as all exhibit a normal,

wild type appearance. There can also be various mutant alleles (in wild populations and in lab strains) that vary from wild type in their appearance, each with a different change at the DNA sequence level. Such collections of mutations are known as an **allelic series**.

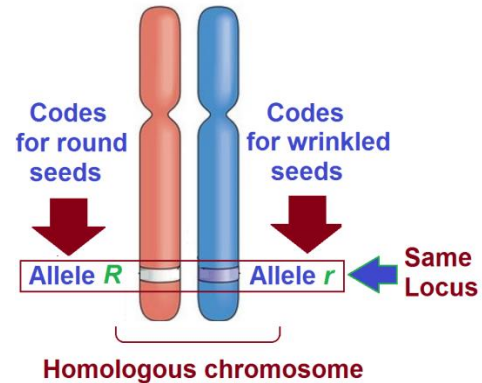


Figure 2: Allele resides at the same locus on homologous chromosomes.

Dominant allele: The allele which is able to exert its effect on phenotype in heterozygous state. For e.g. When the genotype is *Tt*, 'T' allele is sufficient enough to give a tall phenotype.

Recessive allele: The allele which is not able to exert its effect in phenotype in heterozygous state. For e.g., under condition '*Tt*', *t* allele is not able to exert its dwarf effect on phenotype.

Genotype: Combination of alleles in an individual which determines one of its phenotype or trait. Thus an individual's genotype is the totality of that individual's hereditary material. For e.g. *TT*, *Tt* or *tt*. Genotype is inherited while phenotype is not.

Phenotype or trait: The appearance or manifestation of character. A phenotype can refer to any type of characteristic: physical, physiological, biochemical, or behavioral. Thus, the condition of having round seeds is a phenotype, a red eye color is a phenotype, and having sickle-cell anemia is one of the phenotypes. The phenotype depends on the genotype and further it may be influenced by the environment.

Penetrance: The frequency (in percent) with which a dominant or homozygous recessive gene or gene combination manifests itself in the phenotype of the carriers.

Phenocopy: A nonhereditary, phenotypic modification (caused by special environmental conditions) that mimics a similar phenotype caused by a gene mutation

Homozygous: The situation in which similar alleles are present at the locus for characteristics in diploid organisms. For e.g. AA or aa.

Heterozygous: The situation in which two different alleles are present at locus for characteristics. eg. Aa or Tt.

Hemizygous: When one or more genes is present in only one, instead of two copies, eg, human males are hemizygous for most genes on the X and Y chromosomes.

Homomorph: Morphologically identical members of a homologous pair of chromosomes. For example, the sex chromosome is homomorph in human females (XX).

Heteromorph: A chromosome pair with some homology but differing in size, shape, or staining properties. The X and Y chromosomes are different in human males and are termed as heteromorph.

Reciprocal cross: A cross between two sets, in one case, a male exhibiting the trait of interest is mated with a female that does not exhibit that trait, and in the other set, a female exhibiting the trait is crossed with a male lacking that trait (**Figure 3**). Its purpose is to observe the effect of parental sex on the inheritance of a trait. In autosomal inheritance transmission of trait is not affected by parental sex while reciprocal crosses given variable results in case of sex-linked inheritance, cytoplasmic inheritance and genomic imprinting.

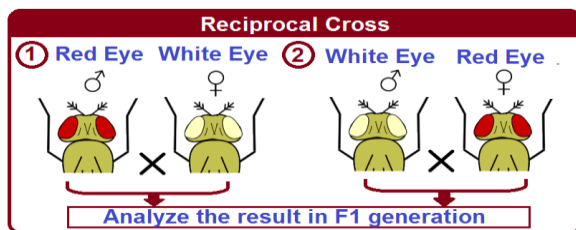


Figure 3: Reciprocal cross is performed to assess the influence of parental sex on inheritance

Back cross: It is a crossing of a F1 hybrid with one of its parents to achieve offspring with a genetic identity closer to that of the parent. When an F1 individual is crossed with a dominant parent then it is termed out cross. The generation obtained from this cross, all possess dominant character.

Test cross: It is a specific type of back cross, which involves the crossing of an F1 individual with a parent who had a homozygous recessive phenotype and genotype (**Figure 4**). The characteristics of the progeny produced from such a breeding would help in determining the zygosity (homozygous or heterozygous) of the parent with respect to a particular gene or trait. If all progeny shows dominant phenotype it means unknown dominant genotype is homozygous, if only 50 % progeny shows dominant trait and rest show recessive trait (1:1), it means unknown dominant genotype is heterozygous.

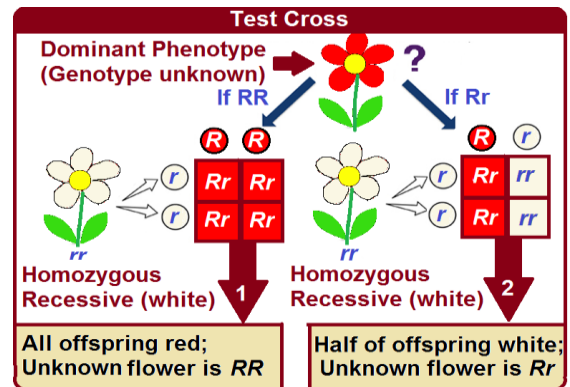


Figure 4: Test cross is used to test homozygosity or heterozygosity of unknown dominant phenotype.

Genome: In eukaryotes, the basic (monoploid) chromosome set, consisting of a species-specific number of linkage groups and the genes contained therein. For example, in humans male, the genome consists of the 24 different chromosomes (22 autosomes, X and Y chromosomes). The mitochondrial DNA is usually considered to be a separate “mitochondrial” genome.

Linkage: It refers to the observation that two or more genes located on the same chromosome are inherited together. The ratio of being transmitted together versus being separated allows an estimate of their distance from each other (recombination fraction).

Crossing over: The exchange of genetic material between non-sister chromatids of homologous chromosomes.

Haplotype: A combination of alleles at closely linked gene loci that are inherited together.

Linkage disequilibrium: Alleles at different loci that are inherited together more frequently or less frequently than expected by their individual frequencies are said to show linkage disequilibrium.

1.3 Monohybrid Cross

When we consider the inheritance of one character at a time by a cross is called a monohybrid **cross**. Mendel began his research on a large number of pea varieties (at least 34) before selecting varieties for his experiments. He confirmed that each variety was genetically pure (homozygous for each of the traits) by growing the plants for two generations, allowing self-pollination, and confirming that all offspring were genetically identical to their parents.

After that, he performed **cross pollination** between two true-breeding pea plants with different traits. Pea is naturally self-pollinating, so cross is done by manually transferring (dusting) pollen from the anther of mature pea plant to the stigma of another mature pea plant. Mendel carefully removed all of the anthers from the female flowers before they matured (**emasculation**), to prevent the self-pollination and affecting his results. (**Figure 5**).

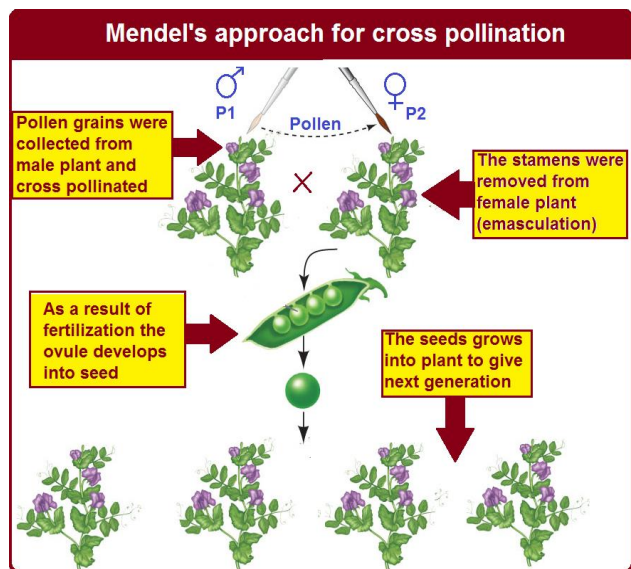


Figure 5: Cross pollination between selected varieties.

Mendel began his research with monohybrid crosses—crosses between parents that differed only in a single characteristic. In one of the experiments, Mendel crossed pea plants of parental generation (P) where one plant is homozygous pure for round seeds (RR) and one plant is homozygous pure for wrinkled (rr) seeds. (**Figure 6**). Then he observed the offspring that resulted from the cross. The offspring from the parents of the P generation are termed as the F₁ (first filial) generation. When Mendel examined the F₁ of this cross, he found that progeny expressed only

one of the phenotypes present in the parental generation: **all the F₁ progeny had round seeds.**

Mendel repeated the experiment several times and always got the same result. He also performed reciprocal crosses, but the results were the same: all of the F₁ were round. At the time, common general belief was that the hybrid seeds would be intermediate, or that the hybrid would have an equal number of round and wrinkled seeds. That is, the offspring were expected to blend the contrasting parental traits. But surprisingly Mendel's findings revealed that the wrinkled trait had disappeared completely in the F₁ generation.

Importantly, Mendel did not stop his experimentation there. He allowed the F₁ plants to self-fertilize producing a second generation (the F₂ generation). Both of the traits from the P generation emerged in the F₂; Mendel counted 5474 round seeds and 1850 wrinkled seeds in the F₂ (**see Figure 6**). Mendel observed that the number of the round and wrinkled seeds constituted approximately a 3 to 1 ratio; that is, in F₂ seeds $\frac{3}{4}$ were round and $\frac{1}{4}$ were wrinkled.

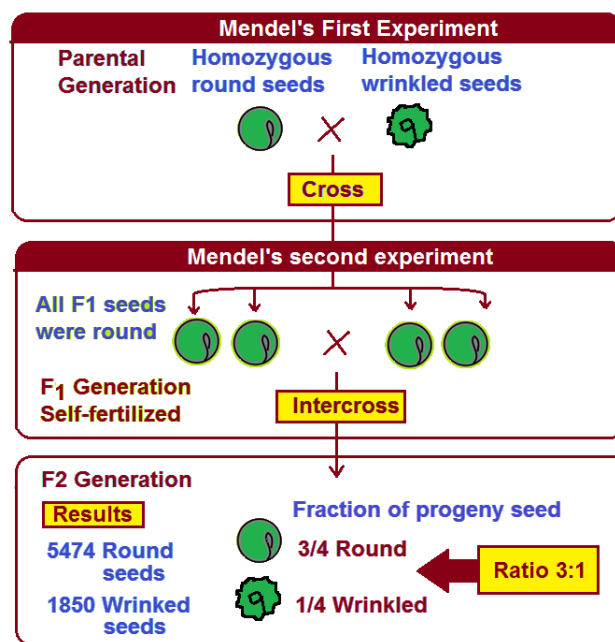


Figure 6: Result of Mendel's monohybrid cross

For the other six characteristics Mendel examined, the F₁ and F₂ generations behaved in the same way as they had for seed shape. One of the two traits would disappear completely from the F₁ generation only to reappear in the F₂ generation at a ratio of approximately 3:1.

1.4 Conclusion from Monohybrid Cross and Law of Segregation

The important conclusion drawn from Mendel from the results of his monohybrid crosses are as follows

- Mendel proposed that each genetic character is influenced by a pair of **unit factors** found at locus. The factors (alleles) identified by Mendel are denoted by letters. The allele for round seeds, for example, is R , while the allele for wrinkled seeds is r . The round-seeded parent and the wrinkled-seeded parent had two identical factors in the parental generation of cross: RR in the round-seeded parent and rr in the wrinkled-seeded parent (**Figure 7a**). He came to the conclusion that, while F_1 plants have phenotypes similar to one of their parents, they must **inherit genetic factors from both parents** because they pass both phenotypes on to the F_2 generation. The presence of both round and wrinkled seeds in the F_2 could be explained only if the F_1 plants possessed factors (alleles) both for the round and wrinkled phenotype inherited from the parental generation.
- Mendel's second conclusion was that when gametes are formed during monohybrid crosses, the **two factors** in each plant **separate**, and each gamete has only one factor (**pure**). When two gametes (one from each parent) fuse to form a zygote, the male parent's factor combines with the female parent's factor to produce the offspring's genotype. Thus, Mendel's F_1 plants inherited a R factor from the round-seeded plant and a r factor from the wrinkled-seeded plant (**Figure 7b**).
- When two dissimilar factors are present together in an F_1 generation, only the trait encoded by round factor (R) is expressed, and all of the F_1 progeny have round seeds. The trait which appeared (round) in the F_1 heterozygous offspring was termed as **dominant** and the traits that disappeared (wrinkled) in the F_1 heterozygous offspring was called **recessive**. From these observations Mendel proposed the concept of **dominance**.

Concept of dominance states that when two different alleles are present in a genotype (heterozygote), rather than both alleles contributing to a phenotype,

only one of the alleles contribute to the trait for that characteristic. The allele exerting its effect in phenotype is termed as the dominant allele. The recessive allele will remain "hidden," but will be transmitted to offspring by the same manner in which the dominant allele is transmitted. The recessive trait will only be expressed by offspring that have two copies of this allele; these offspring will breed true when self-crossed.

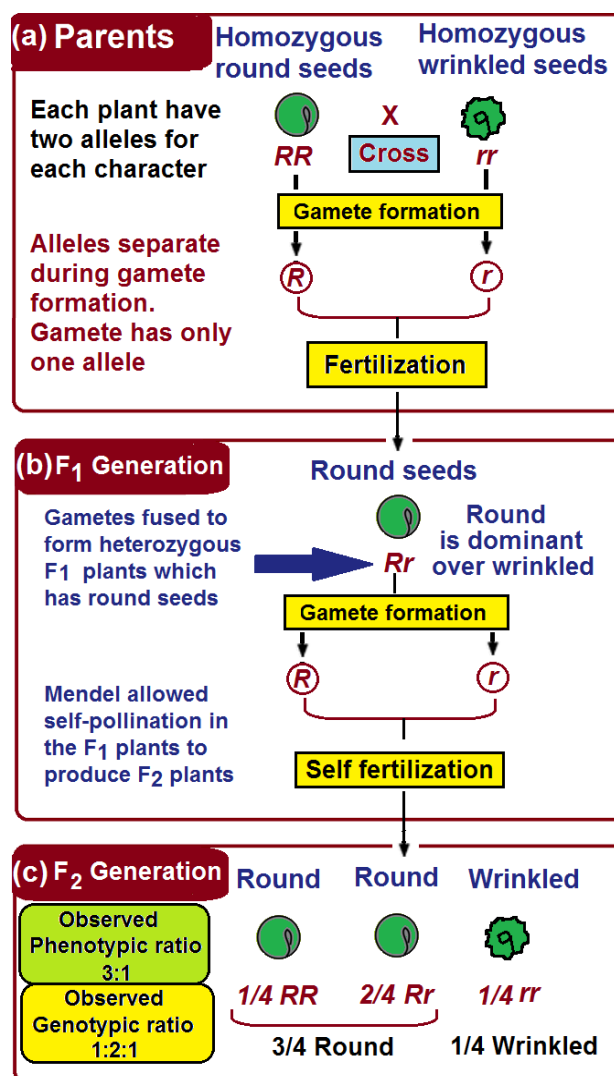


Figure 7: Conclusion of monohybrid cross.

- Last conclusion drawn by the Mendel from monohybrid cross was that when plants of the F_1 (with genotype Rr) produced gametes, half of the gametes received the R allele for round seeds and half received the r allele for wrinkled seeds. Thus, **two alleles of an individual plant must separate with equal probability into the gametes**. The gametes produced from the F_1

plant then paired randomly to produce the following genotypes in equal proportions among the F_2 : RR , Rr , rR , rr (**Figure 7c**). Since, the round (R) factor is dominant over wrinkled (r), there were three round progeny in the F_2 (RR , Rr , rR) for every one wrinkled progeny (rr) in the F_2 . This 3:1 ratio of round to wrinkled progeny that

Mendel observed in the F_2 could occur only if the two alleles of a genotype separated into the gametes with equal probability. On the basis of above observations and conclusions from his monohybrid crosses, Mendel developed the Law of segregation.

The Law of segregation: Mendel's first law of segregation states that each diploid individual possesses two factors (alleles) for a particular characteristic. These two alleles segregate (separate) when gametes are formed, and one allele moves to each gamete (gamete is always pure). Moreover, the two alleles segregate into gametes with equal probabilities.

1.5 Physical Basis for Law of Segregation

Up to now we have studied the results of monohybrid crosses and Mendel's principle of segregation. But the question may come to mind: what are the factors or alleles? Where do they reside? How they separate and move to gametes, what T , t , TT or Tt really means? Answers to these questions can only be given if we look at the behavior of chromosomes during meiosis.

Up until 1900, when Mendel's work was rediscovered, the relationship between genes and chromosomes was unknown. **Walter Sutton** in 1902 and **Theodor Boveri** in 1903 proposed, independently, that genes are located on chromosomes. Boveri was studying sea urchins, in which he found that all the chromosomes had to be present for proper embryonic development to take place. Sutton's work with grasshoppers showed that chromosomes occur in matched pairs of maternal and paternal chromosomes which separate during meiosis.

During a careful examination of chromosome behaviour during meiosis, Sutton and Boveri discovered that each homologous pair of chromosomes consists of one maternal chromosome and one paternal chromosome, and that these pairs segregate independently into gametes during meiosis. They concluded that **behavior of Mendelian**

factors (alleles) and that of homologous chromosomes during meiosis is similar, so probably the Mendelian factor resides on a pair of homologous chromosomes. These conclusions provided a physical basis to heredity. This groundbreaking work prompted E.B. Wilson named the **chromosome theory of inheritance** the "**Sutton-Boveri Theory**" in his classic text.

Each chromosome in a diploid organism has a pairing partner termed as the homologous chromosome. For a homologous pair in a diploid cell, one chromosome is inherited from the mother and the other from the father. Similarly, diploid cells have two alleles at each locus, which make up the genotype for that locus and according to the principle of segregation, one allele of the genotype is inherited from each parent. The similarity in the number of chromosomes and alleles is not coincidental—the two alleles of a genotype are located on homologous chromosomes.

The symbols used in genetic crosses, such as T and t , are simply shorthand notations for specific DNA sequences (alleles) in chromosomes that code for specific phenotypes. The two alleles of a genotype are found on different but on the same locus on homologous chromosomes.

Each chromosome replicates during the S stage of the cell cycle, producing two copies of each allele, one on each chromatid (**Figure 8a**). During anaphase I of meiosis, homologous chromosomes segregate, whereas chromatids separate during anaphase II.

If **no crossing over occurs** during prophase I, the mendelian factors or alleles **will separate during anaphase I**, when homologous chromosomes separate (alleles reside at the same locus on homologous chromosomes) (**Figure 8b**). If **crossing over occurs** during prophase I of meiosis, the two chromatids of each replicated chromosome are no longer identical, and different factors or **alleles segregate during anaphase II**, when two chromatids separate (**Figure 8c**).

Mendel's heredity principles are entirely based on the outcomes of the crosses he performed. However, it is important to remember that Mendel's principles work because they are based on the behaviour of actual chromosomes during meiosis for gamete formation.

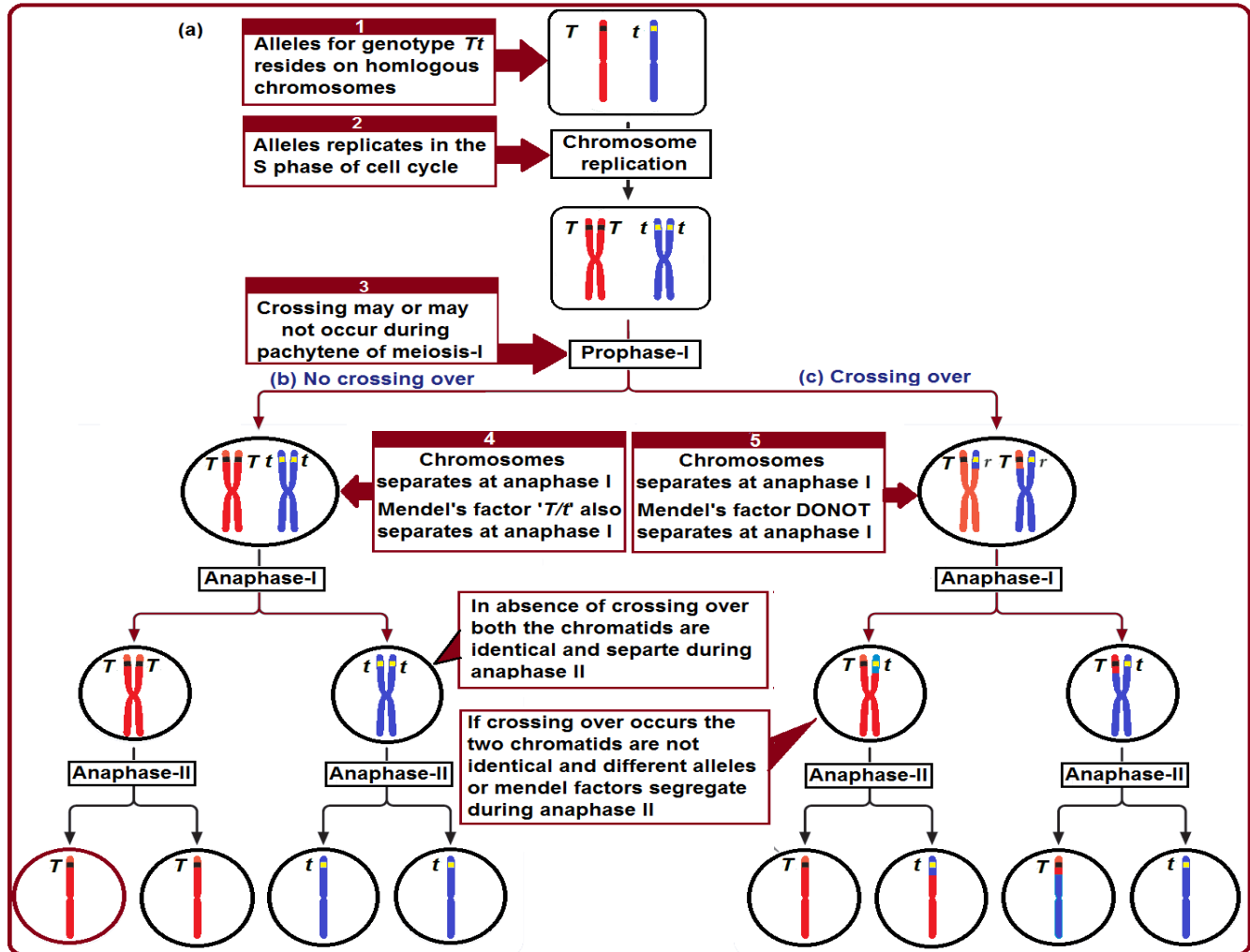


Figure 8: Physical basis of heredity: Behavior of homologous chromosomes and alleles during meiosis.

1.6 Assessment of Results of Monohybrid Crosses

During experimentation Mendel devised multiple ways to predict the outcome of crosses between plants with different phenotypes. Mendel discovered that tall (T) peas were dominant over dwarf (t) peas by crossing two varieties of peas that differed in stem height (t). Mendel confirmed that F_1 progeny showing dominant phenotype are heterozygous (Tt) for allelic combination using **test cross**.

A **test cross** is a back cross between an F_1 genotype and homozygous recessive parental genotypes (tt). To predict the types of offspring that will result from test cross, we must first determine which gametes will be produced by heterozygous F_1 parent and homozygous recessive parent according to law of segregation (**Figure 9a**). The tall plant in this cross is heterozygous (Tt); so $\frac{1}{2}$ of its gametes will

receive a tall allele (T) and the other $\frac{1}{2}$ will receive a dwarf allele (t). All gametes from the homozygous tt dwarf plant will receive a single dwarf (t) allele.

We can use **Punnett square** to explain the results of Mendel in a simple way. The Punnett square is a square diagram that is used to predict the genotypes of a particular cross or breeding experiment. It is named after **Reginald C. Punnett**, who devised the approach in 1905. To understand the importance of Punnett square, let's examine another cross that Mendel carried out.

A Punnett square is built by drawing a grid, placing the gametes formed by one parent along the upper edge and the gametes formed by the other parent down the left side (**Figure 9b**). Each block (a cell within the Punnett square) contains an allele from each of the corresponding gametes, generating the genotype of the progeny produced by

fusion of those gametes. In the upper left-hand block of the Punnett square in **Figure 9b**, a gamete containing T from the tall plant unites with a gamete containing t from the dwarf plant, giving the genotype of the progeny (Tt). It is worthwhile to write the phenotype expressed by each genotype; in this case the progeny will be tall, because the tall allele is dominant over the dwarf allele. This process is repeated for all the blocks in the Punnett square.

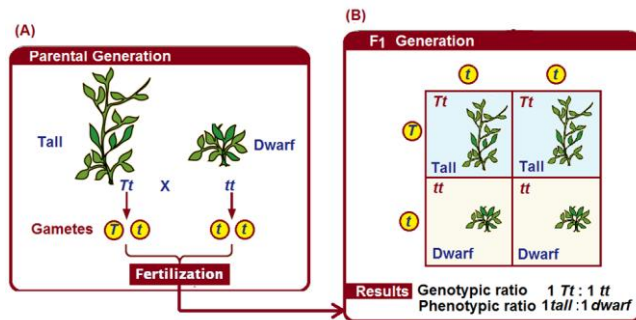


Figure 9: Use of Punnett square to interpret the result of the test cross.

By counting blocks simply, we can determine the types of progeny produced and their ratios. In **Figure 9b**, two blocks contain tall (Tt) progeny and two blocks contain dwarf (tt) progeny; so the genotypic ratio expected for this cross is 2 Tt to 2 tt (a 1:1 ratio).

Genetic Symbols:

The primary purpose of a gene or locus name and symbol is to be a unique identifier so that information about the gene in publications, databases and other forms of communication can be unambiguously associated with the correct gene. These guidelines, therefore, are intended to aid the scientific community as a whole to use genetic information

Different alleles in genetic crosses can be represented by symbols in a variety of ways. For example, upper case letters can represent dominant alleles, while lower case letters represent recessive alleles. If T is the dominant gene for plant height, t will be the recessive gene for dwarf height.

Another useful system was developed in genetic studies of the fruit fly *Drosophila melanogaster* to discriminate between wild-type and mutant traits. The most common allele for a character is known as the **wild type** because it is the most common allele in the population. This system

uses the initial letter, or a combination of several letters, from the name of the mutant trait. If the trait is recessive, lowercase is used; if it is dominant, uppercase is used. The contrasting wild-type trait is denoted by the same letters, but with a superscript $+$. For example, ebony is a recessive body color mutation in *Drosophila*.

The normal wild-type body color is gray. Using this system, we denote ebony by the symbol e , while gray is denoted by e^+ . The responsible locus may be occupied by either the wildtype allele (e^+) or the mutant allele (e). A diploid fly may thus exhibit one of three possible genotypes (the two phenotypes are indicated parenthetically):

e^+ / e^+	gray homozygote (wild type)
e^+ / e	gray heterozygote (wild type)
e / e	ebony homozygote (mutant)

Sometimes a forward slash is used to indicate that the two symbols are alleles of the same gene, but on homologous chromosomes. AA or A/A , both these representations are identical: it represents a homozygous dominant. If we consider a mutant allele that is dominant over the normal wild-type allele, such as the Wrinkled wing in *Drosophila*, the genotypes Wr/Wr , Wr/Wr^+ , and Wr^+/Wr^+ are the three possible genotypes.

The mutant wrinkled-wing phenotype is expressed in the first two genotypes. One advantage of this system is that it allows for additional abbreviation when necessary: The $+$ symbol can be used to represent the wild-type allele. The designations of the three possible genotypes in ebony, for example, become

$+ / +$	gray homozygote (wild type)
$+ / e$	gray heterozygote (wild type)
e / e	ebony homozygote (mutant)

Superscripts and subscripts are sometimes added to distinguish between genes: Lfr_1 and Lfr_2 represent dominant alleles at different loci that produce lacerate leaf margins in opium poppies; E^R represents an allele in goats that restricts the length of the ears.

To identify genes in various organisms, many different systems of genetic nomenclature are used. Typically, the symbol chosen reflects the gene's function or a disorder caused by a mutant gene. In yeast, for example, *cdk* is an

abbreviation for the cyclin-dependent kinase gene, the product of which is involved in cell-cycle regulation. In bacteria, leu^- denotes a mutation that disrupts the biosynthesis of the amino acid leucine, while leu^+ denotes the wild-type gene. The symbol *dnaA* denotes a bacterial gene that is involved in DNA replication (and DnaA, without italics, is the protein made by that gene). Italicized capital letters are used to name genes in humans: *BRCA1* is one of the genes linked to breast cancer susceptibility. Although these various systems appear to be complex, they are effective ways of representing genes.

1.8 The Dihybrid Cross

Mendel's law of **independent assortment** states that genes at one locus do not influence genes at other locus, with regard to the sorting of alleles into gametes, and every possible combination of alleles for every gene is equally likely to occur. In other words, segregation at one locus is independent of another locus. The independent assortment of genes can be illustrated by the dihybrid cross.

A dihybrid cross describes a mating experiment between two true-breeding parents that express different traits for two characteristics. A hybrid organism is one that is heterozygous, which means that it carries two different alleles at a particular genetic position, or locus. Therefore, a dihybrid organism is one that is heterozygous at two different genetic loci.

Mendel wanted to observe the effect of one character on another character. Consider the characteristics of seed shape and seed color for two pea plants, one that has round, yellow seeds (*RRYY*) and another has green, wrinkled seeds (*rryy*) (Figure 10a).

Because each parent is homozygous, the law of segregation indicates that the gametes for the wrinkled green seeded plant are all *ry*, and the gametes for the round/yellow seeded plant are all *RY*. Therefore, the F_1 generation of offspring all are *RrYy* (Figure 10b).

When Mendel self-fertilized the F_1 plants to produce the F_2 , the alleles for each locus separated, with one allele going into each gamete. This is where the principle of independent assortment becomes important.

Each pair of alleles can separate in two ways:

- (1) *R* separates with *Y* and *r* separates with *y* to produce gametes *RY* and *ry* or
- (2) *R* separates with *y* and *r* separates with *Y* to produce gametes *Ry* and *rY*.

The principle of independent assortment tells us that the alleles at each locus separate independently; thus, both kinds of separation occur equally and all four types of gametes (*RY*, *ry*, *Ry*, and *rY*) are produced in equal proportions (Figure 10b).

When these four types of gametes are combined to produce the F_2 generation, the progeny consist of 9/16 round and yellow, 3/16 wrinkled and yellow, 3/16 round and green, and 1/16 wrinkled and green, resulting in a 9:3:3:1 phenotypic ratio (Figure 10c).

A common misunderstanding is that principles of segregation and independent assortment refer to two distinct processes. Actually, the principle of independent assortment is an extension of the principle of segregation. The principle of segregation states that the two alleles at a locus separate in equal probability during gametes formation; the principle of independent assortment states that, when these two alleles separate at one locus, their separation is independent of the separation of alleles at other loci.

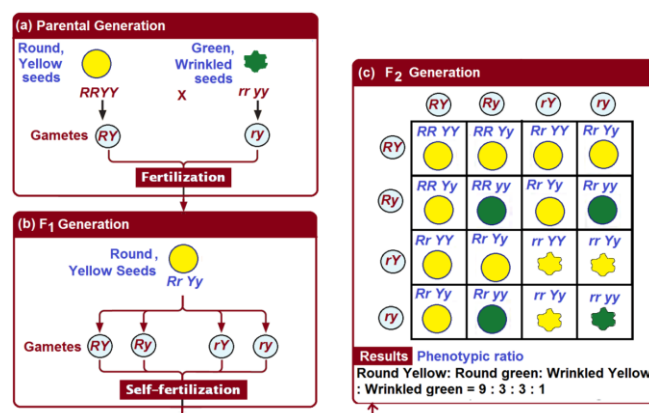


Figure 10: Mendel's dihybrid cross and independent assortment.

1.9 Physical basis for the law of independent assortment

The physical basis for the law of independent assortment is also found in **meiosis I**, where different homologous pairs align in random orientations. Because the orientation

of tetrads on the metaphase plane is random, each gamete can contain any combination of paternal and maternal chromosomes (and thus alleles on them). In anaphase I of meiosis, each pair of homologous chromosomes separates independently of all other pairs, so genes located on different pairs of homologs will assort independently. Independent assortment can produce 2^n types of variant gametes, where n is the number of chromosome pairs. Humans, for example, have 23 pairs of chromosomes, so each parent can produce 2^{23} different types of gametes. The major cause of variation among progenies of same parents is independent selection.

For independent assortment to work, it is necessary that genes responsible for the different characters must be located on different chromosomes. We know that each chromosome contains thousands of genes, thus there are chances that two genes under investigation may be on same chromosome. If genes are located on the same chromosome, they will move together during anaphase I of meiosis and will arrive at the same pole and within the same gamete (unless crossing over takes place). Thus, genes located on the same chromosome, do not assort independently (unless they are located sufficiently far apart that crossing over takes place every meiotic division) and most often shows **linkage**.

1.10 Use of Probability and the Branch Diagram to Dihybrid Crosses

The Punnett square is a valuable tool, but it's not ideal for every genetics problem. For instance, suppose you were asked to calculate the frequency of the recessive class not for an Aa x Aa cross, not for an AaBb x AaBb cross, but for an AaBbCcDdEe x AaBbCcDdEe cross. If you wanted to solve that question using a Punnett square, you could do it – but you'd need to complete a Punnett square with 1024 boxes. The five-gene problem above becomes less threatening once you realize that a Punnett square is just a visual way of representing probability calculations. At some point, it becomes quicker (and less error-prone) to simply do the probability calculations by themselves, without the visual representation of a clunky Punnett square.

Probability: Probabilities are mathematical measures of likelihood. In other words, they're a way of quantifying (giving a specific, numerical value to) how likely something

is to happen. A probability of 1 for an event means that it is 100 % going to happen, while a probability of 0 for an event means there is no chance for it to happen. A simple example of probability is having a $1/2$ chance of getting heads when you toss a coin.

The product rule: One probability rule that's very useful in genetics is the product rule, which states that the probability of two (or more) independent events occurring together can be calculated by multiplying the individual probabilities of the events.

For example, if you roll a six-sided die once, you have a $1/6$ chance of getting a six. If you roll two dice at once, your chance of getting two sixes is: (probability of a six on die 1) x (probability of a six on die 2) = $(1/6) \times (1/6) = 1/36$.

In general, you can think of the product rule as the “and” rule: if both event X and event Y must happen in order for a certain outcome to occur, and if X and Y are independent of each other (don't affect each other's likelihood), then you can use the product rule to calculate the probability of the outcome by multiplying the probabilities of X and Y.

We can use the product rule to predict frequencies of fertilization events. For instance, consider a cross between two heterozygous (Aa) individuals. What are the chances of getting an 'aa' individual in the next generation?

The only way to get an aa individual is if the mother contributes an a gamete and the father contributes an a gamete. Each parent has a $1/2$ chance of making a gamete. Thus, the chance of an aa offspring is: (probability of mother contributing a) x (probability of father contributing a) = $(1/2) \times (1/2) = 1/4$.

The sum rule of probability: In some genetics problems, you may need to calculate the probability that any one of several events will occur. In this case, you'll need to apply another rule of probability, the sum rule. According to the sum rule, the probability that any of several mutually exclusive events will occur is equal to the sum of the events' individual probabilities.

For example, if you roll a six-sided die, you have a $1/6$ chance of getting any given number, but you can only get one number per roll. You could never get both a one and a six at the same time; these outcomes are mutually exclusive. Thus, the chances of getting either a one or a six