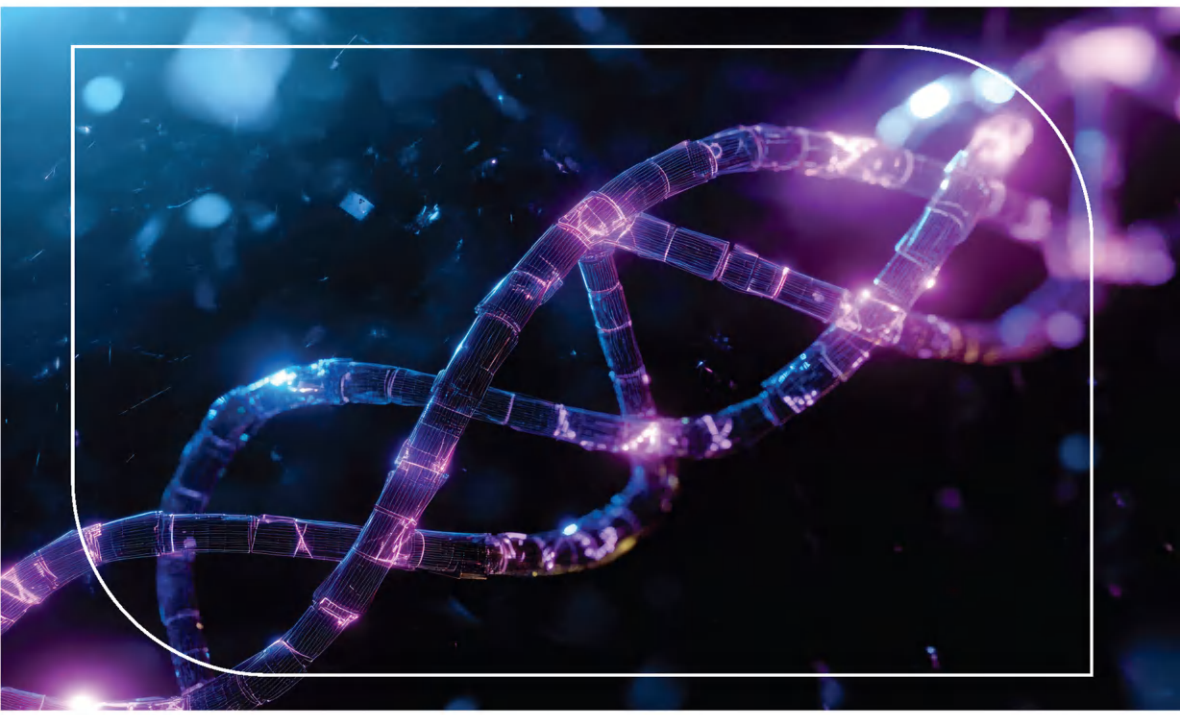
Molecular Genetics, Structures, Mechanisms, and Functions

Volume 2

Applications and Exploring the Nucleus



Tariq Ahmad Bhat
Abdul Rauf Shakoori
Jameel M. Al-Khayri
Editors

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Contents

Contributors ..... *xi*

Acknowledgment ..... *xiii*

Preface ..... *xv*

1. Mendel's Laws of Inheritance **1**
Maria Butt, Mahnoor Imran, Muhammad Naveed Shahid, and Uzma Qaisar

2. Linkage, Crossing Over, and Recombination **19**
Faiza Akhtar, Khadija Hafeez, Nadia Yasmeen, and Shagufta Perveen

3. Structure of Gene **65**
Wajahat Waseem Tantray and Sumira Farooq

4. Genome Diversity and Evolution **81**
Shagufta Perveen, Faiza Akhtar, Nadia Yasmeen, and Khadija Hafeez

5. Recombination and Evolution **121**
Shagufta Perveen, Nadia Yasmeen, Khadija Hafeez, and Faiza Akhtar

6. Mutations, Mutagens, and Types **157**
Mohd Gulfishan, Ajmat Jahan, Rayees Afzal Mir, Salma Praveen, and Tariq Ahmad Bhat

7. Genetic Engineering and Agricultural Science **175**
Rohit Patel, Krina Mehta, Sameera Sarma, Paritosh Parmar, Dweipayan Goswami, Meenu Saraf, and Arpit Shukla

8. Protein Synthesis **209**
Umbreen Bibi, Sara Zafar, Nazia Aslam, and Manzar Abbas

9. Transposable Elements **247**
Sara Zafar, Hina Zahid, Nazia Aslam, and Manzar Abbas

10. Chromosomal Aberrations **277**
Maida Wahid, Saima Iftikhar, and Muhammad Shahid Nadeem

Index ***309***



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Preface

Molecular Genetics, Structures, Mechanisms, and Functions comprises two volumes. Volume 2 is titled *Applications and Exploring the Nucleus*. The main motivation for writing this book was to explore the nucleus, understand its mechanisms, and put together its applications in applied fields for beginners, experts, and those venturing into the fascinating science of molecular biology.

This book has ten chapters that deal with the concept of the gene, its relationship with chromosomes, and its functional manifestation to the benefit of society in general. Some chapters particularly deal with the linkage, crossing over and recombination, and diversity, evolution, and recombination that have helped mankind to explore the hidden amazing genetic mechanisms. These concepts have paved the way for creating favorable biological understanding and opened new horizons in the applied fields. This technology has made a profound effect on the society, with applications for better health through improved disease diagnosis, human gene variation, improved drug and pharmaceutical production, better understanding of specific crime-scene forensics, and agriculture.

The book has chapters on mutagenesis and its mechanisms, protein synthesis, transposable elements, diversity of evolution, and chromosomal aberrations with in-depth mechanisms and repercussions in improving health, preventing genetic diseases, enhancing food resources, environmental bioremediation, etc.

Volume 1: Principles of Gene Manipulation and Genomics provides an overview of the future of genetic engineering and delves into the role of biotechnology and its applications in genetic engineering. It discusses the tools of recombinant technology, which have brought about revolution in our understanding of various complex biological phenomena. Chapters cover mutagenesis, construction, and sequencing of DNA libraries along with applications of genetic engineering for improving health, preventing genetic diseases, enhancing food resources, managing environmental bioremediation, and more. Topics include genetic engineering tools for restriction enzymes and vectors, gene and cell division, mutation detection and screening in plants, population genetics, sexuality in bacteria, and more. Several chapters focus on the tools of recombinant technology, such as restriction enzymes,

vectors, etc., that have paved the way for creating organisms of choice and opened new horizons in the fields of medicine, agriculture, and industry for human welfare.

All the chapters included in these two volumes have been contributed by brilliant young and upcoming biologists and highly experienced senior molecular biologists from different reputed universities and research institutes.

CHAPTER 1

Mendel's Laws of Inheritance

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ABSTRACT

Inheritance can be defined as the process of transferring genetic information from the parents to offsprings in living organisms. The whole process of heredity is dependent upon inheritance and it is the reason that the offsprings are similar to parents in their morphological, physiological, and biochemical traits. This simply means that due to inheritance, the members of the same family possess similar characteristics. It was only during the mid-19th century that people started to understand inheritance in a logical and scientific way. This understanding of inheritance was made possible by a scientist named Gregor Mendel, who formulated certain laws to understand the mechanism of inheritance. As a tribute to Mendel's contribution, he was given the title of "Father of Genetics" and his laws are known as Mendel's laws of inheritance. In this chapter, a thorough review of the principles of genetic inheritance formulated by him is presented.

1.1 INTRODUCTION

The members of a family may have some of the common facial features, hair color, or susceptibility to any health-related issue like diabetes, cancer, and other genetic disorders. The features that are common within families are having genetic basis, it means that they are determined by the genetic

Molecular Genetics, Structures, Mechanisms, and Functions, Volume 2: Applications and Exploring the Nucleus. Tariq Ahmad Bhat, Abdul Rauf Shakoori, and Jameel M. Al-Khayri (Eds.)
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information a human inherits from the parents. In 19th century, a scientist named Gregor Mendel highlighted the key rules of inheritance with the help of a very simple and popular system, the pea plant (Biffen, 1905).

Johann Gregor Mendel, who is considered as the "Father of Genetics" was born on 20th July 1822 in the Silesian part of the Austrian Empire (today's Czech Republic). He was a teacher, a learner, a scientist, a mathematician, and a man of faith. It would be justified to say that Mendel had a lot of determination. He proceeded through difficult situations to make some vital discoveries in the field of biology. In 1856, Mendel started a research project to study the patterns of inheritance. Luckily, the organism he chose as a model system proved to be the best system to understand the mechanism of inheritance. By observing a model system, researchers can understand those principles that apply to other organisms that are difficult to study or major biological systems like humans (Fairbanks and Rytting, 2001).

1.2 MENDEL'S EXPERIMENTS

Mendel understood the inheritance of seven different features in peas that included plant height, flower color, seed color, seed shape, pod shape, pod color, and position of flower. For this purpose, he first generated and raised pea lines with two different forms of a feature like tall vs short-heighted plants. He raised these lines for generations until they became pure-breeding always producing offspring identical to their parent, then bred them with each other and studied how the traits were passed in the generations (Marks, 2008). Moreover, to calculate how the plants in every generation looked, he counted the exact number of plants that showed each trait. Surprisingly, he obtained very similar patterns of inheritance for all of the seven features he studied.

One form of a feature like the tall always masked the other form like the short in the very first generation after the cross. Mendel called the observable form the **dominant trait** and the concealed form the **recessive trait**. In the second generation, after plants were allowed to self-fertilize (pollinate themselves), the hidden form of the trait reappeared in a minority of the plants. Specifically, there were always about 333 plants that showed the dominant trait (e.g. tall) for every 111 plants that showed the recessive trait (e.g. short), making a 3:1 ratio. Mendel also found that the features were inherited independently, that is, one feature such as plant height, did not influence the inheritance of other features like flower color or seed shape. When a tall and short plant was crossed, all of the offspring were tall. When the offsprings were self-fertilized, they produced tall and short plants in a ratio of 3:1 in the next generation.

1.3 MENDEL'S FINDINGS

In 1865, Mendel displayed the results of his experiments with approximately 30,000 pea plants to the local Natural History Society. Based on the inheritance patterns he observed, the numerical data he obtained, and a mathematical analysis of his results, he proposed a model of inheritance in which the characteristics like flower color, plant height, and seed shape were in a control of pairs of heritable factors that came in different versions. One version of a factor (the dominant form) could hide the presence of another one (the recessive form). The two paired factors separated during the production of gametes like each gamete whether it is a sperm or an egg, randomly received just one of the factors. The factors controlling different characteristics were inherited independently of one another. In 1866, Mendel published his observations and his model of inheritance, under the title **Experiments in Plant Hybridization** in the proceedings of the Natural History Society of Brünn. At that time, Mendel's work was not accepted by the scientific community and he was not able to convince the community during his lifetime. His colleagues failed to identify the worth of his work because his findings were opposite to the famous ideas of a single factor controlling each trait in inheritance. Additionally, his mathematical approach to fuse it with the biology was an innovative and pioneer idea. It was novel, innovative, and little bit confusing to other biologists of that time (Plomin, 1990).

In the middle of the 19th century, when he was performing his experiments, most of the biologists pitch into the idea of **blending inheritance**. It was not a proper scientific hypothesis but a very general model in which inheritance involved the permanent blending of the parent's characteristics in their offspring (producing offspring with an intermediate form of a characteristic). The blending model was suitable with some explanations of human inheritance. For example, children are often similar to both of their parents. However, the blending model was unable to explain why Mendel crossed a tall and a short pea plant and obtained only tall plants and why self-fertilization of one of those tall plants would produce a 3:1 ratio of tall to short plants in the next generation. Instead if the blending model was right, a tall plant crossed with a short plant should be raising a medium plant which will keep producing more medium plants. He found that all the offspring of the cross were tall and that when they self-fertilized they produced tall and short plants in a ratio of 3:1 (Castle, 1933).

As it turns out, both pea plant height and human height (along with many other characteristics in a wide range of organisms) are controlled by pairs of

heritable factors that come in distinctive versions just as Mendel proposed. In humans, however, there are many different factors like genes that contribute fractionally to height and vary among individuals. This makes it difficult to see the contribution of any one factor and produces inheritance patterns that can resemble blending. In Mendel's experiments in contrast there was just one factor that differed between the tall and short pea plants, allowing Mendel to clearly see the underlying pattern of inheritance (Porter, 2014).

In 1868, Mendel became abbot of his monastery and largely set aside his scientific pursuits in favor of his pastoral duties. He was not recognized for his extraordinary scientific contributions during his lifetime. In fact, it was not until around 1900 that his work was rediscovered, reproduced, and revitalized. Its rediscoverers were biologists on the brink of discovering the chromosomal basis of heredity and realized that Mendel's "heritable factors" were carried on chromosomes (Castle, 1921).

1.4 MENDEL'S MODEL SYSTEM—THE PEA PLANT

Gregor Mendel carried out his key experiments using the garden pea *Pisum sativum*, as a model system. Pea plants make a convenient system for studies of inheritance and they are still used by some geneticists today. Pea plants have multiple traits which occur in pairs and can be easily identified. For example, the flowers can be purple or white. The peas can be yellow or green or smooth or wrinkled. The pea pods can be inflated or constricted or yellow or green. The flower position can be axial or terminal. The stem length can be tall or dwarf. These morphological pairs of characters have been illustrated in Figure 1.1.

Useful features of pea plants include their rapid life cycle and the production of lots and lots of seeds as progeny. Pea plants also typically self-fertilize meaning that the same plant makes both the sperm and the egg that come together in fertilization. The morphology of the flower does not allow the pollens from other flowers to reach the stigma of a different flower. So, the pollens from the same flower can only fertilize the eggs of the same flower. Mendel took advantage of this property to produce **true-breeding** or pure-breeding pea lines. Pure breeding just means that the plant will always make more offspring like itself when self-fertilized over many generations. He self-fertilized and selected peas for many generations until he got lines that consistently produced offspring identical to the parent plant (e.g. always short). Pea plants are also easy to cross-fertilize or mate in a controlled way.

This is done by artificially transferring pollens from the anthers (male parts) of a pea plant of one variety to the carpel (female part) of a mature pea plant of a different variety. To prevent the recipient plant from self-fertilizing, Mendel painstakingly removed all of the immature anthers from the flowers before the cross. Once Mendel had established true-breeding pea lines with different traits for one or more features of interest (such as tall vs short height), he began to investigate how the traits were inherited by carrying out a series of crosses.

Because it is very easy to work on peas and also very productive in the seed production that he would be able to perform many crosses and then observe many different individual plants. He was sure that his results were consistent and accurate (Dipple and McCabe, 2000). As it turned out, the 3:1 was a crucial clue that let Mendel crack the puzzle of inheritance. He presented the two laws of inheritance. First is the law of segregation, describing how individual traits are inherited. And second, the law of independent assortment, describing how two or more traits are inherited relative to one another.

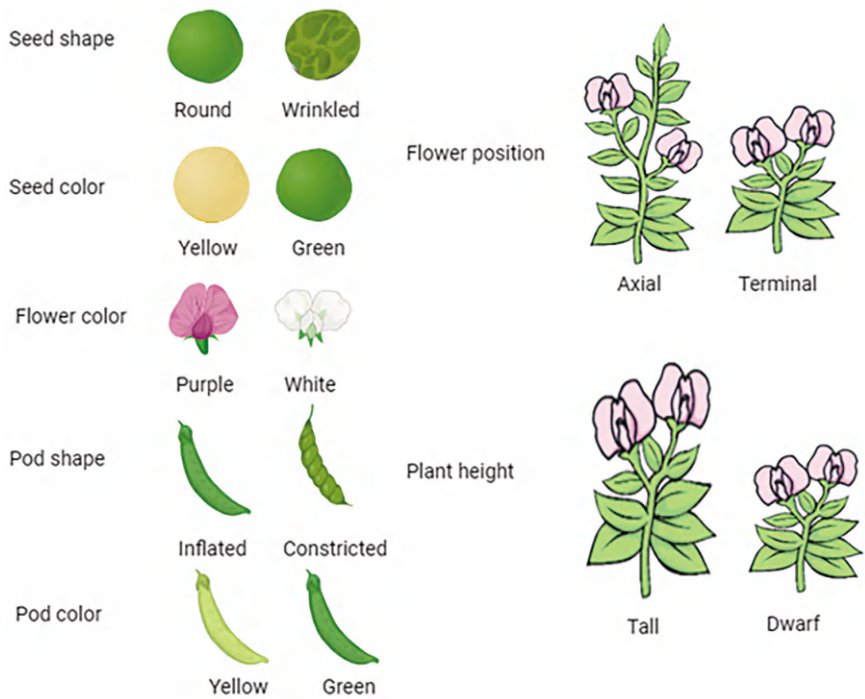


FIGURE 1.1 Illustration of seven characteristics of Mendel's pea plants. ⏎

1.5 MENDEL'S FACTORS

Based on his results, he came up with a model for the inheritance of individual characteristics. In Mendel's model, parents pass along "heritable factors," that are called as **genes** that determine the traits of the offspring. Each individual has two copies of a given gene, such as two genes for seed color. These copies of a gene controlling one trait are called alleles. If these copies or **alleles** of the gene represent different versions in one individual, then it is called **heterozygous**. Heterozygous organisms are also known as hybrids. One allele could be the **dominant** one and the other allele could be the **recessive** one. For seed color, the dominant yellow allele *Y* hides the recessive green allele *y* (Correns, 1950). When both alleles controlling a trait are similar in an organism, then it is called **homozygous**. Homozygous organisms are also known as true breeding or pure breeding.

The set of alleles carried by an organism is known as its **genotype**. Genotype determines **phenotype**, an organism's observable features. When an organism has two copies of the same allele (say, *YY* or *yy*), it is said to be **homozygous** for that gene. If, instead, it has two different copies (like *Yy*), we can say it is **heterozygous**. The phenotype can also be influenced by the environment in many of the real-life cases but this did not have an effect on Mendel's work.

1.6 LAW OF DOMINANCE

Mendel crossed a pure-breeding violet-flowered plant to a pure-breeding white-flowered plant. When he gathered and planted the seeds produced in this cross, he found that 100% of the plants in the next generation were producing flowers purple in color. Conventional wisdom at that time would have predicted that the hybrid flowers should be pale violet because the parent's traits should blend in with the offspring. Instead, Mendel's results showed that the white flower trait had completely disappeared. He called the trait that was visible in the generation (violet flowers) the **dominant trait** and the trait that was hidden or lost (white flowers) the **recessive trait**.

Importantly, Mendel did not stop his experimentation there. Instead, he let the F_1 plants self-fertilize. Among their offspring, called the F_2 generation, he found that 705 plants had violet flowers and 224 had white flowers. This was a ratio of 3.15 violet flowers to one white flower or approximately 3:1 (Lyttle, 1993). This 3:1 ratio was not fake because for the other six characteristics that Mendel examined, both the F_1 and F_2 generations behaved in the same way they did for flower color (Fig. 1.2). One of the two traits would disappear completely from the F_1 generation, only to reappear in the F_2 generation in a

ratio of roughly 3:1. It is known as the law of dominance. Figure 1.2 illustrates a cross between pea plants that are true-breeding for purple flower color and plants that are true-breeding for white flower color. This cross-fertilization of the P generation resulted in an F_1 *generation with all violet flowers. Self-fertilization of the* F_1 generation resulted in an F_2 generation that consisted of 705 plants with violet flowers and 224 plants with white flowers.

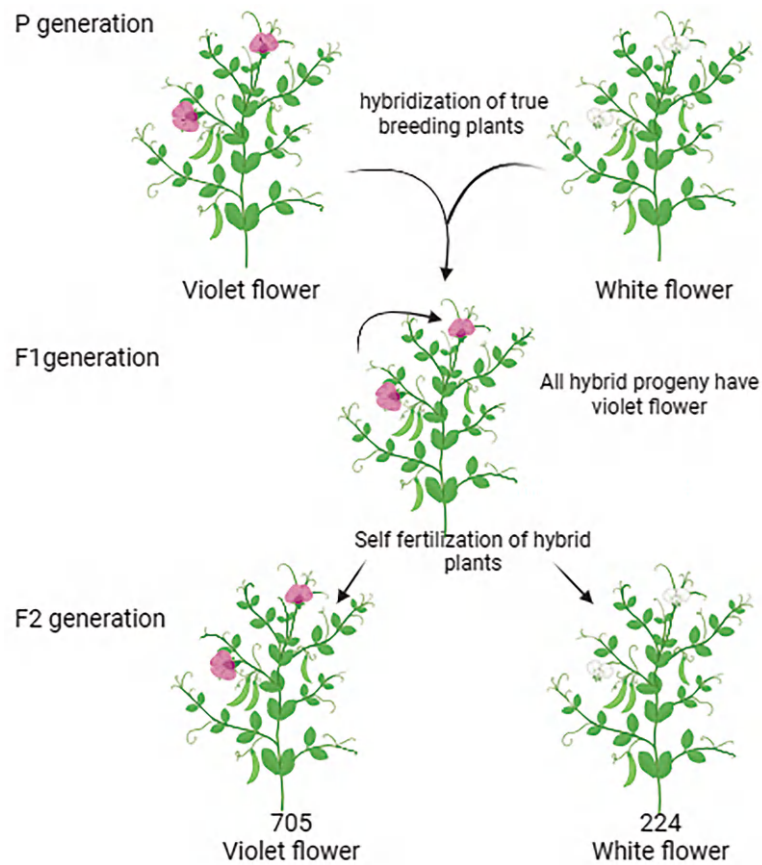


FIGURE 1.2 Self-fertilization of true breeding lines.

1.7 PHENOTYPES AND GENOTYPES

Each living organism inherits two alleles for each trait, one from the father and one from the mother. In homozygous conditions, since alleles are the same so the outward expression reflects the allele that the organism

possesses. If both alleles are (TT), then the plant would be tall but if alleles are (tt) then the plant is dwarf. So the genetic makeup (genotype) and the outwardly expression (phenotype) coincide with each other. But if both alleles are different from each other (heterozygous condition) then only one allele (the dominant) expresses itself while the other allele which is recessive remains hidden. Recessive alleles only express their phenotype when they are in homozygous form (tt). Similarly, a pure-bred pea plant producing yellow seeds (YY) is crossed with a pure-bred green seed-producing plant (yy). Both of these plants are homozygous by genotype (Fig. 1.3). The F1 progeny which is the first generation of off springs is 100% yellow pea plants. But these offsprings are hybrid (Yy) because they inherited Y allele from one parent and y allele from the other parent. In the hybrid, only the dominant allele (Y) can express itself so the progeny is yellow by phenotype. When these F1 plants (Yy) self-fertilize, the second generation of offsprings (F2) is produced with varying genotypes and phenotypes. Below is a graphic representation (Fig. 1.3) of this cross showing two columns, the first with the "Phenotype" and the second with the "Genotype." In the phenotype column, one yellow pea plant cross-fertilizes with one green pea plant. After self-fertilization of these yellow pea offspring, 75% of the second-generation offspring have yellow peas and 25% have green peas. The genotype column shows the first-generation offspring as 100% Yy, and the second generation as 25% YY, 50% Yy, and 25% yy.

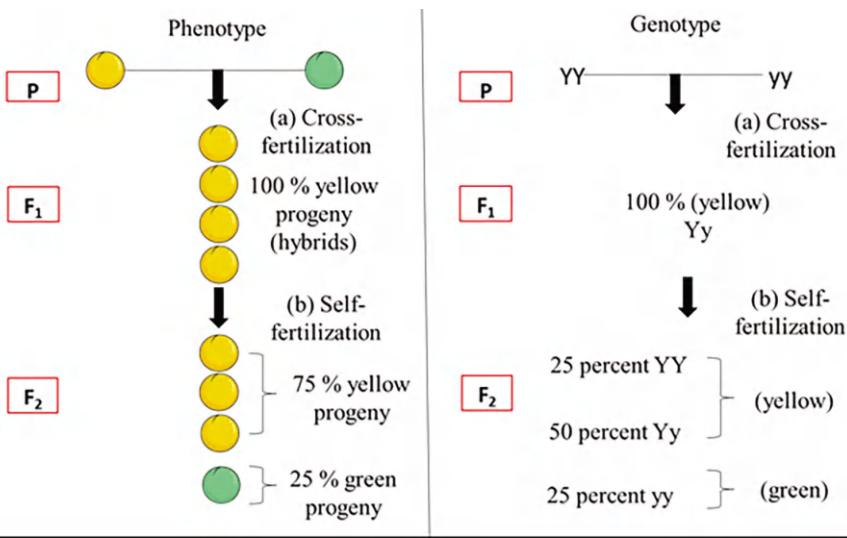


FIGURE 1.3 Comparison of phenotype and genotype.

1.8 THE LAW OF SEGREGATION

As a conclusion of Mendel's experiments, he presented two important laws of inheritance which are still considered the basis of modern genetics. According to the **law of segregation**, only one of the two gene copies (alleles) present in an organism is distributed to each gamete (egg or sperm cell) that it makes as a result of meiosis (Fig. 1.8) and the allocation of the gene copies is random. When an egg and a sperm combine in fertilization, they form a new organism, whose genotype consists of the alleles contained in each gamete. According to this law, no gamete can contain more than one allele. During meiosis, homologous chromosomes which carry alleles separate as a result of disjunction and one daughter cell contains only one allele (Fig. 1.8). These daughter cells develop into gametes, egg in female and sperms in male parts.

Figure 1.4 illustrates a monohybrid cross. In the P generation, one parent has a dominant yellow phenotype and the genotype YY and the other parent has the recessive green phenotype and the genotype yy. Each parent produces one kind of gamete, resulting in an F_1 *generation with a dominant yellow phenotype and the genotype Yy. Self-pollination of the* F_1 generation results in an F_2 generation with a 3 to 1 ratio of yellow to green peas. One out of three of the yellow pea plants has a dominant genotype of YY and two out of three have the heterozygous genotype Yy. The homozygous recessive plant has the green phenotype and the genotype yy.

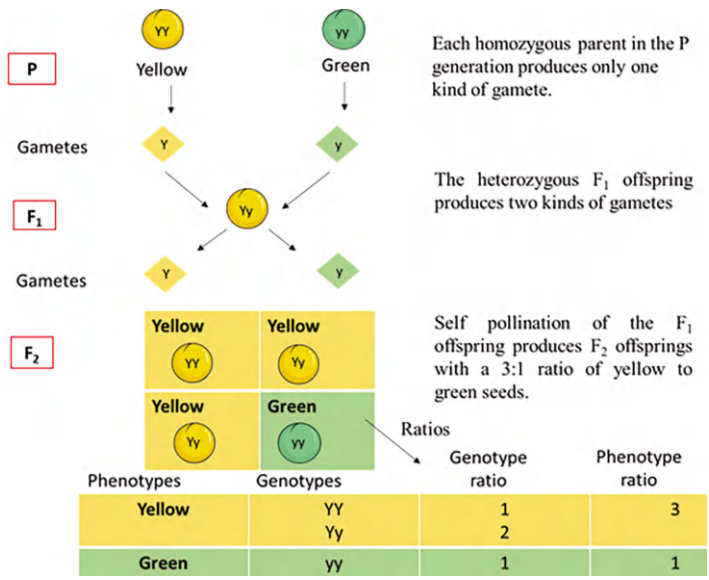


FIGURE 1.4 A monohybrid cross. ⏎

The four-squared box shown for the F_2 generation (Fig. 1.4) is known as a **Punnett square**. To prepare a Punnett square, all possible gametes made by the parents are written along the top (for the father) and side (for the mother) of a grid. Here since it is self-fertilization, the same plant is both mother and father. The combinations of egg and sperm are then made in the boxes in the table representing fertilization to make new individuals. Because each square represents an equally likely event and in this way genotype and phenotype ratios can be determined by counting the squares (Wang and Xu, 2019).

1.9 THE TEST CROSS

Mendel also found that an organism having a dominant phenotype (such as a yellow-seeded pea plant) can be a heterozygote (*Yy*) or a homozygote (*YY*). Because for the dominant phenotype, presence of only one dominant allele is enough but for the recessive phenotype, two (homozygous) recessive alleles are required. So recessive phenotype reflects homozygosity of genotype but dominant phenotype could be either homozygous or heterozygous. In order to figure out the genotype of dominant phenotypic organism, Mendel used test cross. In a test cross, the organism having a dominant phenotype is crossed with an organism that is homozygous recessive and the progeny phenotype is analyzed. If the parent with the unknown genotype is homozygous dominant, all the resulting offspring will have at least one dominant allele. If the parent with the unknown genotype is heterozygous, 50% of the offspring will inherit a recessive allele from both parents and will have the recessive phenotype. Thus, if in the progeny, all offspring display dominant phenotype, then the parent of the questioned genotype would be considered homozygous. But if recessive phenotype is seen in the progeny then the questioned parent would be considered heterozygous. This technique is called a **test cross** as it can test the genotype of the dominant parent and it is still used by many of the plant and animal breeders today.

Figure 1.5 represents a test cross explaining the genotypes and phenotypes of parents and progeny outcomes. If the organism with the dominant phenotype is homozygous, then all of the F_1 offspring will get a dominant allele from that parent and show the dominant phenotype. If the organism with the dominant phenotype organism is instead a heterozygote, the F_1 offspring will be half heterozygotes (dominant phenotype) and half recessive homozygotes (recessive phenotype). The fact that getting a 1:1 in this second case is another confirmation of Mendel's law of segregation.

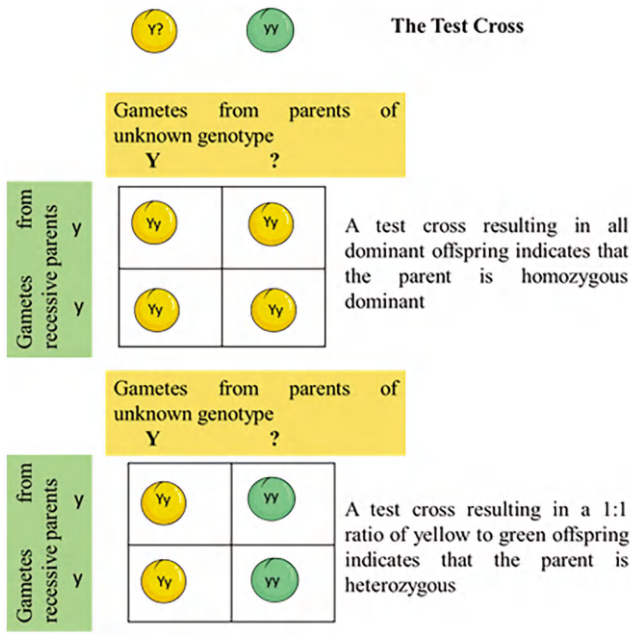


FIGURE 1.5 A test cross. ⏎

1.10 LAW OF INDEPENDENT ASSORTMENT

Mendel's Law of Segregation applies to the inheritance of alleles controlling a single trait. But how the alleles controlling one trait and the alleles controlling another trait are inherited during gamete formation. Do they interfere with the inheritance of each other or not? Do the dominant alleles go together in the daughter cells or they can separate independently? In order to answer these questions, Gregor Mendel performed some crosses with pea plant and analyzed the results. He found that genes controlling different traits were inherited independently of one another. On the basis of these results he proposed the law of independent assortment. Mendel's **law of independent assortment** states that the alleles of two (or more) different genes get sorted into gametes independently of one another. In other words, the allele a gamete receives for one gene does not influence the allele received for another gene. Example: Pea color and pea shape genes.

Mendel crossed two pure-breeding pea plants, one with yellow, round seeds (*YYRR*) and one with green, wrinkled seeds (*yyrr*). Because each parent is homozygous, the law of segregation tells that the gametes made by the wrinkled, green plant all are *ry*, and the gametes made by the round, yellow plant are all *RY*. That gives F_1 offspring that are all *RrYy* with phenotype yellow and round (Fig. 1.6).

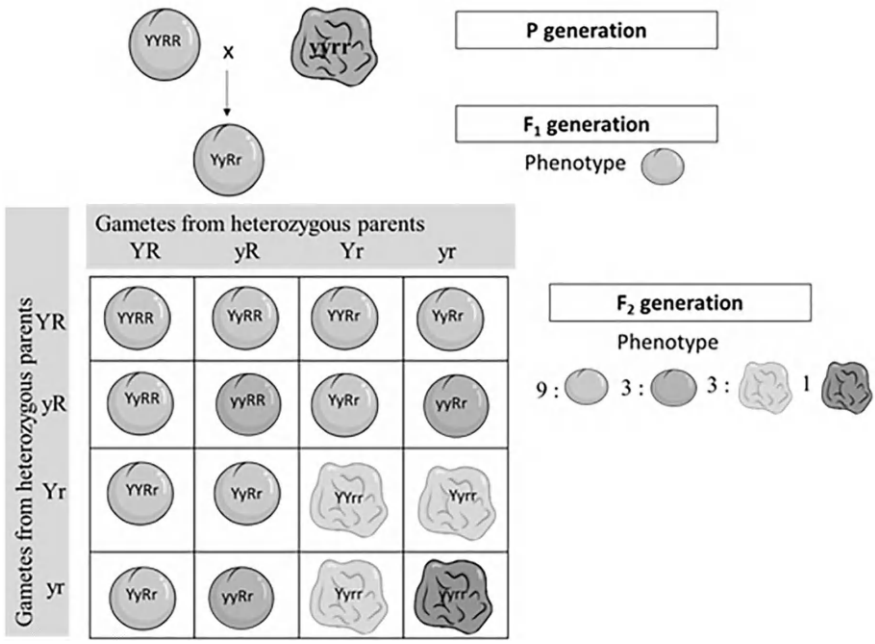


FIGURE 1.6 A dihybrid cross. ⏎

The F_1 plants can produce four different types of gametes: YR, Yr, yR, and yr when genes for shape and for color are assorting independently. It can be predicted that the genotypes of the F_2 plants by placing these gametes along the top and side axes of a 4 × 4 Punnett square and filling in the boxes to represent fertilization events. Punnett square predicts four different phenotypic classes of offspring, yellow/round, yellow/wrinkled, green/round, and green/wrinkled, in a ratio of 9:3:3:1. This is the prediction of the model in which the seed shape and seed color genes assort independently. The allele specifying yellow seed color is dominant to the allele specifying green seed color and the allele specifying round shape is dominant to the allele specifying wrinkled shape as shown by the capital and lower-case letters. This means that the F_1 plants are all yellow and round. Because they are heterozygous for two genes, the F_1 plants are called **dihybrids** (*di* = two, *hybrid* = heterozygous). A cross between two dihybrids is known as a **dihybrid cross shown in Figure 1.6**. When Mendel did this cross and looked at the offspring, he found that there were four different categories of pea seeds: yellow and round, yellow and wrinkled, green and round, and green and wrinkled. These **phenotypic** categories that are defined by observable traits appeared in a ratio of approximately 9:3:3:1 (Ruch, 1998). Y and R alleles of the yellow, round

parent and the y and r alleles of the green, wrinkled parent are not inherited as units. Instead the alleles of the two genes are inherited as independent units.

This ratio was the key clue that led Mendel to the law of independent assortment (MacLeod et al., 2005). That is because a 9:3:3:1 is exactly what was expected to see if the F_1 plant made four types of gametes (sperm and eggs) with equal frequency: *YR*, *Yr*, *yR*, and *yr*. In other words, this is the result that was predicted if each gamete randomly got a *Y* or *y* allele, and, in a separate process, also randomly got an *R* or *r* allele (making four equally probable combinations).

1.11 INDEPENDENT ASSORTMENT VS LINKAGE

If there was no independent assortment, then the genes for seed color and seed shape might have always been inherited as a pair. That is, the yellow and round alleles (both dominant) might always have stayed together and the green and wrinkled alleles (both recessive) would be paired. The color and shape genes are physically stuck together and cannot be separated as represented by the boxes around the alleles in Figure 1.7.

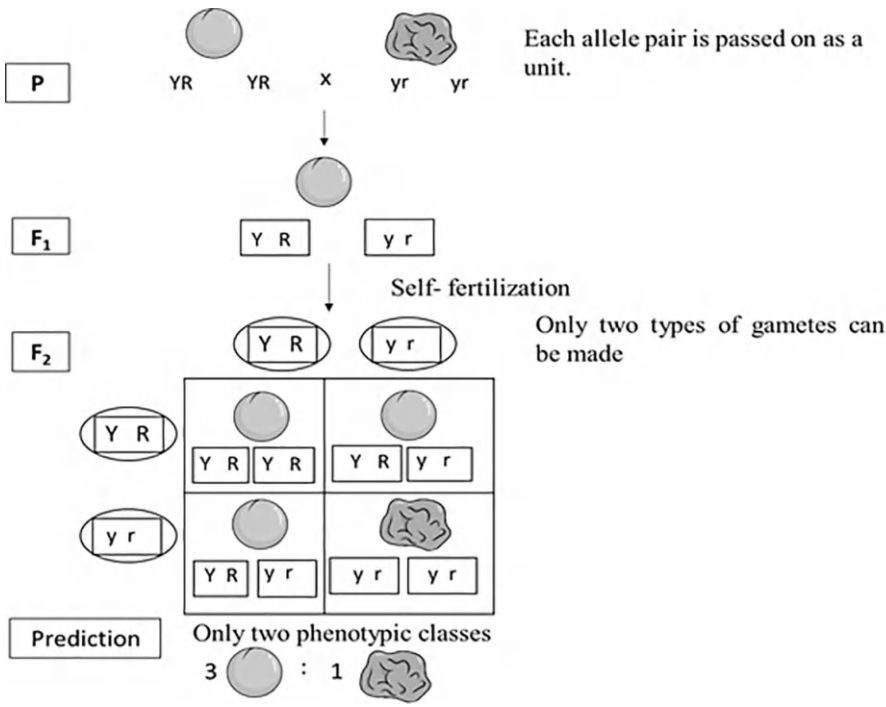


FIGURE 1.7 Complete linkage. ⏎

In this diagram, the Y and R alleles are represented as a unit (Y-R), as are the y and r alleles (y-r). Homozygous dominant parent (Y-R)(Y-R) yellow, round is crossed with a (y-r)(y-r) green, wrinkled parent. Each parent produces just one type of gamete, containing either a (Y-R) unit or a (y-r) unit. F_1 generation would be dihybrid heterozygous for both genes with yellow, round phenotype. The allele pairs would remain as indivisible units in the F_1 dihybrid: (Y-R)(y-r). When the F_1 dihybrid self-fertilize, it can produce two types of gametes: a gamete containing a (Y-R) unit and a gamete containing a (y-r) unit. These two types of gametes will each be produced and we can predict the genotypes of the F_2 offspring by listing the two gamete types along the axes of a 2 × 2 Punnett square and then filling in the boxes to simulate fertilization events. As a result, we would get three different genotypes in a 1:2:1 ratio: (Y-R)(Y-R), (Y-R)(y-r), and (y-r)(y-r). These genotypes correspond to a 3:1 ratio of yellow, round: green, wrinkled seeds. This is the prediction of the model in which the seed shape and seed color genes are completely linked and do not assort independently.

If the seed color and seed shape genes were in fact always inherited as a unit or completely linked, a dihybrid cross should produce just two types of offspring, yellow/round and green/wrinkled, in a 3:1. Mendel's actual results were quite different from this the 9:3:3:1, telling him that the genes assorted independently (Dahlberg, 1943).

1.12 PHYSICAL BASIS FOR INDEPENDENT ASSORTMENT

Two copies of a gene carried by an organism (such as a *Y* and a *y* allele) are located at the same spot on the two chromosomes of a **homologous pair**. Homologous chromosomes are similar but non-identical and an organism gets one member of the pair from each of its two parents. The physical basis for the law of independent assortment lies in meiosis I of gamete formation when homologous pairs line up in random orientations at the middle of the cell as they prepare to separate. The gametes can be obtained with different combos of "mom" and "dad" homologues because the orientation of each pair is random (Fig. 1.8).

To visualize this, compare chromosome arrangement 1 (top) and chromosome arrangement 2 (bottom) at the stage of metaphase I in Figure 1.8. In one case, the red "mom" chromosomes go together while in the other, they split up and mix with the blue "dad" chromosomes. If meiosis happens many times, as it does in a pea plant, both the arrangements will get and

thus *RY*, *Ry*, *rY*, and *ry* classes of gametes with equal frequency. Homologous pairs of chromosomes line up at the metaphase plate during metaphase I of meiosis. The homologous chromosomes with their different versions of each gene are randomly segregated into daughter nuclei, resulting in a variety of possible genetic arrangements.

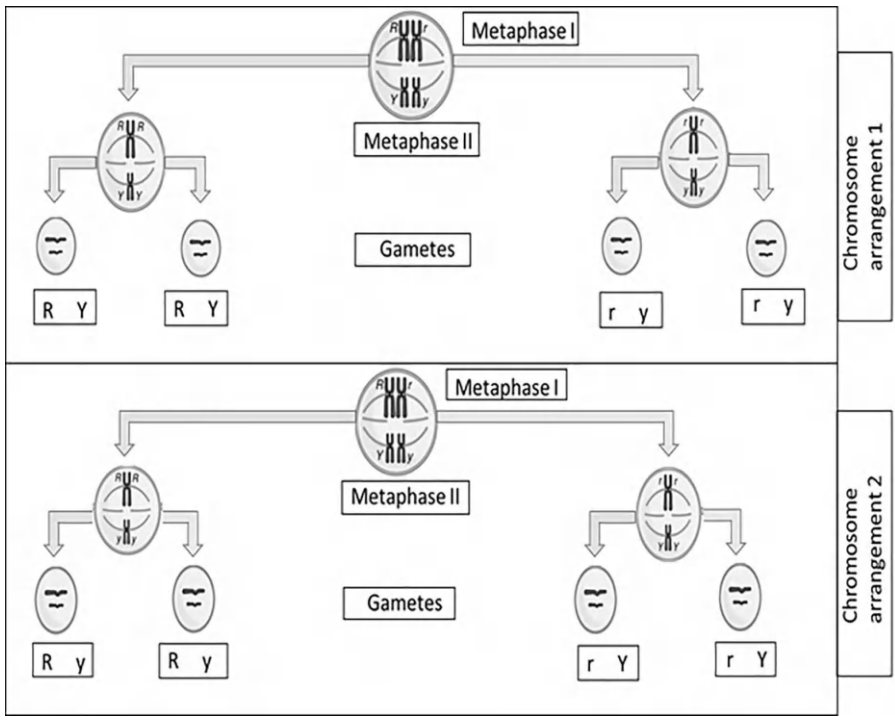


FIGURE 1.8 Metaphase arrangement of homologous chromosomes. ⏎

Genes that are on different chromosomes (like the *Y* and *R* genes) assort independently. The seed color and seed shape genes are on chromosomes 1 and 7 of the pea genome, respectively. Genes that are far apart on the same chromosome also assort independently because of the crossing over or exchange of homologous chromosomes that occurs early in meiosis I.

There are, however, gene pairs that do not assort independently. When genes are close together on a chromosome, the alleles on the same chromosome tend to be inherited as a unit more frequently than not. Such genes do not display independent assortment and are said to be **linked** (Green, 1981).

1.13 CONCLUSION

Based on his experiments he concludes some of the things like the inheritance of each trait is determined by certain "factors" which are now known as genes, that are passed on to future generations unchanged. An individual inherits one factor from each parent for each trait. Certain traits may not be seen in an individual or a progeny but can still be passed on to the next generation. There are some exceptions to Mendel's principles, which have been discovered as per the knowledge of genes and inheritance has increased. The principle of independent assortment does not apply if the genes are close together (or linked) on a chromosome. Also, alleles do not always interact in a standard dominant/recessive way particularly if they are codominant or have differences in expressivity or penetrance.

KEYWORDS

- **inheritance**
- **morphological**
- **physiological**
- **biochemical**
- **genetic information**
- **offsprings**

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CHAPTER 2

Linkage, Crossing Over, and Recombination

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ABSTRACT

It is important to note that all genes residing on a single chromosome are considered linked to each other. The mechanism responsible for the inheritance of linked genes within the same linkage group is referred to as "linkage." In contrast, crossing over leads to the emergence of new traits through the exchange of genetic segments between chromosomes, serving as a source of genetic diversity. However, it is worth noting that linkage tends to decrease the likelihood of variability in gametes unless crossing over takes place. Genetics encompasses the investigation of hereditary traits and the diversity that arises from them. Recombination is the mechanism through which DNA fragments break and recombine, resulting in the appearance of novel varieties of alleles. This method fosters genetic diversity at the genetic level, reflecting variations in DNA patterns among different organisms. RNA recombination is a crucial factor in genetic changes associated with the emergence of RNA viruses. Genomic recombination significantly presents the development of entirely new lineages, the development of host tropism, environmental changes, virulence, and disease. It encompasses both intra- and intrachromosomal recombination. During sexual reproduction, the interaction of genomic data between different chromatids of homologous chromosomes, understood as a chromosomal crossover, shows the formation of recombinant chromosomes. Gene change appears when one DNA

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sequence replaces a homologous sequence, resulting in its identical nature. Recombination often exhibits sex dimorphism in both sex chromosomes and autosomes. Viral recombination takes place when viruses from distinct parent strains contaminate the identical host tissue and combine their genetic material during replication, giving rise to progeny viruses that inherit genes from both parents. The process of evolution causes changes in the genetic makeup of populations over time. Genetic diversity and alterations in allele frequencies are necessary for evolutionary processes to occur.

2.1 INTRODUCTION

Linkage in genetics refers to the tendency of genes located close together on the same chromosome to be inherited together. This concept is crucial for understanding inheritance patterns and genetic mapping. There are two types of linkage: complete linkage in which recombination does not occur and incomplete linkage in which recombination occurs. Crossing over occurs during meiosis when homologous chromosomes exchange segments. If genes are located far apart, crossing over is more likely to occur, leading to new combinations of alleles and disrupting the linkage between genes. Recombination is the production of new genetic combinations through various mechanisms. Four main types of naturally occurring recombination include: general (homologous) recombination, illegitimate (nonhomologous) recombination, site-specific recombination, and replicative recombination.

2.2 LINKAGE

"A linkage refers to a phenomenon in which two or more genes that are interconnected are consistently passed down together in the same genetic combination for multiple generations, spanning beyond two generations."

These observations contributed to the formulation of the concept of genetic linkage, which explains how two genes closely located on the same chromosome tend to be inherited together more frequently. In essence, the closer two genes are positioned on a chromosome, the higher the likelihood of their coinheritance or linkage. Conversely, genes located farther apart on the same chromosome have a greater probability of being separated during recombination, the process that shuffles DNA during meiosis. Consequently, the degree of linkage between two genes is contingent upon the distance that separates them on the chromosome.

2.3 HISTORY OF LINKAGE

In 1905, a significant observation was made by William Bateson, Edith Rebecca Saunders, and Reginald C. Punnett regarding sweet pea plants. They noticed that traits related to flower color and pollen shape in these plants appeared to be linked, rather than assorting independently in accordance with Mendel's Law of Independent Assortment. Several years later, in 1911, while studying heredity in fruit flies, Thomas Hunt Morgan made a noteworthy observation. He observed that the eye color of a fruit fly was correlated with the fly's sex and speculated that these two traits were interconnected.

The chromosomal theory of inheritance emerged considerably later than Mendelian genetics, primarily due to the absence of advanced technology and empirical evidence, which initially led to the rejection of Mendel's genetic concepts. Nonetheless, subsequent experiments ultimately validated Mendel's findings.

Biologists subsequently formulated the chromosomal theory of inheritance, with T.H. Morgan playing a pivotal role in advancing and substantiating this theory. This breakthrough gave rise to the understanding of gene linkage and recombination.

2.3.1 REGINALD CRUNDALL PUNNETT THEORY

Reginald Crundall Punnett conducted a study involving different sweet pea varieties. He crossed one variety with red flowers and round pollen grains with another variety bearing blue flowers and long pollen grains. The F1 generation exhibited blue flowers with long pollen grains, as these blue and long traits were dominant over red and round traits, respectively. However, when these blue long heterozygous hybrids were crossed with double recessive red round homozygous individuals (a test cross), they did not produce the expected 1:1:1:1 ratio in the F2 generation. Instead, the outcome yielded four distinct combinations in a ratio of 7:1:1:7, comprising 7 blue long, 1 blue round, 1 red long, and 7 red round progenies.

2.4 TYPES OF LINKAGE

Here are the key terms associated with linkage, rephrased:

2.4.1 LINKED GENES

Genes that do not undergo independent assortment and are inherited as a group due to their close proximity on the same chromosome are referred to as linked genes.

2.4.1.1 X-LINKED GENE

Sexual dimorphism in animals arises from distinct patterns of gene expression that are biased toward one sex. These expression patterns are under the control of genetic sex determination hierarchies, which dictate an individual's sex. In this study, we demonstrate that the male-biased wing expression pattern of the *Drosophila biarmipes* gene yellow, located on the X chromosome, operates independently of the fly's sex determination hierarchy.

Rather, our findings reveal that a regulatory interaction occurs between yellow alleles situated on homologous chromosomes, a phenomenon known as transection. This interaction effectively suppresses the activity of a yellow enhancer that functions within the wing. Consequently, this enhancer can remain active in males (XY) but not in females (XX). The process of enhancer silencing through transection relies on the presence of the yellow intron and the participation of the chromatin architecture protein Mod(mdg4). Our results suggest that transection may play a broader role in influencing the sex-biased expression of X-linked genes in general (Galouzis and Prud'-homme, 2021).

2.4.1.2 Y-LINKED GENE

The emergence and early evolutionary history of sex chromosomes have been proposed to revolve around the association of factors with contrasting effects on male and female functions. Garden asparagus (*Asparagus officinalis*) serves as an ideal species for exploring this hypothesis, as its X and Y chromosomes appear cytologically similar and evolved from a common ancestral autosome pair concurrently with the transition from hermaphroditism to dioecy.

Through mutagenesis screens and the application of single-molecule fluorescence in situ hybridization, we directly implicate Y-specific genes that play distinct roles: suppressing female (pistil) development and being essential for male (anther) development. When we compare the assembled sequences of the contiguous X and Y chromosomes, we observe that the loss of recombination between the genes responsible for inhibiting female organ formation (known as suppressor of female function) and promoting male function aspTDF1 is a result of hemizygosity. We have also conducted experimental demonstrations of aspTDF1's functionality. These findings offer direct evidence supporting the idea that sex chromosomes can operate by linking two sex determination genes (Harness et al., 2020).

2.4.2 UNLINKED GENES

Genes positioned at a greater distance from each other on a chromosome or on the two chromosomes of a homologous pair are known as non-linked genes. These genes exhibit independent assortment.

Chromosomes are believed to have originated in early evolution through the assembly of initially unlinked genes. This genetic linkage provides several advantages, such as reducing the assortment load and mitigating intragenomic conflicts within reproducing protocell models. Interestingly, these advantages can allow chromosomes to become prevalent even when they experience a replicative disadvantage compared to unlinked genes, which is directly proportional to their length.

In our numerical analysis, we demonstrate that chromosomes can spread within protocells, even when considering the presence of recurring deleterious mutations affecting replicating genes, such as ribozymes. The concept of dosage effect plays a crucial role in selecting the optimal genomic composition within protocells, and this selection influence carries over to the genetic composition of emerging chromosomes.

In the absence of a precise segregation mechanism, protocells continue to benefit from the stochastic corrector principle, now operating at the chromosome level, ensuring the survival of early replicators. An intriguing outcome of this process is the emergence of multigene families on chromosomes, with genes present in optimal proportions. Furthermore, the formation of chromosomes brings an added advantage: an increase in the size of the genome that can be selectively maintained (the number of different genes). This increase is primarily attributed to the significant reduction in the assortment load.

The establishment of chromosomes is subject to strong positive selection within protocells that harbor unlinked genes. The linkage effect raises the error threshold of replication, allowing for larger genome sizes. This is because deleterious mutations affecting protocell metabolism, and consequently fitness, exhibit antagonistic (diminishing return) epistasis. This result reinforces the benefits conferred by chromosomes on protocells, enabling the fixation of highly specific and efficient enzymes (Szilagyi et al., 2020).

2.4.3 LINKAGE GROUP

A collection of all the genes found on a pair of homologous chromosomes constitutes a linkage group. The number of linkage groups in an organism corresponds to the organism's haploid chromosome count or the number of chromosome pairs.

Numerous genes responsible for valuable breeding traits, such as pollen fertility restoration and resistance to fungal diseases like downy mildew and rust, have been incorporated into the genome of the cultivated sunflower *Helianthus annuus* L. through introgression from wild relatives. These genes are specifically located on linkage group 13. In sunflowers and various other plant species, it is possible to hinder recombination within the chromosomal regions housing these resistance genes. This suppression of recombination is advantageous for marker-assisted selection, where molecular markers linked to these loci can be effectively applied. The coinheritance of two loci, Rf1 (identified by the fertility restoration trait) and Pl5/Pl8 (associated with resistance to multiple races of *Plasmopara halstedii*), marked by the STS marker Ha-P1, was examined in the F2 generation of hybrids. These hybrids were produced by crossing a sterile line, VIR 116A, with pollen fertility restorers from the VIR sunflower collection. In the case of the VIR 116A × VIR 740 cross, the recombination frequency between the Pl5/Pl8 and Rf1 loci was found to be 8.61, whereas it was twice as high in the crosses VIR 116A × VIR 195 and VIR 116A × VIR 80. In all studied F2 populations, there was an absence of the phenotypic class characterized by sterile plants possessing the paternal Ha-P1 marker variant.

Cytological analysis indicated that the pollen fertility indices are more likely determined by the plant's genotype rather than the allelic makeup of the male gametophyte. The lack of a recombinant F2 class was proposed to be linked to embryonic lethality, meaning the failure of zygotes carrying the recessive allele of the fertility restoration gene and a specific allele of the resistance (susceptibility) gene inherited from the male parent to develop successfully (Anisimova et al., 2023).

Various tilapia stocks have exhibited distinct sex-determining loci, including linkage groups 1, 20, and 23. Among these, the “genetically improved farmed tilapia” (GIFT) stock is of particular significance in the realm of tilapia aquaculture. The GIFT stock originates from diverse Nile tilapia (*Oreochromis niloticus*) populations, some of which may have experienced introgression with O. mossambicus. The intricacies of the sex-determining mechanism within the GIFT stock were not previously understood, primarily due to its complex and diverse origins.

To address this, a modified approach known as bulk segregant analysis (BSA) combined with double-digest restriction-site associated DNA sequencing (BSA-ddRADseq) was devised and employed. This method was used to identify and locate sex-linked single nucleotide polymorphism (SNP) markers in 19 GIFT strain breeding nucleus families. Two additional Stirling

families were included as control groups, with a single XY locus already mapped to LG1 in these families. The analysis uncovered approximately 1500 SNPs per family dispersed throughout the genome. In Stirling families, phenotypic sex exhibited a strong correlation with LG1, whereas, in the majority of the GIFT families, only SNPs located in LG23 demonstrated a clear connection with sex determination. No other genomic regions associated with sex determination were evident. The validity of this discovery was confirmed using a set of LG23-specific DNA markers, including the five SNPs most strongly linked to sex from this study, the LG23 sex-associated microsatellite UNH898, ARO172, and the recently identified amhy marker for individual fish (n = 284).

Despite its diverse origins, sex determination in the GIFT strain breeding nucleus was notably linked to a specific locus within LG23. BSA-ddRADseq proved to be a cost-effective approach for analyzing multiple families, reinforcing this finding. This technique holds potential for application in the study of other complex traits. The sex-linked SNP markers identified have practical utility for potential marker-assisted selection (MAS) aimed at controlling sex ratios in GIFT tilapia, thereby mitigating unwanted reproduction during the growout phase (Taslima et al., 2020).

2.4.4 LINKAGE VALUE

The degree of the association between two genes on the same chromosome is termed the linkage value. This value is influenced by the distance separating the linked genes.

2.4.5 LINKAGE MAP

A genetic map that depicts the arrangement of genes on a chromosome, aiding in the determination of how frequently two gene loci are inherited together.

Linkage mapping, a traditional mapping technique, relies on genetic recombination during the development of mapping populations. In recent twenty years, linkage mapping has found widespread application in numerous plant species, leading to the cloning or tagging of numerous QTLs (Price, 2006). Nonetheless, linkage mapping comes with drawbacks, including comparatively limited mapping precision, reduced allele diversity, and slower mapping progress.

A genetic linkage map for mungbean was established by incorporating 430 simple sequence repeat (SSR) and EST-SSR markers derived from mungbean and its closely related species. These markers were successfully positioned across 11 linkage groups, collectively spanning a total distance of 727.6 cm. This novel mungbean map marks a significant milestone, as it aligns precisely with the haploid chromosome count of mungbean for the first time. Within this map, we pinpointed a total of 105 QTLs (Quantitative Trait Loci) and genes associated with 38 traits linked to the domestication of mungbean. Notably, unlike other Vigna crops, numerous linkage groups played pivotal roles in mungbean domestication. Particularly, striking was the distribution of high-impact QTLs across seven of the 11 linkage groups. Additionally, we identified a substantial number of QTLs with minor contributions. The accumulation of both major and minor contribution mutations has significantly contributed to distinguishing wild from cultivated mungbean varieties. Remarkably, we uncovered valuable QTLs responsible for traits such as seed size, pod dehiscence, and pod maturity, which were previously unidentified in other *Asian Vigna* species. These QTLs hold a great potential as novel genetic resources for enhancing the traits of other *Asian Vigna* species. In essence, these findings lay the groundwork for the enhancement of mungbean and related legumes through selective breeding and genetic improvement efforts (Isemura et al., 2012).

2.5 CLASSIFICATION OF LINKAGE

Linkage can be categorized based on various inheritance parameters. These classifications are outlined as follows:

2.5.1 CROSSOVER-DEPENDENT LINKAGE

Linkage can be distinguished into two types with regard to the occurrence of crossing over:

2.5.1.1 COMPLETE LINKAGE

In this scenario, two adjacent genes situated on the same chromosome remain closely linked and are passed down together through generations because crossing over does not occur. Although rare, Thomas Hunt Morgan demonstrated complete linkage in male *Drosophila* through his experiments.

2.5.1.1.1 Thomas Hunt Morgan

In 1910, Thomas Hunt Morgan conducted experiments at Columbia University with the aim of demonstrating that sexual reproduction leads to variations in offspring. To conduct his experiments, Morgan chose to work with fruit flies, scientifically known as *Drosophila*. During his investigation, amidst a population of thousands of red-eyed fruit flies, he encountered a solitary male fly with white eyes. After conducting numerous observations, Morgan drew the following conclusions:

1. Eye color and sex were controlled by the same chromosome, indicating that chromosomes carried the genes responsible for transmitting parental traits to the offspring.

Morgan's groundbreaking experiment was subsequently documented in an article titled "Sex Limited Inheritance in *Drosophila*."

There were several compelling reasons why Morgan selected fruit flies as the ideal subjects for his experiments:

1. Males and females could be easily distinguished from each other.
2. Fruit flies had a relatively short lifespan, approximately two weeks.
3. A single mating could produce a significant number of offspring.
4. They were straightforward to cultivate.

Notably, Thomas Hunt Morgan's experiment also revealed that the white-eye trait was exclusively present in males. This observation was attributed to the fact that the white-eye trait resided solely on the X chromosome, illustrating that the inheritance of traits could differ between sexes. Females did not exhibit white eyes because this trait was located on only one of their X-chromosomes. Consequently, Morgan's experiment provided a comprehensive explanation of concepts such as genetic linkage and crossing over.

Morgan selected *Drosophila* melanogaster as his research subject for the following reasons:

1. Observation of a White-Eyed Male *Drosophila*: Initially, he noted the presence of an atypical, white-eyed male *Drosophila* among the typical red-eyed ones.
2. Small Size: *D. melanogaster* is of diminutive stature.
3. Short Lifespan and Rapid Reproduction: These fruit flies exhibit a brief lifespan, enabling the study of numerous generations within a relatively short period. Additionally, they reproduce at a high rate.

In his experiments, Morgan commenced by crossing a purebred, white-eyed male with a purebred red-eyed female, adhering to Mendel's laws. As anticipated, the F1 offspring displayed red eyes. Subsequently, when the F1 generation was interbred, the ratio of red-eyed to white-eyed progeny was 3:1. Intriguingly, no white-eyed females were observed in the F2 generation.

To delve deeper into this phenomenon, he conducted a cross involving a heterozygous red-eyed female and a white-eyed male, resulting in a progeny ratio of 1:1:1:1 (comprising 1 white-eyed female, 1 red-eyed female, 1 white-eyed male, and 1 red-eyed male). This observation prompted Morgan to contemplate the linkage between the traits and the sex chromosomes. He proceeded with a series of crosses and eventually established that the gene responsible for eye color was situated on the X chromosome.

Cotton fiber length holds significant importance in the textile industry, as longer fibers are more efficiently processed into yarns, resulting in the production of higher-quality fabrics. Typically, there exists an inverse relationship between crop yield and fiber quality characteristics, such as length. A comprehensive understanding of the mechanisms governing fiber length regulation could serve as a valuable tool for cotton breeders aiming to enhance fiber length while preserving high yields.

One noteworthy cotton mutation, known as Ligon lintless-2 (Li 2), is governed by a single dominant gene (Li 2) that leads to notably shorter fibers compared to those of the wild-type cotton (*Gossypium hirsutum* L.). When in a near-isogenic state alongside a wild-type cotton line, Li 2 serves as an ideal model system for studying fiber elongation. Two near-isogenic lines of Ligon lintless-2 (Li 2) cotton, one carrying the mutant gene and the other with the wild-type gene, were established through five generations of backcrossing (BC5). An F2 population was subsequently created by crossing these two near-isogenic Li 2 lines, facilitating the development of a linkage map for the Li 2 locus located on chromosome 18.

Within the Li 2 locus region, five SSR markers were closely mapped, with two of these markers flanking the Li 2 locus at positions 0.87 and 0.52 centimorgans. Notably, there were no observable distinctions in the initiation of fiber growth and early fiber elongation between mutant and wild-type ovules. Microarray-based gene expression profiling provided insights into the potential roles of maintaining reactive oxygen species (ROS) homeostasis and regulating cytokinin's in the Li 2 mutant phenotype. This comprehensive gene expression dataset ultimately led to the successful identification of an EST-SSR marker (NAU3991) that displayed complete linkage with the Li 2 locus (Hinchliffe et al., 2011).

2.5.1.2 INCOMPLETE LINKAGE

This phenomenon occurs when specific and well-established traits, instead of consistently appearing together for generations, generate new combinations in the F2 generation. A dihybrid organism produces four types of gametes, with the parental types being more common than the non-parental types. Incomplete linkage results in the formation of novel combinations due to the exchange of chromosome segments during crossing over. This type of linkage is prevalent in various organisms.

Example

When purebred dominant sweet pea plants with a homozygous genotype for blue flowers and long pollen (BBLL) were crossed with double recessive plants with a homozygous genotype for red flowers and round pollen (bbll), an interesting pattern emerged.

In the F1 generation, all the offspring plants displayed a hybrid genotype, specifically being heterozygous for blue flowers and long pollen (BbLl).

Subsequently, when these F1 hybrids were subjected to a test cross with double recessive parents (bbll), the resulting F2 generation produced all four possible progeny types (BbLl, BbLl, bbLl, and bbll) in a ratio of 7:1:1:7, rather than the expected 1:1:1:1 ratio. Remarkably, the parental phenotype, characterized by blue flowers and long pollen (BbLl), and the red flower and round pollen phenotype (bbll), collectively accounted for 87.4% of the offspring, while the recombinant phenotype, involving blue flowers and round pollen (Bbll) and red flowers and long pollen (bbLl), constituted only 12.6%.

This experiment serves as evidence that the genes governing flower color and pollen shape are linked genes situated at a significant distance from each other on a chromosome. During gametogenesis, these linked genes tend to stay together, but due to their relative distance and the process of crossing over, they occasionally separate, resulting in the observed recombinant phenotypes.

2.5.2 CHROMOSOME-BASED LINKAGE

Linkage can be classified into two types based on the type of chromosomes involved:

2.5.2.1 AUTOSOMAL LINKAGE

This refers to the linkage of genes situated on autosomes, which are non-sex chromosomes. Humans possess 22 autosomal linkage groups.

2.5.2.2 ALLOSOMAL LINKAGE

Allosomal linkage involves genes located on sex chromosomes. Humans have two types of sex chromosomes: X and Y.

2.5.3 GENE CONFIGURATION-BASED LINKAGE

Depending on the presence of dominant and recessive alleles within a gene pair, linkage can be categorized into two phases:

2.5.3.1 COUPLING PHASE (CIS PHASE)

In this phase of linkage, either both dominant alleles or both recessive alleles of the gene pair are located together on the same chromosome and are inherited as a unit.

2.5.3.2 REPULSION PHASE (TRANS-PHASE)

In the repulsion phase of linkage, the dominant allele of one gene is paired with the recessive allele of another gene on the same chromosome.

2.6 LINKAGE ANALYSIS

Linkage analysis is a molecular technique that looks for chromosomal regions via families that segregate with the disease phenotype (Sivalingam, 2023). Both binary and quantitative features can be mapped using it (Sivalingam, 2023). If we are aware of the correlation involving phenotypic and genetic similarity, linkage analysis can either be parametric or non-parametric. The traditional method is called parametric linkage analysis, and it studies the likelihood that a gene that is essential to a disease is associated with a genetic marker using the LOD score, which determines whether a given pedigree where the disorder and the genetic marker are segregating is due to linkage (with a provided linkage value) or random chance. The non-parametric linkage analysis, in the meantime, examines the likelihood of an allele be identical by descent with itself.

The most popular method for identifying genes in monogenic Mendelian diseases in humans has been linkage analysis. Both human as well as animal

genome is searched for chromosomal locations indicating co-segregation with the desired trait using a top-down, hypothesis-free, genome-wide method. Linkage analysis has two subtypes: parametric linkage analysis and non-parametric analysis of linkage. A model of disease inheritance within a family, such as recessive or dominant, is specified in a parametric linkage analysis, and then evidence of illness segregation within a family in accordance with that model is sought after. In order to conduct linkage studies on a genome-wide scale, between four hundred and eight hundred DNA markers (microsatellites) spaced at frequent times across the genome must be genotyped. Microsatellite markers have been substituted in recent years by arrays with 10–50,000 SNPs. Non-parametric linkage, which assumes the sharing on inherited alleles with respect to the sharing of the illness phenotype, has been used increasingly frequently for the evaluation of complex features and diseases. It does not specify an inheritance model. We infer that a DNA marker is firmly linked to the disorder-causing mutation by finding a high correlation between phenotypic variation and genetic variation of relatives for a particular DNA marker, which identifies a chromosomal location for the disorder's locus.

LOD scores, which can be defined as the log of the probabilities that a disease locus as well as marker locus are connected on the chromosome, are used to express the findings of linkage studies. Linkage is considered significant in parametric analysis while the LOD score is >+3.3 and "suggestive" in non-parametric analysis when the LOD score is >+1.9. These methods have the drawback of requiring a large number of such related individuals, especially when mapping complicated features and disorders, and relying on the occurrence of a single mutation with a very strong effect that causes the disease (which is highly penetrant variations). Despite linkage analysis's great success in locating the genes responsible for rare monogenic disorders, it has mostly failed to locate the genes responsible for complex common diseases and features that are observed in the population as a whole (Uitterlinden, 2018).

2.6.1 PARAMETRIC LINKAGE ANALYSIS

Established by Newton Morton (Morton, 1955), the LOD score is a statistical test that is frequently used for linkage analysis in populations of people, animals, and plants. The odds to get the test data, assuming that both loci are actually related, is compared to the probability of simply witnessing the same

data. In contrast to negative LOD scores, which suggest a lower likelihood of linkage, positive LOD scores favor the presence of linkage. An easy method for analyzing complex family pedigrees to find the relationship between Mendelian characteristics (or between a trait and a marker, or between two markers) is computerized LOD score analysis. Strachan and Read go into more depth about the process. It functions as follows, in brief:

1. Create a pedigree.
2. Calculate the frequency of recombination in various ways.
3. Determine the LOD score for every estimate.
4. The best estimate will be the one with the greatest LOD score.

2.6.2 LIMITATIONS

The type-1 error rate and mapping power of human QTLs can both be greatly increased by a number of technical and theoretical restrictions of linkage analysis (Ferreira, 2004). Linkage analysis has been successful in identifying genetic variations that are linked to rare disorders like Huntington disease, but it has not been as successful in applying to more widespread conditions like heart disease or other cancers (MacDonald, 1992). This can be explained by the fact that some rare disorders are caused by genetic pathways that are distinct from those affecting typical diseases (Hirschhorn and Daly, 2005).

2.7 SIGNIFICANT OF CROSSING OVER

1. Linkage poses a challenge for breeders aiming to amalgamate desirable traits into a single variety.
2. Due to the impediment of recombination by linkage, associated characteristics persist across multiple generations.

In the realm of genetics, a linkage group encompasses all the genes residing on a specific chromosome. These genes are inherited as an integral unit, functioning and segregating together during cell division rather than independently. Because of the presence of linkage groups, certain traits do not adhere to Mendel's law of independent assortment, which asserts that this principle is applicable only when genes are located on distinct chromosomes. When a chromosome fractures and subsequently recombines with its counterpart, alterations can occur in the gene content of the chromosome if the breaks and reunions happen at corresponding locations. This process is known as crossing over, which involves the exchange of genes between chromosomes.

2.8 CROSSING OVER

"Crossing over is the genetic recombination process resulting from the exchange of genetic material between the homologous chromosomes within a pair. It involves the reciprocal swapping of segments of genetic material between the non-sister chromatids of two homologous chromosomes, leading to the creation of novel gene combinations."

"Meiotic crossing over is the primary process responsible for generating a fresh array of allelic combinations within individual chromosomes and plays a crucial role in ensuring the accurate segregation of homologous chromosomes into gametes. The characteristics of meiotic recombination that have evolved over time are shaped by natural selection and may not align perfectly with the objectives of selective breeding research" (Strelnikova and Komakhin, 2023).

"Crossing over plays a crucial mechanical function during the initial meiotic division, guaranteeing the separation of homologous chromosomes" (Hunter, 2015).

Meiosis is a cellular division process fundamental to sexual reproduction. It enables species to preserve a consistent set of chromosomes across multiple generations by ensuring the accurate separation of homologous chromosomes during prophase I of meiosis. Simultaneously, meiosis serves as a wellspring of genetic diversity. This diversity arises from various mechanisms, including the recombination of entire chromosomes, the exchange of segments between homologous chromosomes during crossing over, and conversions that occur when unpaired nucleotide bases undergo repair at the sites of programmed double-strand breaks (DSBs) in DNA (Mercier et al., 2015).

The essential role of meiotic crossing over in selective breeding becomes apparent when observing the incorporation of specific economically valuable genes from the chromosomes of wild species into those of cultivated plants (De Muyt et al., 2009). Regulating the positioning of meiotic recombination sites and the rate of crossover events offers the potential to more efficiently engineer a novel allelic composition of chromosomes (Wijnker and de Jong, 2008; Lambing et al., 2017; Blary and Jenczewski, 2019).

2.9 THEORIES OF CROSSING OVER

The discovery of chiasma formation and the related phenomenon of crossing over was initially made by F. Janssens, while Morgan was credited with identifying the concepts of linkage and recombination. There are two theories that aim to elucidate the relationship between crossing over and chiasma formation:

2.9.1 CLASSICAL THEORY (TWO-PLANE THEORY)

This theory, also known as the two-plane theory, was originally put forth by L.W. Sharp. According to this theory, chiasma formation is considered the cause of crossing over, rather than its consequence. It posits that chiasmata form prior to the occurrence of genetic crossing over.

2.9.2 CHIASMA TYPE THEORY (ONE-PLANE THEORY)

This theory, also referred to as the one-plane theory, was initially proposed by Jannsen and later developed by Belling and Darlington. In contrast to the classical theory, this theory contends that chiasma formation results from the process of crossing over, with crossing over taking place before chiasma formation. It is worth noting that the chiasma type theory is widely accepted within the scientific community.

2.10 TYPES OF CROSSING OVER

Crossover products were categorized into three distinct groups: simple, mixed, and complex. These categories were exemplified with illustrations representing both A-to-D and D-to-A orientations.

2.10.1 SIMPLE CROSSING OVER

This occurs when chromatids break and recombine at one specific location, resulting in the formation of one chiasma.

2.10.2 MIXED CROSSING OVER

In this case, chromatids break and recombine at two distinct sites, giving rise to two chiasmas.

2.10.3 COMPLEX CROSSING OVER

Complex crossing over takes place when chromatids undergo breaks and recombination at numerous locations, leading to the formation of multiple chiasmas.

Simple crossover products were characterized by having a single exchange interval. In contrast, for mixed and complex crossover products, we evenly distributed half of an exchange to each of the intervals bordering the mixed or complex region. Within the complex crossover products, we identified three subtypes:

1. Those that featured a haplotype switch between the exchange intervals but lacked any mixed polymorphisms.
2. Those that contained at least one mixed polymorphism.
3. Those that exhibited more than two haplotype switches yet did not include any mixed polymorphisms.

2.11 MECHANISMS OF CROSSING OVER

The process of crossing over involves the following sequential steps:

2.11.1 SYNAPSIS

The pairing of homologous chromosomes, known as synapsis, occurs during the zygotene phase of meiosis. This paired set of homologous chromosomes is referred to as a synapsis.

2.11.1.1 PARACENTRIC SYNAPSIS

In paracentric synapsis, the pairing of homologous chromosomes starts from the middle (centromeric region) and then extends toward the telomeres. This type of synapsis ensures that the centromeres of homologous chromosomes align correctly, which is essential for proper chromosome segregation during meiosis.

2.11.1.2 PRETERMINAL SYNAPSIS

Preterminal synapsis is the opposite of paracentric synapsis. Here, the pairing of homologous chromosomes starts from the telomeres (the ends of the chromosomes) and proceeds toward the centromeres. While this is less common, it can occur in some species or under specific conditions.

2.11.1.3 RANDOM SYNAPSIS

Random synapsis, as the name suggests, does not follow a strict pattern. Pairing may start from anywhere along the chromosomes, neither specifically

at the centromeres nor the telomeres. This type of synapsis can lead to variations in chromosome alignment and may result in genetic diversity among offspring.

The type of synapsis that occurs can depend on the species, the specific chromosome, and the stage of meiosis. The accurate pairing and alignment of homologous chromosomes are crucial for the proper segregation of genetic material during meiosis and the production of genetically diverse gametes.

2.11.2 TETRAD FORMATION

Each chromosome consists of two chromatids, termed dyads. When two synapsed homologous chromosomes come together, a group of four homologous chromatids, consisting of two dyads, forms a structure called a tetrad. It is important to note that the two chromatids within the same chromosome are known as sister chromatids, while the two chromatids from different chromosomes, one from each homolog, are termed non-sister chromatids. During the zygotene stage of meiosis I, an intricately organized structure of filaments called the synaptonemal complex develops between the paired homologous chromosomes. This complex helps maintain the closely paired state of the homologous chromosomes.

2.11.3 EXCHANGE OF CHROMATID SEGMENTS

The non-sister chromatids come into contact at specific points, referred to as chiasmata, where gene exchange occurs between the two non-sister chromatids within a tetrad. These chiasmata are regions where homologous chromosomes are physically connected and exchange portions of chromatids. During synapsis, the non-sister chromatids of homologous chromosomes break and recombine, resulting in the formation of chiasmata. The exchange of genetic fragments is facilitated by the development of recombination nodules during the pachytene stage. The segments that remain unchanged on the chromatid are termed non-crossovers, while the altered segments are referred to as recombinants.

2.11.4 TERMINALIZATION

The chromatids progressively separate from the centromere toward the chiasma, eventually detaching from each other. This process is known as

terminalization. Terminalization of chiasmata initiates during the diplotene stage following crossing over and culminates in the diakinesis stage.

2.12 SIGNIFICANCE OF MEIOTIC CROSSING OVER IN EVOLUTION AND NATURAL SELECTION

A comprehensive historical overview of the evolution of the theory of meiotic recombination involving chromosomal DNA repair, along with the identification of key proteins like SPO11, RAD51, the ZMM complex, and others responsible for meiotic crossing over in most eukaryotes, can be found in a recent review authored by Bogdanov and Grishaeva (2020). In this chapter, we will briefly touch upon the fact that meiotic crossing over is governed by meiosis-specific genes, specifically referred to as meiotic recombination genes (Youds and Boulton, 2011; Bogdanov and Grishaeva, 2020). These genes are typically repressed in somatic cells that undergo mitotic division. The transition of diploid cells from mitotic division to meiotic division occurs through a process of negative regulation, where the genes responsible for initiating meiosis suppress the genetic program for mitosis, subsequently activating the previously dormant meiotic genetic program (Turner, 2007; Bogdanov and Grishaeva, 2020).

In flowering plants, the occurrence of meiotic crossing over takes place within specialized cells, namely microsporocytes and megasporocytes. This process involves several sequential steps, including the generation of programmed DNA DSBs and their repair through homologous recombination, with a preference for utilizing a homologous chromosome as a template. The products of these crossover events are chromosomes carrying novel combinations of allelic gene variants (Mieulet et al., 2018).

The physical manifestation of crossover exchange is observed as a visible crossing between chromosomes under a microscope, known as a chiasm. Apart from facilitating crossover exchange, chiasmata also serve a structural and mechanical role by holding chromosomes as bivalents during prophase and metaphase I of meiosis. Consequently, it is these bivalents, rather than individual chromosomes, that align on the spindle equator during metaphase I, allowing for the segregation of homologous chromosomes in the initial stage of meiotic division and subsequent cell tabloidization (Bogdanov and Grishaeva, 2020).

It is worth noting that many cellular proteins essential for DSB repair in the DNA of somatic cells also play a role in meiotic crossing over. However, their functions may undergo modifications depending on their localization,

posttranslational changes, and interactions with meiosis-specific proteins (Villeneuve and Hillers, 2001). This implies that, during evolution, the process of crossing over diverged from its original role of solely repairing somatic damage and acquired a specific function tailored to meiosis. Consequently, meiotic crossing over represents an evolutionary adaptation of somatic repair functions to ensure successful sexual reproduction during the transition from the diploid to haploid phase of the life cycle. It is suggested that the evolutionarily established parameters of meiotic crossing over are subject to regulation by natural selection to maximize the adaptability of organisms to changing environmental conditions across multiple sexual generations (Zhuchenko and Korol, 1985; Wijnker and de Jong, 2008). In this context, the parameters of the evolutionarily established mechanism of meiotic crossing over can constrain the selection process, which may require the generation of maximum genetic diversity among sexual offspring, even at the expense of adaptability to specific natural habitat conditions.

2.13 EXPLORING THE CROSSOVER AND NON-CROSSOVER ROUTES OF MEIOTIC RECOMBINATION: A RESEARCH JOURNEY THROUGH TIME

Initiation of meiotic recombination begins with the creation of programmed DSBs (Lam and Keeney, 2014). Following resection, one end of the DNA engages in homologous invasion, forming a displacement (D) loop. Subsequently, this D-loop captures the second DNA end, resulting in the formation of a double Holliday junction (Gray and Cohen, 2016). Although these two steps involve different biochemical processes, both lead to the creation of heteroduplex DNA. An earlier proposal by Szostak et al. in 1983 suggested that the resolution of double Holliday junctions generates two crossover and two non-crossover configurations, dependent on the location of paired nicks at each junction. However, subsequent research conducted in yeast and mice has revealed that the majority of non-crossovers originate from mechanisms distinct from double Holliday junction resolution (Gilbertson and Stahl, 1996; Allers and Lichten, 2001a; Wu and Hickson, 2003; McMahill et al., 2007; Cole et al., 2014). Nevertheless, it is still widely acknowledged that double Holliday junction resolution plays a pivotal role in crossover formation (Collins and Newlon, 1994; Schwachman and Kleckner, 1995; Allers and Lichten, 2001b).

In 1964, Holliday introduced a model to elucidate how recombination can lead to the generation of either crossover or non-crossover products during the process of repairing DNA breaks. His proposal posited that, prior to the synapsis of homologous chromosomes, breaks occurred in one of the polynucleotide DNA strands, referred to as nicks. The central element of this model was an intermediate molecular structure in which the single-stranded tails of cleaved DNA infiltrated the double-stranded DNA of the homologous chromosome, giving rise to a four-stranded intermediate configuration. This complex is now recognized as the Holliday structure or Holliday junction (Hj).

Holliday's model suggested that these complexes were cleaved by DNA repair enzymes, which are presently referred to as resolvases. This cleavage process resulted in the division of the four-stranded structure into two double-stranded structures, contingent upon which of the strands was broken. This process, termed "resolution" in English-language literature, led to either a crossover (cross) or dissociation without a crossover. Investigations in fungi demonstrated that crossover and non-crossover products of recombination (recombination repair) emerged in equal proportions, aligning with the predictions of the Holliday model.

Holliday initially proposed that recombination commenced with symmetrical nicks in one polynucleotide strand of homologous chromosomes, although this did not align with experimental observations (Sthal, 1994). To reconcile the model with empirical data, Szostak et al. (1983) suggested that meiotic recombination commenced with DSBs in one chromatid (DNA molecule). In the revised recombination model, which was based on DNA double-strand break repair (DSBR model), they posited that the intermediate recombination product contained two Holliday junctions. This proposition was founded on the detection of DNA DSBR in mitotically dividing cells.

According to Szostak et al. (1983) model, each of the two Holliday junctions in the intermediate recombination product (precrossover intermediate) should be realized independently. However, the results should mirror those of the Holliday model, with crossover and non-crossover products appearing in equal proportions. This model garnered robust support from physical experiments conducted on intermediate and final meiotic recombination products in *S. cerevisiae* yeast. In these investigations, intermediate recombination products, termed joint molecules between homologous chromosomes, were identified (Schwacha and Kleckner, 1994). These joint molecules exhibited many characteristics predicted for double Holliday junctions, which constituted the central intermediates of the new model (Schwacha and Kleckner, 1994). The outcomes of these studies have been widely accepted as evidence

of the existence of double Holliday junctions. The term "joint molecules" denotes the physical structures detected in the experiments, while "double Holliday junctions" (dHj) designates the intermediate products anticipated by the model.

Meiotic recombination stands as a fundamental cellular and evolutionary process. Within the context of meiosis, DSBs typically undergo repair through reciprocal exchange between homologous chromosomes, resulting in either crossovers or non-crossover gene conversion events (Baudat and De Massy, 2013; Hunter, 2015). In many species, crossovers and the interconnections they create between non-sister chromatids, known as chiasmata, play a pivotal role in signaling proper chromosome segregation during the first meiotic division (Oliver et al., 2008; Nagaoska et al., 2012). Beyond their immediate cellular function, meiotic recombination and crossovers also wield significant influence in the realm of evolution. They directly give rise to new combinations of alleles and, indirectly, elevate genetic diversity within species while accelerating the rate of adaptations (Hadany and Comeron, 2008; Felsenstein, 1974). Given the profound dual role of meiotic recombination and crossing over, it comes as no surprise that both processes are ubiquitous across the vast majority of eukaryotic organisms.

2.13.1 ENHANCEMENT OF MEIOTIC RECOMBINATION DURING THE PHASE OF D-LOOP RESOLUTION

A. The established model for DSB repair involves either a break occurring on the D chromosome or the A chromosome. This initial DSB event triggers a series of processes, including resection, strand invasion, and second end capture. This intermediate stage may progress into a fully ligated double Holliday junction (dHJ), or it may undergo further modifications by resolvases while retaining nicks.
B. The D-loop migration model is proposed to explain the abundance of simple crossovers. Initial strand invasion is illustrated separately from each end, yielding distinct crossover products. Following strand exchange, repair synthesis, and strand unwinding, co-directional D-loop migration occurs, positioning the D-loop on one side of the break. Subsequent second end capture by the D-loop results in the creation of a new heteroduplex tract, and resolution of the dHj according to configuration generates one simple crossover product and another with a heteroduplex DNA tract. This simple model,

particularly with frequent D chromosome breaks at the A3 hotspot, can produce the observed crossover patterns, including numerous simple crossovers, D-to-A crossovers with a mixed tract on the left side of the hotspot, and A-to-D crossovers with a mixed tract on the right side.

C. Modifications to the model described in (B) that shift the locations of simple crossover exchanges away from the center of the hotspot.

   i. Strand exchange occurs at an interstitial position within the DMC1/RAD51 nucleoprotein filament, followed by cleavage of the distal 3' single-stranded DNA end. Repair synthesis then proceeds from the clipped end, resulting in a shift of the exchange in the simple crossover product away from the center of the hotspot.
   ii. The repair DNA polymerase can detect mismatches at the 3' end of the invaded strand, ultimately causing a modest shift in the exchange point away from the center in the resulting simple crossover. The crossover with heteroduplex DNA has an exchange point defined by the 3' end of the non-invading broken strand or by the position/length of the D-loop before resolution.

Crossing over takes place when non-sister chromatids exchange genetic material between chromosomes to form gametes. This process rearranges the alleles found on parental chromosomes, leading to the creation of gametes that contain a blend of genetic material from both the mother and father. In simpler terms, it is the swapping of genetic information within the reproductive cell lineage.

During meiosis, the formation of egg and sperm cells involves the alignment of pairs of chromosomes. This alignment brings together corresponding DNA sequences from these paired chromosomes. This crucial mechanism is responsible for introducing genetic diversity in sexually reproducing organisms and plays a vital role in ensuring accurate chromosome segregation.

2.14 DISTRIBUTION OF CROSSOVER

The occurrence rate and spatial distribution of crossovers represent critical factors that facilitate the emergence of novel and selectively advantageous genetic variations during meiosis (Zhuchenko and Korol, 1985; Wijnker and de Jong, 2008). Numerous studies have demonstrated that the positioning of meiotic crossovers on chromosomes is not random; instead, it follows a

strict pre-determined pattern, exhibits non-uniformity, and is independent of genome size (Mercier et al., 2015; Mézard et al., 2015; Lambing et al., 2017; Wang and Copenhaver, 2018; Blary and Jenczewski, 2019). Crucially, the number and distribution of crossovers are under tight regulation, with three interconnected phenomena at the forefront: the centromere effect, crossover interference, and crossover assurance. These factors collectively govern the patterning of crossovers along chromosomes and their distribution among tetrads or bivalents (Wang et al., 2015; Hughes and Hawely, 2020).

2.14.1 CENTROMERE EFFECT

The centromere effect, first observed in *Drosophila*, refers to the classic phenomenon where crossovers do not occur in regions near the centromeres (Beadle, 1932; Hawley, 1980).This phenomenon has been documented in various organisms, including *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, and humans (Lambie and Roeder, 1986; Mathani and Willard, 1998). While the precise causes of the centromere effect remain incompletely understood, it appears to be influenced by factors such as distance from the centromere and highly repetitive heterochromatin (Wang et al., 2015; Mather, 1939; Sturtevant and Beadle, 1936; Hawley, 1980; Hartmann et al., 2019). A similar, albeit weaker, pattern is also noted near the telomeres in *D. melanogaster* (Wang et al., 2015; Beadle, 1932; Hawley, 1980). Notably, the extent of the centromere effect varies among species, even within the genus *Drosophila*. For instance, it is less pronounced in *D. simulans* compared to *D. melanogaster* and is even milder, or possibly absent, in *D. mauritiana* based on crossover data utilizing P-elements as markers (Ture, 1996). Studies involving sister species like *D. pseudoobscur*a and *D. persimilis* also suggest a tendency toward a detectable yet weaker centromere effect compared to *D. melanogaster* (McGaugh et al., 2012; Kulathinal et al., 2008). Remarkably, all these *Drosophila* species exhibiting a weaker centromere effect than *D. melanogaster* display higher crossover rates and longer genetic maps.

2.14.2 CROSSOVER INTERFERENCE

Crossover interference occurs when the occurrence of one crossover inhibits the formation of a second crossover in nearby regions. This results in a lower number of double crossovers that are farther apart than expected if each crossover along a chromosome arm occurred independently (Sturtevant,

1915; Muller, 1916). Initially identified in *D. melanogaster* (Sturtevant, 1915; Miller et al., 2016) this phenomenon has been observed in numerous other organisms, although not universally (Copenhaver et al., 1998; Housworth and Stahl, 2003; Berchowtiz and Copenhaver, 2010). Intriguingly, two species that lack an apparent crossover interference mechanism, namely fission yeast and *Aspergillus nidulans*, also do not possess a synaptonemal complex (Kohli and Bahler, 1994; Egel-Mitani et al., 1982). Supporting the notion of a direct link between the synaptonemal complex and crossover interference, mutants for the synaptonemal complex protein ZIP1 in *S. cerevisiae* show a lack of interference (Sym and Roeder, 1994). However, in *D. melanogaster*, the complete synaptonemal complex is not essential for interference (Page and Hawely, 2001). It is noteworthy that neither the centromere effect nor crossover interference could be observed in *D. melanogaster* Blm (Bloom syndrome helicase) mutants, suggesting a potentially unified pathway for governing crossover distribution across chromosomes (Hatkevich et al., 2017).

2.14.3 CROSSOVER ASSURANCE

Crossover assurance, also known as "obligate chiasma," refers to the observation that each tetrad or pair of homologous chromosomes undergoes at least one crossover, regardless of chromosome length or the total number of crossovers across the genome (Wang et al., 2015; Page and Hawely, 2001; Lopez et al., 2012). As crossover formation is critical for proper chromosome segregation during meiosis I, crossover assurance is subject to stringent regulatory control. In line with expectations, most species with a limited number of crossovers per meiosis exhibit some degree of crossover assurance, reducing the likelihood of tetrads with zero crossovers. A striking example is *Caenorhabditis elegans*, where the proportion of tetrads with no crossovers (referred to as E0 tetrads) is less than 1% (Hillers and Villeneuve, 2003). However, it is important to note that faithful segregation of homologous chromosomes does not always necessitate crossover formation. For instance, in *Drosophila*, including most dipteran species, males exhibit accurate chromosome segregation despite the absence of crossing over during meiosis (Morgan, 1910; Novitski, 1978).Females also achieve proper a chiasmate chromosome segregation for the small (dot) chromosome (known as the Muller F element or chromosome 4 in the *D. melanogaster* subgroup) through alternative mechanisms that involve centromeres and heterochromatic DNA (Hawley et al., 1993; Puro and Nokkala, 1977). To

gain a comprehensive understanding of the diverse mechanisms governing meiosis, quantifying E0 tetrads and, more broadly, the full range of Er tetrad classes (where r represents the number of crossovers in a tetrad) proves valuable.

2.14.4 E0 TETRADS

Nonetheless, directly measuring E0 tetrads is feasible only in systems where all four meiotic products are recoverable. In species where analysis is restricted to one of the four chromatids produced by meiosis (typical in most species, including *Drosophila*), Er can be estimated based on specific meiotic models that consider the fraction of products with varying numbers of crossovers. The first and commonly used model to estimate Er was developed by Weinstein (1936) under specific assumptions, including no crossover interference, no chromatid interference, random segregation of chromatids into haploid gametes, and equal viability among all possible meiotic products (Weinstein, 1936, 1918). In *D. melanogaster*, estimates of E0 (computed using Weinstein's model unless otherwise specified) exhibit variability across studies, genotypes, and environmental conditions. However, the prevailing trend suggests that crossover assurance in female meiosis is not absolute (E0 > 0), and its associated references. A study utilizing Whole-Genome Sequencing (WGS), conducted by Miller et al., in 2016 calculated an overall E0 value of 0.112. Interestingly, this value was similar for both the X chromosome and autosomes. In contrast, investigations employing visible markers (based on observed fly phenotypes) and larger sample sizes often yield smaller estimates of E0 for the X chromosome when compared to autosomes (Zwich et al., 1999). In summary, complete or absolute crossover assurance has not been reported for any chromosome in wild-type *D. melanogaster* flies. Intriguingly, studies on *D. virilis* have revealed lower E0 values compared to *D. melanogaster* (Hemmer et al., 2018; Chino and Kikkawa, 1933). This observation has been suggested to result from the higher rate of recombination and longer genetic map in *D. virilis.*

2.15 SIGNIFICANT OF DISTRIBUTION OF CROSSOVER

Understanding the distribution of crossover rates across genomes also presents an excellent opportunity to test hypotheses related to evolutionary processes. Various selection models predict that variations in recombination

rates can impact rates of evolution and levels of genetic diversity within species. These models are based on the premise that selection acting at a particular locus or nucleotide site leads to population dynamics in nearby genomic regions, resulting in a reduction in the local effective population size (Ne) relative to scenarios without selection (Smith and Haigh, 1974). Importantly, this phenomenon can arise from both positive and negative selection pressures (Charlesworth, 1996; Gillespie, 2000; Wiehe and Stephan, 1993; Plagnol et al., 2007). Although the specific effects of selection and linkage on Ne depend on numerous factors, all these models predict a positive association between intragenomic variation in crossover rates and two key aspects:

1. The effectiveness of selection (influenced by the population-scaled selection coefficient, $Ne \times s$) and
2. The level of neutral intraspecific genetic variation or diversity (influenced by the population-scaled mutation rate, $Ne \times \mu$).

These predictions have been observed in many species regarding the effect of variation in crossover rates on neutral diversity (Plagnol et al., 2007; Langley et al., 2012; Johri et al., 2020). However, findings from studies in humans, *Drosophila*, and yeast have provided mixed support for the hypothesis that variation in crossover rates across genomes influences the effectiveness of selection, as estimated through parameters such as codon usage bias or rates of protein evolution (Johri et al., 2020; Charlesworth and Campos, 2014)

2.16 RECOMBINATION

"Recombination is the method of interchanging nucleotide sequences either within or between genomes" (Posada et al., 2002).

It is a process where fragments of DNA are broken and recombined, resulting in the creation of new combinations of alleles. This mechanism of recombination generates genetic diversity at the gene level, showcasing variations in DNA sequences among different organisms. Typically occurring during meiosis in eukaryotic cells, cells with a nucleus and organelles, recombination occurs when gametes (egg and sperm cells) are formed through cell division. In the initial stage of meiosis, homologous pairs of chromosomes from both parents align. Chromosome arms may transiently touch and fuse during this alignment, leading to a crossover event.

2.17 TYPES OF RECOMBINATION

Living organisms have been found to exhibit a minimum of four distinct types of naturally occurring recombination.

2.17.1 HOMOLOGOUS RECOMBINATION

The process of homologous recombination is one where a donor sequence replaces a corresponding region in the acceptor DNA molecule, either homologous (similar) or homologous (similar but not identical) (Lemey and Posada, 2009). In numerous organisms, including yeast and humans, including DSBs, homology recombination (HR) is a tightly controlled biochemical mechanism necessary to maintain genomic integrity. Because it relies on sister chromatids and highly conserved molecular associates for DSB repair and is error-free, HR differs from other techniques such as non-homologous end-joining (NHEJ) and microhomology-mediated end-joining (MMEJ) (Lieber, 2010; Wang et al., 2022).

2.17.2 ILLEGITIMATE OR NON-HOMOLOGOUS RECOMBINATION

In regions with limited sequence similarity, illegitimate or non-homologous recombination becomes apparent, such as conversions between further chromosomes or deletions that eliminate multiple the genes found on a chromosome. Nevertheless, upon analyzing the short regions of similar sequences are seen in the DNA pattern at the boundaries of these events. occasionally discovered. Throughout the majority of the cell cycle, NHEJ functions as the main rebuilding process for any kind of DNA DSB and is necessary for the formation of antigen receptors. Ionizing radiation sensitivity and lymphocyte loss is caused by NHEJ deficiencies. All the following steps of NHEJ depend on synapsis, which is the integration of both of the DNA ends of a DSB. Synopsis, like the final processing phase, appears to be done by a variety of mechanisms, according to recent research. The decision between NHEJ and alternative DSB repair pathways is discussed in the opening section of this review. After that, it incorporates current knowledge of the mechanisms behind NHEJ synapsis and provides updates on other NHEJ steps such as DNA end processing (Zhao et al., 2020).

2.17.3 SITE-SPECIFIC RECOMBINATION

Site-specific recombination occurs when definite short pattern, typically around 12–24 base pairs in length, are involved in recombination between

otherwise dissimilar parental molecules. This type of recombination relies on dedicated enzymatic machinery, with each specific site requiring its own enzyme or enzyme system. Notable examples of site-specific recombination include the integration of certain bacteriophages, a restructuring of antibody genes in vertebrate animals, as well as the introduction of genes, such as *lambda*, and into a bacterial chromosome.

2.17.4 REPLICATION RECOMBINATION

The third type of recombination is replication recombination, which results in the creation of a fresh copy of a DNA segment. Many transportable elements use replication recombination to create a duplicate of themselves at a new location.

2.18 TYPES OF RNA RECOMBINATION

2.18.1 TYPE I (HOMOLOGOUS) RECOMBINATION

This kind of recombination happens when two RNA molecules with a lot of sequence homology or similarity to one another come together. Joining take place at flawless locations on the two RNA molecules, producing recombination RNAs that have the same pattern and anatomical array of the original RNA molecules. As a result, the term "homologous" denotes crossover events that occur at places that are similar or homologous to the two molecules, in addition to the presence of homology in sequence among the two parental RNAs. Sequence homology is necessary both surrounding and within the crossing sites, but it need not be present there specifically. When whole viral genomes are involved in RNA recombination, such as in picornavirus recombination, this sort of recombination is frequently seen.

2.18.2 TYPE II (ABERRANT HOMOLOGOUS) RECOMBINATION

Like I type recombination, II type recombination occurs when two molecules of RNA with same pattern come together. Contrary to I type recombination, II type recombination crossovers take place at unrelated locations. In this kind of recombination, there is no clear reason for the choice of crossover locations. There is typically no pattern likeness near the joining position on the two molecules of RNA in aberrant homologous reintegration. Only RNA reintegration can take place in this manner. Despite the two RNA molecules

in question sharing a sequence, aberrant homologous recombination sometimes serves as the only source of recombination in genetic crosses. When faulty RNAs are used in the recombination process, this sort of recombination occurs more frequently than usual.

2.18.3 TYPE III (NON-HOMOLOGOUS OR ILLEGITIMATE) RECOMBINATION

When there is no pattern likeness between two molecules of RNA, type III recombination occurs. As a result, it is unclear what mechanism underlies the choice of recombination sites. The joining regions on the two RNAs may have identical subordinate structures. In contrast to homologous recombination, this kind of recombination happens much repeatedly in DNA recombination than it does in RNA recombination. Gene insertions, deletions, and rearrangements that are seen in RNA viruses may be caused by type III recombination.

2.19 GENOMIC RECOMBINATION

Genetic recombination, sometimes referred to as genomic reorganization, is the interchange of genomic data across several species, producing offspring that have a distinctive combination of features that are different from the ones of their parents. Meiosis in eukaryotes involves genetic recombination, which can result in the development of a novel genetic structure that can be passed down to the next generations. Generally speaking, there are two types of natural recombination:

1. Intrachromosomal recombination, which involves the independent assortment of alleles located on different but homologous chromosomes during meiosis I.
2. Intrachromosomal recombination, occurs through a process known as crossing over (Daly and Minton, 1995).

2.20 GENETIC RECOMBINATION IN EUKARYOTES

Genetic recombination happens when homologous chromosomes link together during meiosis in eukaryotes. This makes it possible for the chromosomes to communicate genetic data. As an alternative, strands of DNA can be severed and then joined to form new DNA molecules.

In eukaryotes, recombination can also take place during mitosis, frequently including both of the sister chromosomes created following genomic repetition. Under this situation, in novel allele no alternatives are made because the twin genomes are frequently similar. Mitosis as well as meiosis involve homologous sequence recombination, which is the mixing together of DNA molecules with comparable structural characteristics. Chromosomes that are not sisters couple up during meiosis, causing recombination among non-sister homologs.

2.21 IMPORTANCE OF RECOMBINATION

Recombination is necessary for maintaining the stability of the genetic material as it passes from one generation to the next and across somatic cell divisions. It must cooperate with other activities taking place on the template for DNA, such as transcription, replication, and the special functions played by chromosomes at the centromeres and telomeres. It is necessary to coordinate the nucleus's spatial and temporal activity appropriately and to time it to the cell cycle stage. Regulatory mechanisms, including posttranslational modifications (PTMs) that directly affect recombination proteins, as well as chromatin changes that impact the DNA substrate, are involved. While the primary goal of recombination is to maintain genomic stability, it can also influence copy-number variations and overall chromosomal structures. Therefore, quality control mechanisms are necessary throughout the recombination process to ensure high fidelity and prevent genomic instability. Recombination has significant implications for human diseases, particularly cancer and potentially neurological disorders, through its regulatory mechanisms (Heyer, 2015).

2.21.1 MECHANISM

Several unique enzymes help to enable the recombination of genetic material. Recombination's strand transfer step is catalyzed by recombinases, a key class of enzymes. RecA, the main reconnecting enzyme in *E. coli*, is crucial for mending breaks in the double-strand (DSBs). DSB repair is carried out by two recombinases in yeast as well as other eukaryotic species. The RAD51 protein is necessary for both meiotic as well as mitotic reintegration, while DMC1 is solely needed for meiotic recombination. The bacterial RecA protein's archaeal equivalent is called RadA.

2.21.1.1 BACTERIAL RECOMBINATION

Recombination, which involves the transfer of genetic material, frequently takes place in bacterial systems. However, failed or abortive genetic material transfer, often known as non-productive transfer, can also occur. This refers to shift of bacterial DNA from the giver cell to receiver where the coming DNA integrates into their genetic makeup but does not result in a successful transfer. Failed transfers have been observed following transduction and conjugation. Dilution of the transferred fragment often occurs during culture growth (Rieger et al., 2012; In, B. Genetic recombination).

2.21.1.2 CHROMOSOMAL CROSSOVER

Chromosome crossover plays a crucial role in promoting recombination during meiosis in eukaryotes. This process can occasionally lead to the creation of novel chimeric alleles and results in offspring possessing gene combinations that are distinct from those of their parents. Genetic recombination facilitates the movement of genes, contributing to increased genetic diversity.

Chromosomal crossover occurs when the paired chromosomes inherited from each parent undergo recombination, typically during meiosis. In the prophase I at pachytene stage, the four chromatids align closely, allowing homologous regions of two chromatids to exchange genetic material (Alberts et al., 2002).

2.22 GENE CONVERSION

- Among the methods of alike recombination, gene conversion, makes it easier for genetic material to be transferred unidirectionally from a sequence known as the "donor" to one that is extremely homologous, or the "acceptor."
- Gene conversion can happen during mitosis but typically happens during meiosis.
- Gene conversion processes in mammalian cells often result in small gene conversion sequences, rarely longer than 1 kb.
- In particular, selection has an impact on how gene reformation affects the multigene family's evolution.

- Over 25,000 recombination geographical areas have been found in the genome of human, and both non-allelic and allelic hotspots exhibit high gene conversion activity often.
- It is possible to more correctly forecast the exact positions of unstable genomic areas by improving our knowledge of how gene conversion affects the creation or eradication of exchange hotspots in the human genome.
- According to Chen et al. (2007), several human genetic illnesses have gene conversion as one of their contributing factors.

2.23 GENETIC ENGINEERING IN PLANTS

Utilizing genetic engineering to improve plant capacities is essential given the problems posed by the effects of climate change population increase. Farming and molecular farming are both hampered by the labor-intensive nature of present methods and their restriction to a few species. As a result, new methods and equipment are required to increase the variety of species that can be transformed as well as the effectiveness of those transformations. While microbe and animal systems have been transformed by nanotechnology, its use in plants has not yet reached its full potential. However, reports of direct germline editing, plastid and genome of mitochondria modification, as well as nano-mediated delivery for manipulation of genes in plants are beginning to emerge (Squire et al., 2023).

2.24 SAFETY CONSIDERATIONS IN PLANT GENETIC ENGINEERING

In the past, plant breeding depended on the plants' inherent genetic flexibility to produce better types. Early farmers used environmental changes to increase the frequency of desirable qualities in a species by selectively breeding plants with those traits. Expected agronomic impacts are the main foundation for regulatory requirements and safety evaluation studies for transgenic plants. Reliable safety evaluation methods depend on the presence and stable representation of implanted genetic information in subsequent transgenic generations. Humans have substantially altered the genomes of beneficial plants by selective breeding for at over 10,000 years, giving the offspring notable differences from their natural parents. Breeding standards

established by the crop's intended application, the environment in which it may grow, the quantity needed, and the economics were the driving forces behind these adjustments (Kappelli and Aberson, 1998)

2.25 DNA RECOMBINATION

In order to facilitate the transmission of genetic information between chromosomes, repair broken double-strands of (DSBs), and incorporate foreign DNA, DNA recombination activities are essential for meiosis. repair of DSB and foreign DNA insertion take place in vegetative cells and are thought to be managed by the similar reintegration technology, whereas intrachromosomal exchange only occurs in meiotic cells (Mengiste and Paszkowski, 1999; Vergunt and Hooykaas, 1999). For cells to survive, DSBs must be repaired effectively. This can be done either through homologous recombination (HR), which uses a homologous DNA template for accurate repair, or through non-homologous mechanisms, which ligate DNA ends without requiring homology and result in illegitimate recombination (IR).

Although the enzymes essential to these activities have not changed throughout time, their relative activity changes greatly among contrasting individuals. Multicellular eukaryotes generally rely on IR for DSB repair, whereas bacteria and single-celled eukaryotes, such as yeast, mostly use HR (Mengiste and Paszkowski, 1999; Vergunt and Hooykaas, 1999).

As the common DSB reform process also controls the insertion of foreign DNA, this discrepancy has significant consequences for the use of genetics in crop plants. Contrary to IR, which frequently leads to accurate insertion at the identical place of host genetic material and permits gene targeting (GT), HR allows precise absorption at the transgene's random integration site with predictable copy counts. According to Vergunst and Hooykaas (1999), Kumar and Fladung (2001), GT is an important method in functional genomes for decoding unidentified gene functions.

2.25.1 MEIOTIC RECOMBINATION

For homologous chromosomes to be properly segregated during meiosis, crossover recombination is essential. Meiotic recombination is an inherent process started by designed DNA DSBs, unlike spontaneous mitotic recombination. The development of a minimum of one crossover per homolog is ensured by meiosis-specific mechanisms, which counterbalance the core

recombine machinery's propensity to use a sister-chromatid templates and yield non-crossover results (Hunter, 2007)

Chromosome segregation depends on recombination during meiosis because it creates physical links between homologous chromosomes that enable them to align properly on the spindle and separate correctly during the first division (Petronczki et al., 2003).

Recombination failure frequently results in chromosome disjunction failure, which has detrimental effects on gamete development. Meiotic recombination depends on the generation and consequent repair of DSBs, which are caused by the Spo11 protein. DSB generation in *S. cerevisiae* needs the existence of at least nine other proteins, despite Spo11's high degree of conservation. It is yet unclear how exactly these elements play a role. Recombination and the advancement through meiotic prophase are synchronized, ensuring that particular stages in the recombination cycle take place at the correct moment. For example, DSB formation only happens following local DNA replication (Keeney, 2001).

2.26 THE EVOLUTION OF SEX DIMORPHISM IN RECOMBINATION

Male and female meiosis differ in a number of significant ways. Males create four little motile gametes (spermatozoa) while females only make one big gamete from the four meiotic products. Meiosis timing varies greatly between male and female cells, with male meiosis occurring continuously and female meiosis often halting twice. Males and females can experience different levels of genetic recombination after meiosis due to changes in the frequency and location of crossing over.

The first feature, the emergence of heterogamy, has acquired substantial transcendent study. Less consideration has been given to gender-specific recombination differences, particularly in formal models. Recombination dimorphism can happen on the autosomes or the sex chromosomes. Recombination on autosomes may be entirely missing in one sex (achiasmy) or quantitatively different between sexes (heterochiasmy).

2.26.1 ACHIASMY

It is also common, although has gained low observation, to have reintegration dimorphism on the somatic chromosomes, notably achiasmy. One sex in achiasmy appears to be completely devoid of recombination. This is

different from the meiosis loss that is frequently seen during parthenogenesis. In species where parthenogenesis is uncommon or unknown, achiasmy develops.

The "Haldane-Huxley rule" declares that in dioecious species, achiasmy predominately take place in organism having different sex chromosomes. There are, however, some exceptions, such as specific Musca and Culex species.

Pleiotropy and the no-recombination hypotheses can both be used to explain the origin of achiasmy. Both hypotheses have drawbacks and restrictions on how they might explain the patterns of achiasmy that have been observed.

The pleiotropy hypothesis falls short of explaining the extreme pleiotropic effects, the fact that hermaphrodites only have one gender and that achiasmy evolved in a group of organisms with a XX/XO sex determination system.

2.26.2 HETEROCHIASMY

The measurement of heterochiasmy is difficult. Chiasma counts make up the majority of obtained data, which frequently ignore the location of crossing across along chromosomes.

Studies employing distance maps between a few points are made more challenging by the difference in chiasma position across sexes. The amount of recombination that appears to occur more frequently in one sex than the other may not actually exist, depending on where markers are located along chromosomes. The analysis of heteroplasmy data has become more challenging as molecular approaches have shown additional variance in recombination frequency between chromosomes and genotypes (Korol et al., 1994).

It is challenging to detect the presence of phylogenetic inertia and establish the number of species or species groups required for hypothesis testing when analyzing heteroplasmy data because of the unidentified value rate of heteroplasmy evolution, for example.

Both Trivers (1988) and Burt et al. (1991) examined chiasma count data and discovered significant gender variations. Although these data need to be further examined, recent map data likely to confirm their analyses.

Whether they are hermaphrodite or dioecious, about 75% of chiasmata species have recombination rates that differ by more than 5% between the

meiosis of the sexes, as determined by chiasma count or map length. In uttermost circumstances, in female meiosis reintegration can be up to three times greater (as in the case of the zebrafish *Danio rerio* and the planarian *Schmidtea* polychrome) or about 1.5 times lower (as in the case of the monecious species *Pinus taeda*).

According to Trivers (1988), heterogamety does not have a significant impact on heterochiasmy in dioecious species because it tends to demonstrate a tendency toward reduced recombination in male meiosis.

2.27 NEUTRAL HYPOTHESIS

In-depth theoretical research on the evolution of mean recombination rates has been done (as summarized by Barton and Charlesworth, 1998; Otto and Lenormand, 2002), and a few experiments (e.g. Korol and Iliadi, 1994) have shown that it has the ability to evolve. However, the evolution of different recombination rates in males and females has no theoretical nor empirical basis. Burt et al. (1991) hypothesized that heteroplasmy might be neutral when they failed to discover a relationship between the degree of variation in heteroplasmy and the chance for gender distinctions in selection, which, in their view, must be elevated in bisexual creatures, between in asexual vegetation and relatively small in hermaphroditic animals. It is crucial to remember that the absence of evidence for a certain hypothesis does not automatically suggest neutrality, especially when that hypothesis lacks a solid theoretical basis and supporting data.

2.28 EVOLUTIONARY EXPLANATION AND SEXUAL SELECTION

Trivers (1988) suggested that sexual selection might lead to heterochiasmy. He proposed that male genes and combination of genes would, on a typical basis, be better than those handed on by females because of the greater variation in reproductive outcomes among males. According to Trivers, this clarifies that males have lower rates of recombination, with variations being explained by modifications to the system of sexual selection. However, this proposal lacks a theoretical basis for autosomes and is mostly dependent on models of the evolution of sex chromosomes (Nei, 1969). A competing linguistic model has also been put out, which contends that recombination rates should be higher for the sex that has undergone more intensive selection or has a bigger reproductive variation (Burt et al., 1991).

2.29 RECOMBINATION IN VIRUSES

Recombination is a widely observed procedure that generates diversity in the majority of viruses. It involves the fusion of independently emerging variants in the similar viral molecule, opening up novel possibilities for viruses to control selective pressures, adapt to different environments, and adjust to new hosts. As a result, clinical researchers as well as epidemiologists, molecular biologists, and evolutionary biologists are interested in studying viral recombination.

2.29.1 MECHANISM OF RECOMBINATION IN VIRUSES

The molecular procedure of genomic recombination involves crossing over two strands of nucleic acid to create new genomic combinations. We may differentiate between intragenomic recombination—which involves pieces from the similar genetic unit—and inter-genomic recombination—which involves pieces from other origins—based on the origin of the parental strands. The regulation of the expression of genes is linked to intragenomic recombination (Johansonn, 2013). In particular, this is shown in herpesviruses like human Herpesvirus-1 (HSV-1), one of the most well-investigated DNA viruses. To create genetic variety and preserve chromosomal integrity, inter-genomic recombination is an approach. Because it produces new variations with distinct biological characteristics, this type of recombination is particularly pertinent for RNA as well as DNA viruses. This section will concentrate on the process of recombination of inter-genomics.

2.29.1.1 RECOMBINATION MECHANISMS IN DNA AND RNA VIRUSES

Although just a few have undergone in-depth analysis, many recombination mechanisms have been found in both RNA and DNA, viruses. This section will provide comprehensive information on the various methods of recombination, using the HSV-1 virus example of a DNA virus, influenza as an example of an RNA virus, and HIV as an example of a retrovirus, respectively. Since, herpesviruses are significant models for understanding eukaryotic DNA replication, their example of a DNA virus is particularly well-characterized (Weller and Coen, 2012). The first proof of HSV-1 recombination was discovered by Wildy's research in 1955 utilizing two sensitive-to-temperature HSV-1 mutants. There are two generally recognized

recombination methods for RNA viruses: replicative and non-replicative. Replicative and non-replicative recombination are two widely recognized recombination processes in RNA viruses. In contrast to the non-replicative model, which calls for an earlier break in the RNA molecule to enable the development of hybrid genomes, the replicative model entails the exchange of the complex for replication from one RNA template to another (Galli and Bukh, 2014).

2.29.1.2 RECOMBINATION IN DNA VIRUSES

Herpesviruses and bacteriophages both use concatemers, two-component recombinases, and proteins in cells in their mechanistic models. This proposes a prehistoric mechanism in which DNA replication and recombination are linked (Weller and Sawitzke, 2014). Additionally, recombination protein and replication of the common replicative complex shared by all herpesviruses are highly conserved (Renekamp and Lieberman, 2010). Numerous of these proteins are interchangeable with other herpesviruses, showing how one virus can replicate a different one (Sarisky and Hayward, 1996). Recombination is essential for DNA damage repair in DNA viruses, particularly during the latency phase stage Wilkinson and Weller (2004). However, discrepancies in a repair-initiating sequence of DNA among as well as herpesviruses have been discovered by the investigation of the full genomes of different herpesviruses, revealing potential variances in the recombination processes within these subfamilies (Brown, 2014).

2.29.1.3 RECOMBINATION IN RNA VIRUSES

The main subgroups of RNA viruses have dramatically different recombination rates. While (+) ssRNA viruses frequently have high recombination rates, (-) ssRNA viruses typically have low recombination rates. There are significant outliers to this overall tendency, though. Reassortments are common in Orthomyxoviridae, including the human influenza A virus, which is a member of the (-) ssRNA virus family, while recombination is uncommon in flaviviruses. In contrast to flaviviruses, which have low recombination rates (Taucher et al., 2010), influenza A virus recombination rates in natural populations are ten times greater than thought previously (Marshall et al., 2013).

Recombinant genomes can be produced in RNA viruses via two distinct processes. Recombination stricto sensu happens in nearly all RNA viruses, whereas reassortment only happens in segmented RNA viruses. Recombination particularly happens when two nucleotide sequences interact in a way that allows genetic information to be exchanged between them, creating hybrid RNA sequences. A well-known example of this process is the HIV-1 virus (Galetto and Negroni, 2005). Reassortment happens when at least two viruses infect the same host cell at the same time. This exchange of discrete RNA segments occurs when segments from various origins are packaged into some offspring viruses. Influenza A typical illustration of reassortment is a virus' antigenic change (Nelson et al., 2008).

2.30 CONCLUSION

It is important to note that all genes residing on a single chromosome are considered linked to each other. The mechanism responsible for the inheritance of linked genes within the same linkage group is referred to as "linkage." In contrast, crossing over leads to the emergence of new traits through the exchange of genetic segments between chromosomes, serving as a source of genetic diversity. However, it is worth noting that linkage tends to decrease the likelihood of variability in gametes unless crossing over takes place.

Genetics encompasses the investigation of hereditary traits and the diversity that arises from them.

Recombination is the mechanism through which DNA fragments break and recombine, resulting in the appearance of novel varieties of alleles. This method fosters genetic diversity at the genetic level, reflecting variations in DNA patterns among different organisms. RNA recombination is a crucial factor in genetic changes associated with the emergence of RNA viruses. Genomic recombination significantly presents the development of entirely new lineages, the development of host tropism, environmental changes, virulence, and disease. It encompasses both intra- and intrachromosomal recombination. During sexual reproduction, the interaction of genomic data between different chromatids of homologous chromosomes, understood as a chromosomal crossover, shows the formation of recombinant chromosomes. Gene change appears when one DNA sequence replaces a homologous sequence, resulting in its identical nature.

KEYWORDS

- **linkage**
- **complete and incomplete linkage**
- **crossing over**
- **types of crossing over**
- **recombination**
- **types of recombination**
- **types of RNA recombination**

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CHAPTER 3

Structure of Gene

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ABSTRACT

A gene is a fundamental unit of heredity in living organisms, typically comprising DNA sequences that encode the instructions necessary for cellular function, growth, and development. Structurally, a gene consists of exons, which code for proteins, and introns, which are noncoding regions interspersed between exons. Regulatory sequences, such as promoters, enhancers, and silencers, control gene expression by influencing the transcription process. The coding region of the gene is transcribed into mRNA, which is then translated into proteins, the functional products of genes. Various regulatory elements and epigenetic factors, including DNA methylation and histone modifications, also play critical roles in gene activity, determining when and where specific genes are expressed. The complexity of gene structure, along with the interactions between coding sequences and regulatory regions, is vital to understanding gene function, regulation, and the intricate mechanisms underlying genetic inheritance and phenotypic variation.

3.1 INTRODUCTION

Genes serve as the fundamental units of heredity, responsible for the transmission of genetic information from one generation to the next. They are

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integral to the field of genetics and play a crucial role in shaping an organism's traits and characteristics (Carlson, 1991). They are like atoms, which are the fundamental units of physical matter. Mendel, when describing the outcomes of his experiments involving monohybrid and dihybrid crosses, initially conceptualized genes as discrete segments and gave them different names, including hereditary elements. However, his understanding of genes was entirely hypothetical, and he did not know their physical or chemical nature (Allen, 2003).

Traditionally, a gene has been defined as the unit of heredity or the portion of a chromosome that encodes a functional RNA or protein. However, while these definitions align for prokaryotic genes, eukaryotic genes are much more complex. Prokaryotic genes have a simple structure with coding sequences that specify protein synthesis and minimal regulatory sequences that control gene expression (Fogle, 1990). In contrast, eukaryotic genes can be very complex and occupy large portions of chromosomes. This is because eukaryotic genes, particularly those in mammalian cells, are often discontinuous, with coding regions separated by noncoding sequences. Additionally, regulatory sequences responsible for gene expression can be complex and located far from the actual gene sequence (Ong and Victor, 2011). As genes must ultimately be defined in a phenotypic sense, gene expression is critical, and the definition of a functional gene would be those DNA sequences required for normal gene product expression (Jaenisch and Adrian, 2003).

Accurately defining a gene is crucial to comprehend how it functions, including the specific sequences that play a vital role in its controlled expression (Blencowe, 2006). Furthermore, having knowledge of the structure and sequence of a gene is essential to assess and comprehend the molecular basis for gene mutations responsible for causing diseases (Wang et al., 2012).

Furthermore, it will become apparent later that understanding the features of a gene, such as the specific sequences that establish open reading frames, the splice site signals that establish exon/intron boundaries, and the sequences that comprise transcription regulatory signals, is imperative when attempting to identify an unfamiliar gene (Zhang et al., 2000).

Mendel's laws were rediscovered in 1900, in his explanation of the findings from known that chromosomes played a crucial part in inheritance (Gayon, 2016). Since chromosomes were discovered the sole connection between successive generations is through diploid chromosome sets, which are comprised of two sets that look alike, each from mother and father, which occur during fertilization. Soon after, it was discovered that genes and chromosomes behave in a parallel manner (Cimino, 1972).

In the past, various hypotheses were proposed by researchers to decipher the characteristics of genes. For example, De Vries suggested the "single gene for single one character" explanation, which stated that a specific gene controls a specific trait in an individual. Bateson and Punnett suggested that the dominant character in a cross has a determiner, while the recessive character does not. However, all these theories were rejected when Morgan introduced the particulate gene theory in 1926. He viewed genes as chromosomes that are linear structures that contain a series of corpuscles arranged in a specific order. These corpuscles, also known as genes, are the units of heredity that provide instructions for the development, growth, and functioning of living organisms. Therefore, to understand the concept of genes, it is recommended to consider both classical and modern definitions of genes (Portin, 1993).

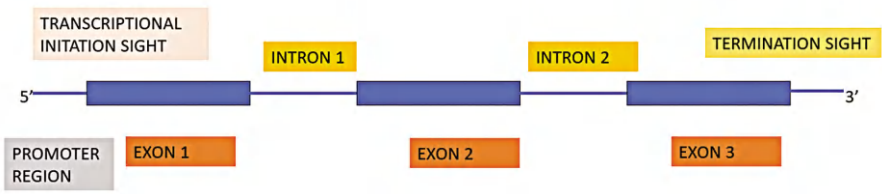


FIGURE 3.1 Structure of gene.

3.2 CONCEPT OF GENE OVER THE YEARS

Since the early 1900s the gene has remained the primary focus of scientific investigation in order to understand the foundation of inheritance. The study of genes has been approached from two primary perspectives: (1) a genetic perspective, and (2) a biochemical (Nicholl, 2023). The following are a summary of these two aspects:

3.2.1 A GENETIC VIEW

The concept of genes from a genetic perspective is primarily based on classical genetics. During his experiments, Mendel referred to genes as factors and concluded that they were the cause or reason for the inheritance of traits (Benson, 2001). The scientists studied and examined the characteristics and actions of chromosomes and genes during mitosis and meiosis in higher plants, which led them to establish the chromosome theory of inheritance. This theory suggests that genes and chromosomes separate and demonstrate

random distribution, proving that chromosomes contain genes at specific locations. Morgan's research on linkage studies in Drosophila further supported this theory and revealed that genes are positioned linearly on chromosomes. He proposed that genes located in proximity on a chromosome are more likely to be inherited together, and that recombination happens as a result of crossing over. The number of linkage groups is equivalent to the chromosomes number (Fraser et al., 2009). This view is also known as the bead theory, and it demonstrates that genes are situated on chromosomes. Below are the key aspects of the bead theory:

1. The gene is considered a structure that cannot be divided by crossing over, while crossing over can take place between genes not within a single gene.
2. It is regarded the fundamental element of alteration or variation, and it undergoes a shift from one allele variant to another. However, there are hardly smaller constituents inside a gene that can undergo modification.
3. The fundamental element of operation is the gene. In the absence of any segment of the gene, it cannot carry out its function.

In the past, chromosomes were seen as a vehicle for genes, serving the purpose of organizing and shuffling them during segregation and recombination. However, the bead theory is no longer considered accurate in regards to these aspects. Recent evidence suggests that genes can be divided, some parts of them can mutate, and certain portions of genes can perform functions on their own.

3.2.2 A PERSPECTIVE FROM A BIOCHEMICAL STANDPOINT

As of right now, the widespread understanding is that a gene refers to a particular nucleotide sequence found in DNA that regulates the synthesis of a single polypeptide chain (Orth et al., 2000). The variations in genes can occur as a result of a single nucleotide changing in more than one location within the gene. When altered nucleotides in a gene undergo crossover, it happens at a very low frequency because the mutant nucleotides are situated very close to each other. If numerous genes that affect the same characteristic are located close together, making crossing over between them rare, they are referred to as a complex locus. Multiple alleles in the nucleotide sequence of a gene can be altered by mutations that occur at various positions within the gene.

3.3 THE GENE IS SPLITABLE

Previously, the notion was held that a gene is a fundamental building block that cannot be separated by crossing over. This implies that crossing over only takes between genes, rather than within a gene. However, it has now been discovered that intragenic recombination occurs in numerous organisms, indicating that a gene can indeed be separated. The intragenic recombination has two primary characteristics (Holliday, 1964).

1. Infrequent events require a significant number of offspring in a test cross to be detected. Benzer had anticipated detecting a low recombination frequency as 10-6, but the lowest frequency he found was 10-4 (equivalent to $0.01 \times 2 = 0.02\%$).
2. Intragenic recombination happens between alleles that are closely located within a gene and have a functional relationship.

3.4 SECTION OF A GENE

In the past, it was commonly thought that the gene was the basic functional unit and that its parts could not operate independently. However, this perspective has been challenged by research on the rll locus of T4 phage, which identified three subcomponents of a gene: the recon, muton, and cistron. The following section offers a brief explanation of each (Portin, 1993).

3.4.1 RECON

Recons refer to the specific segments of a gene where recombination can take place, but not within the recons themselves. A minimum distance between recons within the gene ensures proper recombination. The gene map consists of a linear sequence of these recons.

3.4.2 MUTON

The smallest unit in a gene that can cause a mutation or a mutant phenotype is known as the “gene element.” The bead idea, which claimed that the entire

gene was mutable or changeable, is refuted by this, which suggests that only a section of a gene can undergo mutation or change.

3.4.3 CISTRON

The cistron, which is the functional unit of a gene, is the biggest element. This refutes the bead theory that considered the whole gene as a functional unit. The term "cistron" comes from the cis-trans test, this refers to a method for figuring out whether two mutations are housed in the same cistron or various ones. Here is a description of the test.

3.5 DETAILED OR PRECISE ARRANGEMENT OF NUCLEOTIDES WITHIN A GENE

Benzer categorized the segment into three units—recon, muton, and cistron—which correspond to recombination, mutation, and function within a gene. Each gene is composed of multiple units of these types (Portin, 1993). Structure of a gene involves mapping the locus of individual gene, like how genes are assigned to a chromosome during chromosome mapping. However, for a gene, many alleles can be assigned to the common locus. Intragenic recombination is used to prepare individual gene maps, but this requires a large population due to the low frequency of intragenic recombination. Prokaryotes are often used for this purpose. For example, the lozenge gene in Drosophila has 14 alleles that map to four mutational sites within the same locus, while the rosy eye gene has different alleles that map to ten mutational sites (Portin, 1993).

Genes can be distinguished based on dominance, interaction, character controlled, survival rate, position, sequence, linkage, etc. A brief summary of gene classification based on these criteria is presented below.

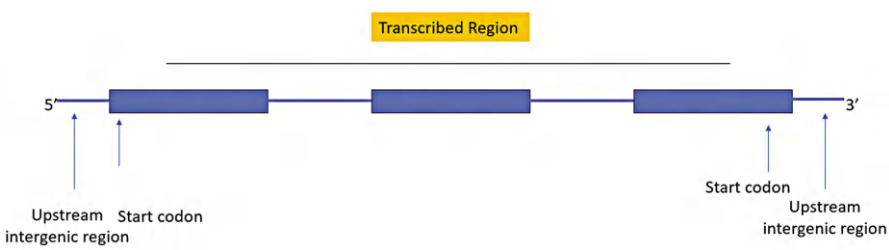


FIGURE 3.2 Fine structure of eukaryotic gene.

TABLE 3.1 Classification of Gene.

1	**Dominance**	
	Dominant genes	Expressed in the F1
	Recessive genes	Suppressed in F1
2	**Based on interaction**	
	Epistatic gene	A gene that exerts a dominant influence over another gene that governs the identical characteristic.
	Hypostatic gene	A gene that is suppressed in its expression by another gene that controls the same trait.
3	**Based on the according to the character control**	
	Major gene	A gene that controls a discrete, qualitative trait that has a noticeable and distinguishable effect on the phenotype.
	Minor gene	A gene that participates in the manifestation of a quantitative trait, but whose impact is not readily observable.
4	**Based on effect on survival**	
	Lethal gene	A genetic trait that causes the death of an individual who possesses two copies of it, known as homozygous condition, and can be expressed in either a dominant or recessive manner.
	Semi lethal gene	A gene that results in the death of over half of the individuals who possess it.
	Subvital gene	A gene that leads to the death of fewer than 50% of the people who carry it.
	Vital gene	A gene that does not cause fatal consequences to individuals who possess it.
5	**Based on position**	
	Normal genes	The class of genes, which includes the majority of genes, that have a fixed position on the chromosomes.
	Jumping genes	A gene that participates in the manifestation of a quantitative trait, but whose impact is not readily observable.
6	**Based on nucleotide sequence**	
	Normal genes	A gene is characterized by a contiguous sequence of nucleotides that encodes a single polypeptide chain.
	Split gene	There are genes found in certain eukaryotes that contain nucleotide sequences which are not continuous. These sequences do not contain information for the synthesis of amino acids.
	Pseudo genes	Genes having nonfunctional nucleotides.
7	**Based on sex linkage**	
	Sex-linked genes	Genes that reside on the sex chromosomes, particularly on the X chromosome.
	Sex-limited genes	Genes that are exclusively expressed in one gender.
	Sex-influenced genes	Genes that exhibit differential expression based on the sex of an individual, such as the gene responsible for hair loss in humans.

TABLE 3.1 *(Continued)*

8	**Based on operon model**	
	Regulator gene	A gene responsible for producing a repressor molecule.
	Operator gene	Within the lac operon, there is a gene that regulates the activity of the structural genes.
	Promotor gene	A gene located in the lac operon triggers the start of mRNA synthesis.
	Structural genes	The genes that regulate protein synthesis by means of mRNA.
9	**Based on mutational role**	
	Mutable genes	Genes that demonstrate a higher frequency of mutations compared to other genes, such as the eye gene in Drosophila.
	Mutator genes	Genes that increase the inherent rate of mutation for other genes, such as the dotted gene found in maize.
	Antimutator genes	Genes that reduce the occurrence of spontaneous mutations in other genes within the same genome can be observed in bacteria and bacteriophages.

3.5.1 SPLIT GENES

Typically, a gene consists of a consecutive series of nucleotides, with no breaks or interruptions in the sequence. This sequence is responsible for coding a single polypeptide chain. Nevertheless, certain genes have been found to have interruptions in their nucleotide sequence, called split genes (Chambon, 1981). There are two types of sequences in these genes: continuous sequences and discontinuous sequences.

1. The mRNA produced from the gene's fragmented DNA has a nucleotide sequence known as the regular sequence. Exons are the sequences that encode for a specific polypeptide chain.
2. Introns are broken-up segments found in a gene's fragmented DNA. The mRNA produced from the gene does not contain these sequences since they do not code for any polypeptide chains. During processing, introns are removed from the mRNA, as they are noncoding sequences and have no functional purpose in the mRNA. In contrast, the coding sequences, that is, exons, are connected by the enzyme ligase.

Split genes were found in other genes, including the ribosomal genes in Drosophila, the tRNA genes in yeast, and the beta globin gene in rabbits and mice (Crick, 1979).

To determine the number and size of intervening sequences in a split gene, the R loop method is used. In this procedure, mRNA and DNA from

the same gene are combined at high temperatures and formamide concentrations. During the pairing process, loops are formed in the DNA noncoding sequences. While the size of the loop denotes the length of the intron, the number of loops correlates to the number of intervening sequences. An electron microscope can be used to see these loops. For instance, the beta globin gene has two intervening segments of 550 and 125 nucleotides in length, but the ovalbumin gene has eight exons and seven introns.

3.5.2 JUMPING GENES

Genetic elements that can migrate from one place in a genome to another are known as transposable elements, jumping genes, or transposons. Barbara McClintock originally found them in maize in 1950. However, her contribution went unrecognized for a considerable period. Transposable elements have since been found in many organisms, including bacteria, and are classified into two main types: insertion sequences and transposons. Insertion sequences are small, simple transposable elements that only carry the genetic information needed for their transposition, while transposons are larger and contain additional genes that can provide benefits to the host organism. Research on transposable elements has significantly advanced our comprehension of genetics and evolution (McDonald, 1993).

1. Insertion sequences are short DNA sequences that are capable of transposition within a genome. They are found in various prokaryotes, including bacteria, bacteriophages, and plasmids, and exist in different types with specific characteristics. Unlike genes, insertion sequences do not contain information to produce proteins, and their main function is to facilitate their own movement within a genome by encoding enzymes that catalyze their transposition. Although they do not directly provide functional molecules, insertion sequences play a significant role in genome evolution and adaptation.
2. Transposons are genetic components that consist of coding sequences capable of generating one or multiple proteins. They are generally extensive DNA sequences that can span thousands of base pairs. These elements have been implicated in chromosomal modifications such as inversion and deletion and are recognized as hotspots for these alterations. They can be valuable tools for studying mutagenesis. Mobile DNA segments have been identified in various eukaryotes, such as maize, yeast, and Drosophila (Daboussi and Capy, 2003).

3.6 REGULATION OF GENE EXPRESSION

Genes that are necessary for maintaining fundamental processes are known as constitutive genes. These genes are unregulated and their expression is constant, unlike regulated genes (Siaut et al., 2007). Research on bacterial genetics has revealed that enzyme structure is determined by genes, such genes are referred to as regulator genes. The idea of regulation of gene was extensively studied by J. Monod and F. Jacob in 1961, who introduced the operon theory.

According to the operon idea, structural genes, operators, promoters, regulator genes, repressor proteins, and inducers all play a role in regulating gene expression in prokaryotes and bacteriophages (Sulavik et al., 1995).

3.6.1 LAC OPERON

The concept of the operon was first introduced in a brief article published in 1960. This led to the development of the "general theory of the operon," which proposed that all genes were regulated through a single-feedback mechanism of repression controlled by operons. Despite this, the concept of the operon remains a significant milestone in the history of molecular biology, even though it has been refined over time (Xie et al., 2003).

An operon is a genetic structure that includes one or more "structural genes" responsible for producing polypeptides, as well as an adjacent "operator-promoter" region that regulates transcriptional activity. The operator and promoter regions are located upstream of the structural genes. This means that an operon is a collection of genes that are closely connected and work in conjunction to produce a variety of enzymes needed for a specific biochemical pathway. The Lac operon is composed of various elements that are briefly outlined below:

3.6.2 STRUCTURAL GENES

The three structural genes z, y, and a make up the lactose operon, which is present in *E. coli*. The ß-galactosidase enzyme, which breaks down lactose into glucose and galactose, is produced by the "z" gene. A permease enzyme is produced by the "y" gene and helps the cell transport lactose. The "a" gene also specifies thiogalactoside transacetylase, which adds an acetyl group to ß-galactoside by transferring it from acetyl co-A. For the metabolism of lactose, all three gene products are necessary. The transcription of these genes

into a single mRNA occurs from a single promoter in a sequential order. The mRNA produced by these genes is known as polycistronic mRNA, and these genes are referred to as structural genes. The signals required for transcription termination are only found in the last cistron of the mRNA (Ganot et al., 2004).

3.6.3 THE OPERATOR REGION

The operator serves as the location where the repressor protein, which is produced by the regulator gene, interacts, and is situated in between the promoter and the structural genes. The repressor protein inhibits RNA polymerase from initiating transcription by binding to the operator. Only when the operator is not bound to the repressor protein, can RNA polymerase bind to the promoter and start synthesizing mRNA.

3.6.4 THE PROMOTER REGION

The promoter gene is a lengthy nucleotide sequence that is uninterrupted and adjacent to the operator gene. Positioned between the operator and regulator genes, the promoter region also consists of palindromic nucleotide sequences that exhibit a two-fold symmetry from a central point. Proteins that have symmetrically arranged subunits recognize these palindromic sequences. The CRP site contains a section with a two-fold symmetry, and this site binds to a protein called CRP (c-AMP receptor protein). The CRP protein is encoded by the CRP gene and is known to bind to a molecule called cAMP, which is found in *E. coli* and other organisms. According to experimental data, a cAMP-CRP complex is formed when the cAMP molecule binds to the CRP gene. This complex is crucial for transcription because it attaches to the promoter and helps RNA polymerase attach to it, which activates the transcription and translation processes. The promoter region, which is upstream of the structural genes and close to the operator area, is where transcription starts. By first attaching to the promoter and then proceeding via the operator and structural genes, the enzyme RNA polymerase catalyzes the transcription of the structural gene into mRNA.

3.6.5 REGULATOR GENE

The regulator gene (1) generates a protein that acts as a repressor. In the absence of lactose (the inducer), the repressor protein attaches to the operator

(o), preventing RNA polymerase from transcribing the three structural genes. On the other hand, when lactose is available, it binds with the repressor protein, causing it to lose its ability to function. This enables the binding of RNA polymerase to the promoter which triggers the initiation of transcription of the adjacent structural genes.

3.6.6 REPRESSOR

Repressor, a protein molecule that can be active or inactive, is a product of the regulator gene. The operator region is where the repressor protein interacts when it is active, suppressing transcription. Contrarily, transcription is unhindered when the repressor protein is inactive.

3.6.7 INDUCER

The repressor is rendered inactive by the binding of the inducer; this results in the prevention of the repressor protein from binding to the operator, allowing RNA polymerase to move forward and transcribe the structural genes. *E. coli* cytoplasm metabolizes a small number of lactose molecules into allolactose, which is a lactose isomer. Inducers that bind to the repressor and trigger the expression of an operon are known as inducers, and operons that are triggered in this way are referred to as inducible operons.

3.7 REGULATION OF LAC OPERON

In an uninduced *E. coli*, the repressor protein binds to the operator and prevents the expression of structural genes. Despite this, *E. coli* does contain a small amount of the enzyme ß-galactosidase. Some lactose molecules progressively enter the cytoplasm over time, where the ß-galactosidase enzyme converts them into allolactose, an inducer. The operator is freed from the repressor protein when an *E. coli* is stimulated because it is bound to the allolactose molecule. This allows RNA polymerase to transcribe the structural genes and produce a polycistronic mRNA. This mRNA produces a permease that speeds up lactose absorption, and many ß-galactosidase molecules in the cytoplasm then break down lactose into glucose and galactose.

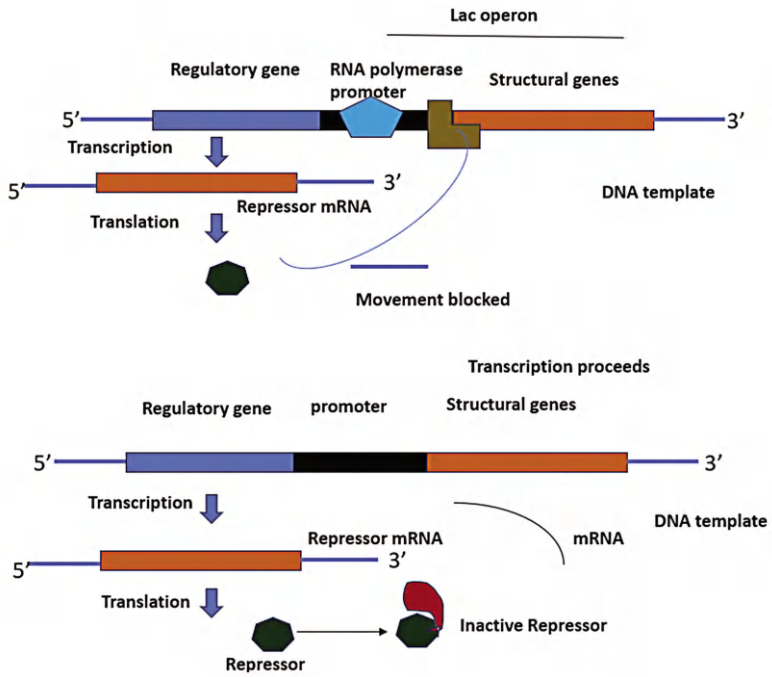


FIGURE 3.3 Lac operon structure and regulation.

3.8 STRUCTURE OF A TYPICAL EUKARYOTIC GENE

The examination of numerous eukaryotic genes has revealed crucial characteristics of their organization. One notable difference, when contrasted with prokaryotic genes, is the remarkable intricacy of eukaryotic genes, especially in more advanced organisms. This complexity frequently encompasses a vast array of disconnected segments that must be put together to form the ultimate mRNA product, along with a complicated set of regulatory sequences that control gene transcription (Black, 2003).

3.8.1 EXONS

Exons are segments of a gene that are present in the functional mRNA, and they may contain both coding and noncoding sequences. The initiation site of transcription is determined by the start of the first exon because the primary transcript's 5' end is not processed. Similarly, the

cleavage site of the primary transcript is determined by the end of the last exon, known as the "polyadenylation site." The polyadenylation site is responsible for generating the 3' end of the mature mRNA. Even though transcription does not end at this location, it carries on for some distance further.

3.8.2 INTRONS

In genes, introns are segments that come in between and are removed during the creation of usable mRNA. Typically, they contain noncoding information, although there are cases where these segments can serve as both introns and exons in alternative processing.

The organization of introns and exons in a gene can vary widely, from a simple arrangement of two exons separated by one intron to a very complex arrangement with many exons forming the final mRNA. The dystrophin gene, which is responsible for Duschenne muscular dystrophy, is an example of a complex gene with at least 70 exons and over one million base pairs of DNA. Some scientists believe that the large size of certain genes may contribute to a higher mutation frequency by providing a larger target for mutations. Although much of the gene's size is made up of intervening sequences, which have no effect on the final protein product, there is a possibility that an actively transcribed region may be more susceptible to mutations.

3.9 CONCLUSION

In conclusion, the structure of typical gene comprises a promoter region, which carries out transcription, and a coding region, which consists of the genetic database for the synthesis of a specific transcript. The coding region is composed of exons, which are the regions that code for the protein, and introns, which do not code for any protein that is spliced during RNA processing. The gene also contains regulatory elements such as enhancers and silencers, which can affect gene expression. Overall, the gene is complex and highly regulated, and variations in this structure can have impressive impacts on an organism's growth and development.

KEYWORDS

- **promoter**
- **exon**
- **intron**
- **transcription start site**
- **enhancer**
- **codon**

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CHAPTER 4

Genome Diversity and Evolution

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ABSTRACT

The genome encompasses the totality of an organism's genetic data, which is essential for its functioning. It is stored within living organisms as chromosomes and large DNA molecules. Genomic diversity refers to the collective set of genetic characteristics that constitute the genetic makeup of a species. It can vary significantly across species and within species and plays a role in the species' survival. Genetic diversity should not be confused with genetic variation, which refers to the propensity of hereditary traits to change. Natural selection acts upon traits that enable populations to adapt to changing environments, utilizing the genetic deviation present in the population. The capacity of a population to adjust to environmental changes depends on the availability of acceptable genetic diversity. A population with more genetic diversity has a higher chance of adapting and surviving, whereas one with less genetic diversity is more susceptible to changes like climate change or the emergence of new illnesses. These evolutionary changes arise from mutations that introduce genomic variations and give rise to individuals with altered biological processes or physical characteristics. Charles Darwin proposed the concept of common ancestry and the ability of species to change over time, resulting in the emergence of new species from existing ones. Each species possesses its own unique set of inherited genetic variations, gradually accumulated over extensive time periods from ancestral predecessors. Darwin's significant works, *On the Origin of Species* (1859) and *The Descent of Man* (1871), highlight his argument that the diversity of

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organisms and their traits can be attributed to natural processes. Speciation, the process of generating new distinct species, occurs through genetic modifications and the development of reproductive barriers between populations. Different types of speciation, such as peripatric, allopatric, and sympatric speciation, contribute to the formation of unique species. Various factors impact the process of evolution, including gene flow (the transfer of genes among populations), genetic drift (the random loss of rare alleles), migration, and natural selection. Mutations occur when a gene in DNA undergoes damage or alteration that changes the genetic information it carries. Mutagens are substances that have the ability to modify the physical properties and genetic information of DNA genes. When a damaged or altered gene is transcribed, the resulting mRNA carries a new message. Natural selection occurs when the environment favors organisms with beneficial features. Beneficial heritable mutations that confer advantages to a species will persist in nature, while unfavorable mutations will eventually be eliminated.

4.1 GENOME

A living cell possesses its complete set of deoxyribonucleic acid (DNA), which is referred to as its genome. In the case of human cells, the genome typically consists of up to three billion base pairs of DNA. Viruses, on the other hand, have ribonucleic acid (RNA) as their genetic material. The genome encompasses all the genetic material of an organism.

4.2 GENE

The terms "gene" and "chromosome" are combined to form the term "genome," as stated in the Oxford Dictionary. However, it was German Botany professor Hans Winkler who first used this phrase in 1920. The word "genome" pertains to the entirety of an individual's genetic makeup within the fields of molecular biology and genetics. Eukaryotes possess a single set of chromosomes that constitute their entire genome. In viruses, the genome is present in either DNA or RNA, while bacteria have their genome located in a single chromosome.

4.3 GENETICS

Genetics involves the examination of genes, genetic variability, and the transmission of traits in organisms (Griffiths et al., 2000; Hartl and Jones, 2005; Retrieved 2018).

4.4 GENOMICS DIVERSITY

4.4.1 GENOMICS DIVERSITY OF MAMMALS

Phylogenetic inference has traditionally been limited to small, tightly constrained sequencing matrices, representing only 2% of the typical mammalian genome. This limitation has hindered our ability to access comprehensive whole-genome sequencing alignments that encompass the full range of mammalian biodiversity. To overcome this sampling bias, we employed an alignment of 241 complete genomes and conducted a thorough analysis of noncoding sequence variations within coalescent and concatenation-based phylogenetic frameworks. We also utilized various types of phylogenetically significant structural variations to validate our findings. By employing this methodology, we were able to construct a reliable time tree for placental animals, assessing age variation at hundreds of genomics loci independent of protein-coding annotations (Foley et al., 2023).

The coalescent and combined phyla inferred from multiple analyses of the data consistently supported more complex taxonomic groups. For example, the evidence pointed to the grouping of primates and colugos with tree shrews (Euarchonta), and the combination of bats, cetartiodactyls, perissodactyls, carnivorans, and pangolins (Scrotifera). This method also revealed patterns such as Feruungulata, which includes all scrotiferans except bats, and the combination of carnivorans and pangolins with perissodactyls (Zooamata). However, it is important to note that these methods assume a single best tree, obscuring phylogenetic conflicts resulting from incomplete lineage sorting and past hybridization. To address this, the researchers deduced phylogenies from tens of thousands of non-coding loci distributed across chromosomes with distinct recombination rates. They observed significant differences between locus trees estimated from autosomes and the X chromosome during the radiation of contemporary orders (including rats, primates, bats, and carnivores), indicating diversification with gene flow. By analyzing the distribution of conflicting evolutionary evidence along chromosomes with varying historical recombination rates, they were often able to resolve previously contentious phylogenetic relationships (Foley et al., 2023).

4.4.2 GENOMIC DIVERSITY OF GREEN PLANTS

Over the past three decades, the rapid advancement of genome sequencing has significantly enhanced our understanding of the remarkable diversity

present in the mitochondrial genomes of green plants. The sizes of angiosperm genomes, for instance, range from several megabases to 20 kilobases in certain green algae. The extent of DNA derived from external sources, such as the plastid, nucleus, and other species, varies, as does the repertoire of genes, including introns, repeats, and RNA editing. Recombination that combines large and small repeats leads to different genomic arrangements within a species, resulting in a loss of synteny between species and making the genome structure dynamic (Mower et al., 2012).

4.4.3 GENOME DIVERSITY OF CHLOROPLAST AND THEIR EVOLUTION

The existence of life on Earth is highly dependent on the crucial role of chloroplasts. This advancement has facilitated the engineering of chloroplast transgenes to enhance plant traits related to agriculture or produce valuable agricultural and biomedical products (Daniell et al., 2016).

Chloroplasts do play a key part in maintaining life on Earth as active metabolic organelles. Their primary function is photosynthesis, which involves releasing oxygen and transferring solar energy into carbohydrates. However, chloroplasts have additional crucial roles in various physiological processes in plants.

One of the key functions of chloroplasts is the production of essential molecules for plant metabolism. They are involved in synthesizing amino acids, nucleic acids, fatty acid, phytohormones, vitamins, and a variety of other metabolites. These molecules are necessary for plant growth, development, and overall functioning.

Chloroplasts also play a role in nutrient assimilation. They are involved in the assimilation of sulfur and nitrogen, which are essential elements for plant nutrition. By incorporating these nutrients into organic compounds, chloroplasts contribute to the overall nutrient balance in plants.

Furthermore, chloroplasts are responsible for producing metabolites that allow plants to interact with their environment and respond to various signals. They help plants adapt to adaptations in factors, i.e., drought, heat, salinity, light conditions, and pathogen invasion. Chloroplasts act as metabolic centers within plant cells, enabling them to synthesize specific compounds and engage in retrograde signaling, which is a communication pathway that allows efficient coordination between chloroplasts and other cellular components.

4.4.4 ADVANCES IN CHLOROPLAST GENOME SEQUENCING

The rapid development of chloroplast genomics can be attributed to advancements in sequencing technologies. Previously, experiments utilized isolated chloroplasts and rolling circle amplification to amplify the entire chloroplast genome (Jansen et al., 2008; Bausher et al., 2006).

Alternatively, chloroplast genome sequences can be used as probes to search bacteria artificial chromosome (BAC) and fosmid libraries. The PCR method encounters difficulties when dealing with species that have extensively rearranged chloroplast genomes or lack relatives with sequenced chloroplast of genomes (Wu et al., 2012; Lin et al., 2015).

The method for the sequencing of plastid genomes has been revolutionized by next-generation sequencing (NGS) methods, which give researchers faster and more affordable options. Initially, the application of NGS in identifying chloroplast genome sequences in Nandina and Platanus was described by Moore and colleagues (Moore et al., 2006). While various NGS platforms exist for chloroplast gene sequencing, Illumina has emerged as the predominant choice due to its compatibility with rolling circle amplification products. The utilization of bioinformatics tools allows for *de novo* assembly, enabling the identification of consensus chloroplast genomes from these assemblies without relying on reference genome sequences (Wu et al., 2012; Pan et al., 2012).

4.4.5 LAND PLANTS GENOME STRUCTURE

Chloroplast genome organization and structure in terrestrial vegetation are mostly maintained. Typically, they are made up of one circular molecule with a quadripartite structure that has a pair of copies and an inverted repeat (IR) portion that divides the huge single-copy (LSC) and small single-copy (SSC) regions. Additionally, the 120–130 chloroplast genomes are mostly engaged in photosynthesis, transcription, and translation processes. Despite the overall structural continuity, the size of chloroplast genomes varies among species and is unrelated to the size of the nuclear genome size ranges from 107 kb through 218 kb in species such Pelargonium and Cathaya argyrophylla, respectively. Extensive changes in structure have been seen in some lineage of land-plant plastid genomes, involving the deletion of entire families of genes and the IR region.

4.4.6 DIVERSITY OF CHLOROPLAST GENOME

Conserved protein-coding sections of the genome of chloroplasts at top taxonomic levels, such as family levels, can be used to undertake phylogenetic research and look into domestication processes (Jansen et al., 2007). Chloroplast DNA sequences were heavily relied on in earlier evolutionary investigations. Although using variable regions or multiple pieces of DNA increased the importance of these analyses, the knowledge contained in these sequences frequently lacked the level of detail required to distinguish between closely associated taxa, particularly when analyzing within-species taxa that have ambiguous taxonomic relationships. In order to understand the relationships between closely associated groups and the general evolution of plant species, it has been extremely helpful to have access to full chloroplast genomes. Chloroplast genomes have useful applications in agriculture as well.

4.4.7 GENOMIC DIVERSITY OF FERNS

Ferns are the second largest species of vascular plants with over 11,000 species extant (PPGI, 2016). Their genomes exhibit distinctive traits. These traits include a high frequency of polyploidy-induced events of speciation (Wood et al., 2009), the existence of numerous chromosomal (Khandelwal, 1990; Clark et al., 2016) comparatively big sizes of genomes (Hidalgo et al., 2017; Pellicer et al., 2018), a beneficial relationship among genome size, chromosomes number, as well as the time required for integration of lengthy ends repeat-retrotransposons (Nakazato et al., 2008; Barker, 2013; Baniaga and Barker, 2019), along with frequent entire genome duplication all over fern evolution (Clark et al., 2016; Huang et al., 2020). The abundance of repetitive components and the occurrence of entire genome duplications repeatedly have been supported by complete and partial sequencing of fern genomes (Wolf et al., 2015; Marchant et al., 2019). Heterosporous ferns, on the other hand, have unusual non-coding DNA compositions and substantially smaller genome sizes (Li et al., 2018).

In addition, ferns display a variety of evolutionary adaptations, such as the capacity to survive in low-light conditions, resilient adaptation strategies, and astonishingly unequal distribution of the diversity of extant species, about the majority (78%) of fern variety centered on three lineages within the family of the Polypodiales (PPGI, 2016). Because of these qualities, ferns are an excellent choice to study the relationship between genetic diversity and lineage diversification.

4.4.8 GENOMICS OF MAIZE

Maize, a staple food grain and one of the oldest cultivated crops, has a long history dating back to ancient times. It is among the top three grains globally and serves as a significant source of both food and alternative energy. The domestication of maize is believed to have begun around 9000 years ago in Central Mexico. Through extensive molecular research and advanced genomic and quantitative genetic techniques, there has been significant progress in understanding the genes of maize. However, the indigenous and germplasm resources are used by maize breeders to develop global varieties with genetic diversity. This highlights the need to explore genomic diversity, connections, and modifications through novel techniques like plant genome sequencing (Pandita et al., 2023).

Genetic diversity allows different crop varieties to adapt to their environments and survive environmental changes. Genetically homogeneous varieties may be more susceptible to abiotic and biotic stressors compared to genetically diverse types. By utilizing genetic diversity, breeders can maintain crossbred variations and preserve desirable qualities, such as quality traits and stress in crops.

PGRs include all genetic material exits in crops, including ornamental, medicinal, and wild relatives of these plants. This genetic data includes plant species' vegetative and reproductive propagation material as well as the alleles of numerous genes. PGRs include a broad range of substances, including recently developed varieties, landraces from cultivated crops, modern varieties, ancient forms, breed inventory, wild forms, invasive forms, as well as genetic exotics such as superior lines, mutants, and breeders' lines. They also include breed stocks, exotic forms, and weedy forms.

4.4.9 BIO-INFORMATICS ABOUT AQUACULTURE SPECIES

Aquaculture, the fastest-growing sector in food production, is poised to become the primary supplier of shellfish and fish for human consumption like crop and livestock production, aquaculture involves a wide range of species. With increasing demand for seafood due to population growth, there is significant potential for genetic modification of production traits. Genomics, aided by advancing sequencing and bioinformatics technology, is increasingly employed to optimize selective breeding for various aquaculture species and stages of domestication. The combination selection of genomics with biotechnological breakthroughs like genome modification and surrogate

brood supply technology holds promise for accelerating aquaculture genetic advancement (Houston et al., 2020).

4.4.10 GENETIC DIVERSITY SUPPORTS TOBACCO AND ALCOHOL USE IN GENETIC DISCOVERY

Alcohol and tobacco use, which pose significantly increased risks for illness and harm, have been identified as heritable behaviors associated with 15% and 5.3% of global fatalities, respectively (Piovani et al., 2022; Santangelo et al., 2022). Despite their widespread use, previous investigations into genome-wide associations have primarily focused on individuals of European ancestry. To investigate the function of loci and genetic information it is necessary to identify genome map loci linked to alcohol and tobacco (Liu et al., 2019)

4.4.11 BACTERIAL GENOME DIVERSITY

One of the biggest plant families, the Leguminosae, has a widespread distribution over many different geographical areas. The main kinds of legumes all have distinct origins that act as breeding grounds for symbiotic bacteria that are "specific" to that species. These non-symbiotic bacteria are connected to these nitrogen-fixing bacteria, such as Rhizobium, Bradyrhizobium, and Azorhizobium. They play an essential part in nitrogen fixation by forming nodule in the root systems or stems of plants. These bacteria are renowned for having a great genetic diversity.(Martínez-Romero et al. 1996).

4.5 GENETIC DIVERSITY

"Genetic" signifies pertaining to characteristics transmitted from one generation to the next.

"Diversity" denotes the presence of a variety of distinct elements.

According to Hughes et al. (2008), the word "genetic diversity" refers to the statistic that quantifies the genetic heterogeneity level within a population.

The full spectrum of genetic traits that make up genetic make-up is referred to as genetic variety. It can affect the species' long-term survival because of its widely varied scope which is determined through the number of species and changes within species (Biological Web Dictionaries, Genetic Diversity,

2019). It needs to be distinct from genetic variation, which is concerned with the propensity of inherited features to change. In a species with abundant genetic diversity, numerous individuals possess a broad spectrum of distinct traits. Maintaining genetic diversity is crucial for a population's ability to adjust to evolving environments.

4.5.1 NEUTRAL AND MODIFICATION OF GENETIC DIVERSITY

Neutral and Modification of hereditary diversity can be distinguished based on their contributions to fitness and adaptation. These genes are not influenced by natural selection (Holderegger et al., 2006). On the other hand, the modification of genetic diversity consists of the genes that enhance healthiness and enable organisms to adapt to environmental changes (Holderegger et al., 2006). Unlike neutral genes, adaptive genes are influenced by natural selection, facilitating evolutionary changes (Holderegger et al., 2006). They tend to evolve at a faster rate compared to neutral genes (McHugh et al., 2013). Species ability to adapt becomes crucial for its survival, particularly in the face of changing environments. Therefore, it is essential for conservation efforts to identify and understand adaptive genetic diversity (Holderegger et al., 2006; Teixeira and Huber, 2020).

4.5.2 IMPORTANCE OF GENETIC DIVERSITY

The existence of different variations in DNA within a species is significantly influenced by genetic diversity (Frankham et al., 2002). Variations in appearance are among the important qualities that are affected by this diversity but may not be immediately apparent to humans (Frankham et al., 2002). These changes are associated with adaptation, the process by which a species adjusts to thrive in its environment. For example, certain apple varieties thrive in hotter climates due to their genetic makeup. The range of traits exhibited by organisms with substantial genetic diversity enhances their ability to effectively respond to environmental changes. A classic example of this can be observed in the case of peppered moths during the industrial revolution (Cook and Saccheri, 2013). This favored their survival and increased their chances of reproducing. The offspring of dark moths inherited the alleles that contributed to their dark coloration, further enhancing their survival prospects. As a result of their increased fitness, the population of dark moths flourished (Cook and Saccheri, 2013).

Genetic diversity is crucial because it provides specific populations, animals, or groups of people with the capacity to adjust to diverse environmental situations. It equips them to withstand the emergence of diseases, epidemics, and the challenges posed by environmental factors. Conversely, limited genetic diversity can make a species more susceptible to biotic and abiotic pressures such as diseases and drought, thereby increasing the risk of extinction over time.

4.5.3 GENETIC DIVERSITY EXAMPLES

Genomic variability is less than increased in asexually reproducing due to the fact that it will be constrained by the gene pool that is available, which in this instance would only be based on one parent.

Genetic diversity is vital in humans. Humans come in a variety of heights, eye colors, hair colors, skin colors, and so on. The diversity of human populations is the key to identification techniques like fingerprinting. When compared to entire human variation, human genetic diversity is notably discovered in isolated populations where population diversity is low.

- Different dog breeds. To obtain the necessary qualities, dogs are selectively bred.
- Many types of wheat, rose flowers, etc.
- In India, there are more than a thousand different species of mangos and more than 50,000 different types of rice.
- The amount of chemical reserpine that the many species of the medicinal plant *Rauvolfia vomitoria* found in various Himalayan regions generate varies.

4.5.4 GENETIC DIVERSITY GENERATED

Mutations refer to alterations in an individual's DNA, which can occur when errors arise during DNA replication, similar to making mistakes while copying a word. These mutations contribute to the genetic diversity within a species. As generations progress, the number of mutations increases due to accumulating errors. However, certain mutations can result in advantageous changes for a species. It is significant to note that the most of mutations are either harmful or have no significant effect. Nonetheless, advantageous mutations can enhance an individual's chances of survival and increase their reproductive success (Frankham et al., 2002).

Adaptability is observed when a child inherits DNA from both parents. During the formation of sperm and eggs, genes in a baby's DNA acquire two copies, one from each parent. These copies, known as alleles, may possess distinct mutations. Recombination and shuffling of alleles occur in each parent, resulting in the presence of only one allele for each gene in each sperm or egg cell. When sperm and eggs combine, the rearranged alleles from the mother and father mix, generating new combinations of alleles in the offspring (Frankham et al., 2002; Emamalipour et al., 2020). This mixing of alleles increases the genetic diversity of a species by allowing for the emergence of novel mutational and phenotypic combinations. Bacteria, for instance, can clone themselves and transfer alleles from an identical clone to their parent (Emamalipour et al., 2020). In this case, the clone inherits all the DNA flaws present in its parent. Interestingly, bacteria can exchange alleles even when they are unrelated to one another. This unique strategy enables basic organisms like bacteria to diversify their genetic makeup without depending on the mixing of parental alleles (Cook and Saccheri, 2013).

4.5.5 SIGNIFICANCE OF GENETIC VARIETY IN THE GENETIC RESOURCES OF PLANTS

Crop improvement relies heavily on genetic variety (Bhandari et al., 2017). Different genetic resources of plants (PGRs) are used by plant breeders to create or improve varieties of crops with desired features. Farmers' choices for high production, quality, and resilience to pests and diseases are all taken into account when developing new cultivars. Because of the intrinsic genetic variability within populations, early humans have historically chosen advantageous genotypes (Govindaraj et al., 2015; Wernberg et al., 2018). Preferences for developing new crop kinds fluctuate as the environment does. To enhance and create current crop varieties, plant breeders use genetic variety through mutation lines that are indigenous species, breeder stocks, and various other sources (Bhandari et al., 2017). For upcoming breeding programs aimed at creating varieties that are climate resilient, it is crucial to preserve unique genes that confer resistance to biotic and abiotic stress. Additionally, preserving the existing germplasm guarantees the survival of vegetative genotypes with desirable features and aesthetically pleasing characteristics. Plant breeders can choose superior genotypes, supplying genetic information for hybridization initiatives and the emergence of new crop varieties, by utilizing genetic variety between and within plant species

(Bhandari et al., 2017). Plants can adapt to various environmental situations by using genetic variety through PGRs (Govindaraj et al., 2015; Wernberg et al., 2018). Additionally, greater genetic variety aids in reducing the harmful consequences of interbreeding on populations. Increased genetic diversity in PGRs supports ecological services, community integrity, and adaptability to environmental changes (Hoban et al., 2021; Hughes et al., 2008). The development of varied agriculture and horticultural crop types is facilitated by including genetically distinct parents in breeding programs (Raffard et al., 2019).

4.5.6 CONSERVATION OF GENETIC DIVERSITY

Plant genetic materials (PGRs) include all of the various gene alleles that make up a crop species' entire genetic makeup as well as that of its wild relatives. These resources are essential as the world's population continues to grow because they play a significant role in boosting agriculture's resilience and profitability. The sustainable maintenance of these varied resources has considerably aided in the conservation of biodiversity. They have been essential in developing new crop types with advantageous characteristics. Novel genes or alleles connected to desired traits are incorporated into commercially cultivated cultivars using biotechnological techniques. As a genetic resource, diversity preservation is essential for the development of novel crop types over the long term. Additionally, improvements in biotechnological techniques like genetic markers, *in vitro* cultivation, cryogenic preservation, and gene banks, as well as next-generation sequencing, assist in proper identification as well as preservation of species that are endangered or rare (Salgotra and Chauhan, 2023).

4.5.7 IN AGRICULTURE

4.5.7.1 IN CROPS

When people first started practicing agriculture, they used selective breeding to keep good features in crops while getting rid of bad ones. Due to selective breeding, this method resulted in the creation of monocultures, which are large areas of virtually genetically similar plants. Such crops are, however, more prone to illness because of the absence of genetic variety, as bacteria are continually adapting and evolving. It is possible for a disease-causing

bacterium to quickly wipe out huge numbers of that species if it evolves to precisely target a single genetic variant. A whole crop can be entirely annihilated if the variety of genes that the bacteria is particularly effective at targeting corresponds to the agricultural diversity carefully chosen by humans for harvesting (An Introduction to Genomic Diversity, Retrieved 2008).

4.5.7.2 IN LIVESTOKE

The genetic variety of livestock species, which offers a variety of habitats and serves various purposes, is essential for facilitating animal husbandry. Cattle herds can respond to climatic changes thanks to it and it serves as the foundation for selective breeding programs (World Food and Agriculture Organisation, 2015). However, breed disappearances and other types of genetic deterioration can also contribute to the decline of livestock biodiversity. According to UN Food and Agriculture Organizations (2015), 7% of the 8774 breeds were already extinct and 17% were near to extinct.

4.6 EVOLUTION

"Evolution is the process through which inherited traits in biological populations undergo change over successive generations."

Over successive generations, heritable traits undergo evolutionary changes as a consequence of the dynamic forces that shape the prevalence of traits within a population. At every level of biological organization, biodiversity emerges as a result of this process of evolution (Hall and Hallgrímsson, 2008; Voet and Pratt, 2016). In the mid-19th century, Charles Darwin and Alfred Russel Wallace separately formulated the evolutionary theory through natural selection, which Darwin's book *On the Origin of Species* (Darwin, 2004). Observable facts about living organisms, such as:

1. The production of more offspring than can survive;
2. Variations in characteristics among individuals in terms of morphology, physiology, and behavior;
3. Differential rates of survival and reproduction associated with different traits (differential fitness);
4. The heritability of traits across generations establishes evolution through natural selection (Lewontin, 1970).

Hence, individuals within a population are more prone to being succeeded in future generations by the progeny of parents possessing advantageous traits. The emergence of the modern synthesis Orthogenesis and other flawed theories of evolution from the beginning of the twentieth century include mutationism. The dependence evolution theory of Darwin on Mendelian variation in genes was demonstrated by this synthesis (Futuyma and Kirkpatrick, 2017).

4.6.1 HISTORY OF EVOLUTIONARY

4.6.1.1 CLASSICAL ANTIQUITY

The hypothesis that one species of life could have descended from another was put forth by early Greek writings for example Anaximander and Empedocles (Kirk et al., 1983). These ideas continued to be discussed during the Roman era, with Empedocles being followed by the Lucretius poet and philosopher in his influential work *On the Nature of Things* (Lucretius) (Sedley, 2003).

4.6.1.2 MIDDLE AGES

Aristotelianism held the belief that all natural things were actualizations of predetermined forms, representing fixed natural possibilities, contrasting with materialistic perspectives (Torrey, 1937; Hull, 1967). Even while Aristotle himself noted that real organisms might not always match up completely with philosophical forms and provided instances of how to unique kinds of organisms might appear, variations of this concept gained widespread acceptance throughout the Middle Ages and were adopted into Christian doctrines (Mason, 1962). Notably, several Arab Muslim philosophers, including Ibn Khaldun, discussed the concept of evolution. In his book *Muqaddimah* from 1377 AD, Ibn Khaldun proposed that humans originated from "the world of the monkeys" through a process in which species become additional multiple (Kiros, 2001).

4.6.1.3 PRE-DARWINIAN

The biological Science of the 17th century challenged the Aristotelian methodology, seeking to elaborate realistic processes through universal physical laws rather than relying on permanent natural types or a higher cosmic order.

However, the field of biology, which held onto the idea of fixed natural types, was slow to adopt this new approach. John Ray, although strictly identifying each living thing as a species and defining them based on perpetuating features across generations, continued to use the term "species," previously associated with fixed natural types, for both plants and animals (Mayr, 1982). In 1735, Carl Linnaeus developed a biological system that acknowledged the hierarchical relationships among species, yet he maintained the belief that all species were predetermined according to a divine plan (Waggoner, 2000). During this era, other naturalists speculated about the evolutionary processes of species based on genuine laws. In 1751, Pierre Louis Maupertuis described how changes occurring during reproduction could accumulate over successive generations, conclusion in the emergence of recent species (Bowler, 2003). Erasmus Darwin suggested that all warm-blooded creatures might have evolved from a sample microorganism, while Georges-Louis Leclerc and Comte de Buffon indicated the possibility of species degenerating into various individuals (Erasmus Darwin, 1731–1802).

4.6.1.4 DARWINIAN REVOLUTION

The notion of consistent biological typological groups or types underwent a fundamental transformation with Charles Darwin and Alfred Wallace's theory of evolution by natural selection, which described changes in populations over time. Rather than using the term "evolution," Darwin utilized the phrase "descent with improvement" (Darwin and Van, 2002). Limited resources result in many offspring not reaching sexual maturity, which elaborates the coexistence of different plants and animals with distinct lineages while still adhering to the same natural principles applicable to all organisms (Sober, 2009; Mayr, 2002; Bowler, 2003; Sokal and Crovello, 1970).

Darwin started working on his "natural selection" idea in 1838 and was about to finish writing a full book about it when Alfred Russel Wallace provided him with an almost identical notion in 1858. At a meeting of the Linnean Society of London in 1858, their individual articles were jointly delivered (Darwin and Wallace, 1858).

4.6.1.5 PANGENESIS AND INHERITANCE

The process of inheritance and the new trait's origin remain a subject of ongoing inquiry. In his pursuit of answers, Darwin put forth his provisional theory of pangenesis (Liu et al., 2009). However, Gregor Mendel's

revolutionary discovery in 1865, which showed the predictable inheritance of features via the separate arrangement known as gene revolutionized our understanding. Darwin's theory of pangenesis was essentially supplanted by Mendel's laws of heredity (Weiling, 1991). August Weismann developed a differentiation between the somatic cells and germ cells, which gave rise to gametes like sperm and egg cells, in order to prove that inheritance only happens through the germ line. Darwin's pangenes, according to Hugo de Vries, were focused in the cell's nucleus and, after being defined, could go to the cytoplasm and affect the shape of cells. This integration of Darwin's Genesis with Weismann's differentiation of embryo and somatic cells was advocated by de Vries, who popularized Mendel's work and believed that heritable changes transmitted through the germline aligned with Mendelian traits (Wright, 1984).

4.6.1.6 MODERN SYNTHESIS

A significant development known as the modern synthesis emerged, integrating Mendelian inheritance-based population genetics with natural selection in the 1920s and 1930s. This synthesis offered a comprehensive framework applicable to various fields within biology. By examining paleontological fossil transitions, it elucidated patterns observed in populations across different species (Bowler, 1989).

4.7 SPECIES

A biological species is characterized as a group of organisms that possess the capability to naturally interbreed and give rise to offspring that are both viable and fertile.

4.7.1 ORIGIN OF SPECIES

The groundbreaking scientific publication *On the Origin of Species* authored by British naturalist Charles Darwin, was issued in England on November 24, 1859. In this influential book, Darwin presented his theory of evolution, which posited that organisms undergo gradual changes through the procedure of "natural selection." According to Darwin's argument, organisms with advantageous genetic variations that are well-suited to their environment have a higher likelihood of producing more offspring, thereby affecting their species' overall genetic make-up.

Darwin's ideas were shaped by the contributions of French naturalist Jean-Baptiste de Lamarck and English economist Thomas Malthus. During his renowned five-year voyage aboard the HMS Beagle in the 1830s, Darwin amassed crucial evidence to support his theory. His explorations of diverse locations, including the Galapagos Islands and New Zealand, provided him with extensive knowledge of various flora, fauna, and geological formations. This wealth of information, combined with subsequent studies on variation and interbreeding conducted in England, played a pivotal role in the modification of Darwin's theory of organic evolution.

While the notion of organic evolution had been proposed earlier by individuals such as Darwin's grandfather, Erasmus Darwin, and Lamarck, it was Charles Darwin who provided a reasonable justification for this sensation. Darwin's idea of realistic selection was formulated by 1844, but he was hesitant to publish it since it was inconsistent with the biblical narrative of creation. Another British naturalist, Alfred Russel Wallace, published an article on his own in 1858 that contained ideas related to Darwin's theory. In 1858, the two researchers presented a joint talk on evolution to the London-based Linnean Society, and Darwin subsequently finished writing his book.

Origin of Species was rapidly out of stock after its November 24 publication. Orthodox Christians viewed the hypothesis as heretical, although the majority of scientists accepted it since it might explain a number of biological riddles.

The release of Darwin's essay "The Descent of Man, and Selection in Relation to Sex" in 1871, which provided proof for the human development of apes, increased the debate surrounding his theories.

Darwin's theory of evolution was widely accepted by the time he passed away in 1882. He was buried in Westminster Abbey alongside other notable personalities from British history as a tribute to his contributions to science. Evolutionary theory has been improved as a result of subsequent discoveries in genetics and molecular science, but Darwin's theories still serve as the basis for the discipline.

4.7.2 EVIDENCE FOR EVOLUTION

4.7.2.1 ANATOMY AND EMBRYOLOGY

Darwin conceptualized evolution as a gradual process known as "descent with modification," where through time, species undergo modifications that give way to new species. He suggested that all species descended from a

single progenitor that existed in the distant past and the evolution's past creates an evolving tree with several levels.. According to this model, closely related species share more recent common ancestors, and they tend to exhibit shared characteristics inherited from their most recent common ancestor. By retracing the shared traits among species, we can employ this concept to discern their interconnections.

4.7.2.2 HOMOLOGOUS FEATURES

If multiple species share similar physical traits, such as complex bone structures or body plans, it suggests that these traits were inherited from a common ancestor. These shared characteristics, resulting from a shared evolutionary history, are referred to as homologous traits.

A well-known example of homology can be observed in the differences in forelimbs among dogs, birds, and whales.

Homologous structures are most evident during embryonic stages. For instance, all embryos of vertebrates, including human embryos, include gill openings and a tail in their early stages of development. These species diverge even more during development, leading to the transformation of the embryonic tail to a tailbone and the gill slits into the mouth and inner ear. These shared embryonic characteristics show that modern vertebrates' developmental strategies are modifications of those of their newest common ancestor.

In rare instances, organisms could have vital organs that resemble those in other living beings but no longer perform their original, ancestral tasks. Vestigial structures are these structures, which are frequently smaller in size. Examples include the human tailbone, whales' hind leg bones, and some snakes' underdeveloped legs.

4.7.2.3 ANALOGOUS FEATURES

Not all resemblances in physical traits can be attributed to shared ancestry, introducing an additional layer of complexity and fascination. Instead, some physical similarities are a result of convergence, where distinct organisms independently evolve similar traits due to similar habitats or shared selective pressures.

An example of this process is observed in the arctic fox and the ptarmigan, a bird species, both inhabiting the Arctic region. Despite being distantly

related, both species alter color with the seasons, going from dark to pristine white. It is improbable that they share an ancestor who possessed this ability to change color with the seasons, indicating that this shared characteristic does not stem from a common ancestry. Rather, both species independently developed this trait as a response to similar selective pressures. In the harsh winters of their environment, where predators rely on keen eyesight, the genetically programmed capacity to shift to lighter pigmentation during winter allowed both foxes and ptarmigans to survive and reproduce.

4.7.2.4 MOLECULAR BIOLOGY

Shared evolutionary ancestry can be revealed not only through structural homologies but also through similarities in biological molecules. At a fundamental level, all living things share certain key characteristics:

- The same genetic makeup, which is composed of DNA.
- Genetic coding that exhibits either identical or highly similar sequences.
- Transcription and translation are the two essential processes of gene expression.
- Common atomic constituents, such as amino acids.

These commonalities demonstrate that all organisms share a typical ancestor that used the hereditary code, DNA as their genomic material, and transcription and translation to describe genes. As a result, all modern organisms possess these traits.

While features like DNA and gene expression mechanisms are crucial for establishing the common origins of life, they may be less informative when it comes to determining the degree of relatedness between different organisms. Instead, molecular traits such as the nucleotide sequences of genes can be employed to identify the closest relatives within a group of organisms.

4.7.2.5 HOMOLOGOUS GENES

Biologists commonly employ gene sequence comparisons in distinct species, known as homologous or orthologous genes, to establish the evolutionary connections between them.

In general, the greater the number of DNA changes in homologous genes between two species, or differences in amino acid patterns of the polypeptide

they encode, the less closely related they are. For example, the insulin proteins in humans and chimpanzees exhibit a higher degree of similarity (around 98% identical) compared to the roughly 64% similar insulin proteins found in chickens and humans. According to this, chickens and humans are more distantly connected than chimps and humans.

4. 7.2.6 BIOGEOGRAPHY

The distribution of life on Earth, when examined in light of tectonic plate movements over geological time, aligns best with the principles of evolution. The breakup of the supercontinent Pangaea around 200 million years ago provides insights into these patterns. Species that existed prior to the split tend to exhibit a global distribution, while those that emerged afterward are typically confined to specific regions.

For example, when Pangaea divided into Laurasia in the north and Gondwana in the south, distinct groups of plants and animals evolved in each hemisphere. The diversity and traits of animals were greatly shaped by this geographic split.

Another example highlighting the interplay of evolution and geography is the evolution of unique species on islands. Australia, for instance, is home to a remarkable variety of marsupials, while placental mammals dominate most other parts of the world. The isolation of Australia from other land masses for millions of years allowed marsupials to evolve independently, occupying various ecological niches. Similarly, endemic species found on islands like the Hawaiian Islands, Galapagos finches, and Australian marsupials have ancestral ties to mainland animals but have undergone distinct evolutionary processes. These island species often descended from mainland ancestors through events such as landmass separation or accidental dispersal, and subsequently diverged as they adapted to the unique island environments.

4.7.2.7 FOSSIL RECORD

Fossils are the preserved remains or traces of organisms from ancient times. However, it is important to note that the fossil record is incomplete and sporadic due to the rarity of fossilization and the limited discoveries made by humans. The skeletal remains that have been gathered, however, offer important insights into the lengthy processes of evolution. Determining the age of fossils presents a challenge. One method involves examining rock

layers, or strata, which accumulate over time. These layers form a chronological sequence, with the uppermost layers representing more recent periods and the lower layers representing ancient times. By examining the position of fossils within the strata at a particular location, scientists can establish a relative timeline. Furthermore, fossils from different sites can be dated by comparing them to "reference" strata that possess distinctive characteristics. Additionally, scientists utilize radiometric dating techniques, which involve measuring the radioactive decay of specific elements, to estimate the age of fossils with reasonable accuracy.

4.8 SPECIATION

The process of speciation, which takes place during evolution, results in the emergence of different species that are unable to reproduce with one another. It entails the creation of isolation for reproduction between populations that had previously interbred. (Burton, 2022).`

4.8.1 ECOLOGICAL SPECIATION

The term "ecological speciation" refers to the scenario where reproductive isolation results from diverse selection. "Nonecological" kinds of speciation include polyploid speciation, speciation by genetic drift, and speciation under uniform selection. The main cause of reproductive isolation is shown to be ecological adaptation, supporting the idea of the "biology for speciation" put forth by Mayr, which includes a variety of mechanisms of speciation (Sobel et al., 2010).

Ecological selection arises from individuals' interactions with their environment while acquiring resources, such as food, attracting pollinators, or avoiding predators. It can also result from interactions with other organisms during these pursuits, such as competition. Divergent selection occurs when contrasting selection pressures act on two populations, including cases of disruptive selection where extreme phenotypes are favored within a single population, as observed in sympatric speciation.

4.8.2 PLANT SPECIATION

Identical to animal speciation, plant speciation includes the development of barriers to genetic exchange among formerly interbreeding populations.

Prezygotic barriers, which prevent interspecies mating or fertilization, are more important in plants than postzygotic barriers, which include selection against hybrid progeny. Prezygotic barriers frequently evolve as a result of evolutionary diversification in adaptation to ecological factors including habitat and pollinators. Numerous reasons, including ecological divergence, regional spread, high fertility, everlasting life history, and self-fertilization or asexual reproduction, may be responsible for this. Last but not least, a variety of biological and geohistorical elements, many of which present ecological potential, are linked to the diversity of plant species (Rieseberg and Willis, 2007).

4.8.3 ECOLOGICAL DIVERGENCE IN PLANTS

Angiosperms, which make up around 90% of all land plant species, are the subject of reviews by Waser and Campbell (2004); Givnish (2010) on the importance of ecology in speciation. It is thought that pollination is essential for ecological diversification in angiosperms. When paired with other factors, changes in floral characteristics & pollination syndromes are thought to be important for reaching reproductive isolation (Kay and Sargent, 2009).

Pollination is critical to plant diversification, according to data from macroevolution. In both general angiosperm groupings (such as figs; Bruun-Lund et al., 2018) and specific land plant lineages (Hernández-Hernández and Wiens, 2020), studies have revealed that lineages using biotic pollinators exhibit greater rates of diversification than those that do not.

4.8.4 TYPES OF SPECIATION

4.8.4.1 SYMPATRIC SPECIATION

The process of sympatric speciation involves the development of reproductive aloneness driven by ecological factors and adaptation to distinct alternative resources or niches. The main driving force for sympatric speciation is the selection against intermediate phenotypes (Nosil, 2012). These intermediate forms are less advantageous because they either receive fewer resources due to density- and frequency-dependent selection or have reduced mating opportunities due to extreme phenotypic preferences (Turner and Burrows, 1995). Disruptive selection has the potential to induce a pattern of reproductive separation among sympatric taxa, which is normally maintained

through assortative mating, or it can result in bimodal distributions of traits (Dieckmann and Doebeli, 1999; Turelli et al., 2001).

In contrast, allopatric speciation occurs when Geographical obstacles totally obstruct the exchange of genes between populations, allowing each to develop independently and eventually arrive at reproductive isolation (Dobzhansky, 1982; Via, 2009). Sympatric speciation, as proposed by Darwin (Darwin, 2004), occurs without geographic obstacles, with one ancestral population giving rise to new species (Mayr, 1974; Coyne and Orr, 1997). If the distribution ranges of two species overlap, sympatric speciation may take place, according to some biogeographic criteria (Mallet et al., 2009). However, any instance in which the spatial organization of progenitor populations affects habitat choice or mating is not regarded as pure sympatric speciation from the perspective of population genetics (Schluter, 2001; Fitzpatrick et al., 2008; Schluter, 2009).

4.8.4.2 ALLOPATRIC SPECIATION

Allopatric speciation occurs when populations are geographically isolated, resulting in no gene flow between them and allowing the single populations to become independent. This geographic isolation is often induced by various barriers such as mountains, rivers, islands, or other factors. While occasional gene flow may occur in some cases, it remains limited, maintaining the process of speciation. Local diseases and other causes may further isolate populations, having effects that are comparable to those of geographical constraints (Ridley, 2009). Since ecological variables, like climatic and niche variations, directly influence speciation by acting as barriers, allopatric speciation may be seen as an example of ecological speciation. In recent decades, allopatry isn't a prerequisite for speciation to occur, although there has been a rising acknowledgment of diversification with gene flow (Feder et al., 2012).

An example illustrating allopatric speciation and adaptive radiation is Darwin's finches. From a single ancestral population that settled on the islands of the Galapagos in South America, about fifteen different kinds of finches have been identified. Because of their geographical separation, each population evolved independently in response to the particular selective stresses each island placed on them. This is evident in the distinct beak shapes of the finches, which vary according to the available food sources on each island and their feeding habits.

4.9 FACTORS AFFECTING OF GENOMIC DIVERSITY AND EVOLUTION

Genomic diversity undergoes changes over time due to various factors. These changes are primarily influenced by gene flow, genetic drift, mutation, and selection. As a result of gradual changes in genome and genotypic rates within the population, both artificial and natural selection play important roles in preferring superior genotypes (Ray et al., 2015). The idea that advantageous genotypes are chosen and passed down to succeeding generations is supported by Darwin's theory of evolution, which was published in 1859 (Acquaah, 2009; Hintum and Jl, 1995; Ellstrand et al., 1999). However, during the process of domestication, desirable genotypes are deliberately chosen by farmers and breeders, while undesirable ones are disregarded. Over several generations, this selection process causes a decline in the frequency of subpar alleles. Throughout the course of evolution, plant species undergo various morphological, physiological, and biochemical changes that can diverge under domestication, depending on the specific plant part utilized. In some cases, plant species may lose their ability to reproduce sexually due to selection for larger tubers or roots, often associated with the preference resulting in sterility for polyploid kinds. During sexual reproduction, some polyploid plant species, including allohexaploid wheat and potatoes, display diploidization behavior. In addition, certain crops have changed from being perennial to being annual. In order to create modern cultivars by natural and artificial selection, wild animals must undergo a full genetic alteration during the domestication process (Ray et al., 2015). Introducing elements against wild plant relatives can prevent some domesticated cultivars from developing diseases and pests that may make them susceptible over time (Salgotra et al., 2021). Throughout the process of domestication, breeders have selected desirable traits based on their preferences (Salgotra et al., 2021; Ross-labarra et al., 2007). However, cultivars of crops with increased gains, stability to biotic and abiotic, wide adaptability, non-shattering qualities, big seed size, early maturation, and positive quality features are typically given priority by plant breeders (Begna, 2020; Rauf et al., 2010).

A population's average fitness may suffer in the present and the future due to genetic variety brought on by processes like mutations, recombination, and gene flow. This negative effect is referred to as "genetic load," and various methods have been used to estimate it in different animal species. It is now possible to assess the genetic load in groups as well as individuals even without using initial measurements of fitness because to recent developments in the sequencing of genomes and computational methods. In this review, we explore various methods to assess genetic limitation utilizing all genome

sequence information based on indicators of evolutionary preservation and annotate both traditional and recent literature on the topic. We also explore the significance of distinguishing the realized load from the masked load of the genetic load. This distinction improves our comprehension of population genetics of harmful mutations and their impacts (Bertorelle et al., 2022).

4.9.1 SELECTION

4.9.1.1 NATURAL SELECTION

Natural selection can be viewed from both a narrow and broad perspective. In a narrow sense, it pertains to the violation of Hardy-Weinberg laws, particularly when the viability or fertility of individuals depends on their genotype. In a broader sense, it is the primary force driving the adaptive and forward-thinking aspect of evolution. A complete theory of evolution would combine ecological processes, developmental processes, and population genetics to understand the diversity of circumstances, the fitness of phenotypes, the influence of genotype on phenotype, and the changes in genetic makeup within populations.

- Selection favors the increase of alleles that provide a fitness advantage, while alleles that are disfavored by selection decrease in frequency.
- The consequences of selection on genetic diversity can be categorized into two aspects: reduction in diversity through directional selection and maintenance of diversity through balancing selection, where heterozygotes have the highest fitness and multiple alleles are favored.

When goats were domesticated, artificial selection for desirable traits led to the emergence of specific alleles, resulting in genetic variation among populations and the establishment of various breeds for different purposes. However, natural selection continues to play a role, albeit potentially with a more subtle and gradual impact over time (Manunza et al., 2023).

4.9.1.2 ARTIFICIAL SELECTION

Artificial selection involves the intentional or unintentional modification of individuals within a population by human intervention, leading to phenotypic evolution in both animals and plants. The domestication and breeding of food crops played an essential role in the advancement of human civilization,

resulting in the urbanization of plant populations and intense selection for agronomic traits governed by specific genes. Studies have identified genes targeted by artificial selection as important regulators of agronomic features in crop varieties. (Vigouroux et al., 2002b; Yamasaki et al., 2005).

Dairy animal breeds have undergone artificial selection over the past 50 years to improve milk production-related traits. Three popular French cattle breeds (Holstein, Normande, and Montbéliarde) were subjected to genomic sequencing to determine the physiological processes and areas impacted by this selection (Flori et al., 2009).

Goldfish breeding for aesthetic purposes can be traced back to ancient Chinese writings, making it one of the earliest fish species subject to artificial selection. Morphologically divergent breeds emerged during the Ming dynasty, indicating the significant impact of artificial selection on goldfish morphology. They are an excellent model for studying fish shape evolution due to their extensive diversity and well-documented history of artificial selection (Ota and Abe, 2016).

The extent to which species can adapt to the current global warming scenario and ensure their persistence is uncertain. The capacity for adaptation among different populations and the limitations that hinder specific adaptations are not well understood. Some evidence suggests that plants in temperate zones have benefited from early flowering as an adaptive response to global warming. A study focused on *Lupinus angustifolius* L. a member of the Fabaceae family, aimed to evaluate its ability to adapt by advancing the onset of flowering. Four populations from different latitudes in Spain were collected, and artificial selection lines were established over three generations to select individuals that flowered earlier. The study found significant advancements in flowering time in the northernmost populations but not in the southern populations (Sacristan-Bajo et al., 2023).

4.9.2 ADAPTATION

Adaptation is the process through which a species becomes better suited to its environment over several generations, as natural selection acts upon heritable variation. Organisms can adapt to their environment in various ways, including changes in their structure, physiology, genetics, locomotion, defense and attack mechanisms, reproduction, and development. The term "adaptation" was coined in the early 17th century to describe the relationship between design and function. In the field of biology, adaptation carries three main meanings. Firstly, it can refer to the physiological adjustment of an animal or plant's response to its immediate environment, such as changing

its metabolism or temperature in reaction to variations in altitude. Secondly, it commonly describes the process of adapting or features of organisms that improve reproductive success in comparison to alternative traits. This process is driven by genetic variations among individuals that become better suited to a specific environmental context, resulting in greater success. One well-known example is the prevalence of throughout the Industrial Revolution in Britain, the pitch black phenotype for the peppered insect (Biston betularia). The black insects blended nicely with the soot-darkened trees, enabling them to evade predation by birds. Adaptation occurs when there is an alteration in gene frequency that confers advantages associated with a selective trait, such as the coloration of moth wings.

4.9.3 MUTATION

Mutations are spontaneous alterations in the nucleotide pattern of DNA that can be inherited and result from anomalies or abnormalities. They are the main sources of genetic interpretation in crop species that affect phenotype. Mutations that result in genetic diversity can have either a beneficial or a neutral effect on a variety of plant species traits. In addition to selection and genetic drift, DNA mutations are the primary reason why allele frequencies have changed within populations. Throughout history, the variation in genes that has improved food security has been mostly a result of natural or accidental mutations (McCouch, 2004).

The primary driver of plant evolution is mutation, especially when plants experience frequent environmental changes. Environmental changes and shifts in demographic locations associated with socioeconomic conditions can accelerate mutation rates. Stress-inducible mutagenesis, triggered by various external inputs, has been observed to accelerate adaptive evolution in plants. Mutagenesis can lead to various genetic changes, such as mobile element movement, deletions, insertions, copy number alterations, and significant chromosomal rearrangements. In the past, the primary means of genetic variation employed by breeders of plants to generate and improve crop varieties was natural mutations. Modern technology has made it easier to induce mutations through mutagenesis, nevertheless. To increase genetic diversity across crop species and improve features like infection and insect pest resistance, abiotic stress tolerance, and nutritious enhancement in crop varieties, mutation breeding was first proposed (Smith, 1989).

The traditional assumption in evolutionary theory has been that With regard to their outcomes, mutations happen at random. However, analysis of plant *Arabidopsis thaliana de novo* mutations has challenged this

assumption. Surprisingly, the study shows that mutations are less common in the genome's functionally restricted regions. Two-thirds of less important genes are present, and there is a 50% decrease in the frequency of mutations inside gene bodies. The study also shows that epigenomic and physical characteristics can account for approximately 90% of diversity in the genome-wide sequence of alteration bias surrounding genes. Patterns of genetic variability in naturally occurring Arabidopsis accessions are reliably predicted by observed mutation frequencies near genes. Additionally, assessments of allele frequencies from the research support the idea that mutational bias is the key factor guiding variations in sequence development around genes in wild accessions. According to the study's findings, genes that are subject to higher selective pressure have a lesser mutation rate, which challenges the notion that change is a random process in evolution (Monroe et al., 2022).

Mutations have the potential to cause the duplication of large segments of a chromosome often through genetic recombination. This process is exemplified by polyketide synthases, large enzymes responsible for producing antibiotics, which contain multiple independent domains, each catalyzing a specific step in the synthesis process, similar to an assembly line.

4.9.4 MIGRATION

- Gene flow refers to the movement of genes among different populations or individuals.
- Migration is becoming more varied and frequent, leading to significant changes in gene frequency.
- The gene frequency change is the dissimilarity in frequency among the receiving population and the average donor population.

Many insect species, including crop pests like *Spodoptera exigua* migrate over long distances, but little is known about the environmental factors that drive their migration patterns (Wang et al., 2011).

- Immigration involves the perpetual transfer of individuals from one population to another.
- Emigration is the transfer of people from one population to another, resulting in a reduction in the gene pool size.

Migration often acts as an evolutionary force, making groups genetically more similar, although in certain cases, natural selection may work in the opposite direction.

For instance, salmon migrate from rivers to the sea for feeding and growth and then return to their birth rivers to spawn.

Migration not only involves individual movement but can also lead to the introduction of new genetic variations (gene flow). Various factors influence the migration of crop species, such as breeding systems, interactions with wild or weedy relatives, pollinators, and seed dispersal.

A population's genetic diversity is directly increased by migration, which improves the population's capacity for environmental adaptation. It also helps in mixing and preventing population divergence.

4.9.5 GENE FLOW

The procedure of genetic transfer that occurs between populations and sometimes between different species is known as gene flow. It can cause a species or population to develop new variants. The migration of individuals between different populations, such as inland and coastal populations of mice or pollen between populations of grasses that have distinct sensitivities to heavy metals, can result in the transfer of genes.

The transfer of genes between species can happen through the formation of hybrid organisms or through a method called horizontal gene transferred. Horizontal gene transfer, which is frequently seen in bacteria, is the convey of genome data from a single individual to another it is not present in progeny. Horizontal gene transfer donates to the development of antibiotics opposition in the medical industry because microbes that obtain resistance genes are able to pass them to other species. Furthermore, it has been demonstrated that bacteria can transmit their genes to eukaryotes like the yeast *Saccharomyces cerevisiae* and the adzuki bean weevil *Callosobruchus Chinensis*. Eukaryotes bdelloid the rotifers additionally acquired a capacity of genes bacteria, plants and fungus through widespread exchanges. By transporting DNA between them, viruses make it possible for genes to be transferred between many types of biological entities.

4.9.6 GENE POOL

The term "gene pool" refers to the collective genetic material of a population at a specific time. It is commonly used when discussing populations composed of individuals related to the similar species, encompassing all genes and combinations genes (alleles) exit within the population.

As a result, the gene pool is modified to better adapt to the specific environmental conditions of the population. For example, when human populations migrated from equatorial regions to colder climates with reduced sunlight exposure, changes in skin pigmentation occurred over time. The skin became lighter in color to facilitate the absorption of vitamin D, which is crucial for proper bone development. The genetic changes responsible for this pigmentation alteration eventually became part of the gene pools of many of these populations.

4.9.7 GENETIC DRIFT

Gene frequencies can undergo changes between generations through a random process named genetic drift. This happens as a result of population having a limited number of individuals, leading to fluctuations in gene frequencies due to chance, similar to how getting more or lesser than 50 "heads" in 100 coin tosses can happen purely by luck.

In both plant and animal breeding, accurately predicting the genetic gain resulting from artificial selection of a specific trait is crucial. The classical breeder's equation (BE), originally developed for artificial selection but applicable to natural selection as well, is commonly used for this purpose. Recent advancements in high-throughput sequencing techniques have enabled breeders to detect both additive and non-additive genetic effects at the individual gene level (He et al., 2023).

Research indicates that the ongoing scale and pace of human impact on landscapes are significantly reducing the adaptive potential of many species. Population subdivision and genetic significance are considered critical factors for long-term evolution. A study focused on koalas presents a rare and pioneering real-time analysis of this process, demonstrating the evaluation of effective mitigation strategies such as species crossings. The study utilized a large genetic dataset from a koala population affected by the construction of a linear transport system, resulting in population separation. The analysis revealed a decrease in effective population size and allelic richness over time, with a potential loss of genetic diversity between 12% and 69% after just 10 generations (Frere et al., 2023).

The founder principle states that whenever an entirely novel population is founded by a small number of people, genetic drift may have large evolutionary repercussions. A tiny percentage of seeds or animals from a species are frequently passively transferred by wind, the fur of bigger organisms, or other means to islands, lakes, and other remote biological locations.

These few colonizers' allelic frequencies are probably different from those of the group they left behind, and these variations have a long-lasting impact on how the new population develops. The founder principle explains why species in adjacent continental areas are frequently less diversified than those on equivalent islands, like the ones in the Hawaiian archipelago.

KEYWORDS

- **genome**
- **genetic diversity**
- **evolution**
- **evidence of evolution**
- **Darwin's theory**
- **the origin of species**
- **speciation**

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CHAPTER 5

Recombination and Evolution

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ABSTRACT

Recombination is the mechanism through which DNA fragments break and recombine, resulting in the appearance of novel varieties of alleles. This method fosters genetic diversity at the genetic level, reflecting variations in DNA patterns among different organisms. RNA recombination is a crucial factor in genetic changes associated with the emergence of RNA viruses. Genomic recombination significantly presents the development of entirely new lineages, the development of host tropism, environmental changes, virulence, and disease. It encompasses both intra- and interchromosomal recombination. During sexual reproduction, the interaction of genomic data between different chromatids of homologous chromosomes, understood as a chromosomal crossover, shows the formation of recombinant chromosomes. Gene change appears when one DNA sequence replaces a homologous sequence, resulting in its identical nature. Recombination often exhibits sex dimorphism in both sex chromosomes and autosomes. Viral recombination takes place when viruses from distinct parent strains contaminate the identical host tissue and combine their genetic material during replication, giving rise to progeny viruses that inherit genes from both parents. The process of evolution causes changes in the genetic makeup of populations over time. Genetic diversity and alterations in allele frequencies are necessary for evolutionary processes to occur. Prezygotic and postzygotic barriers act as obstacles, separating species from each other and preventing copulation or the presentation of possible, productive progeny. Speciation is the proceeding

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through which unique species emerge, driven by the divergence and genetic isolation of populations within a species, resulting in reproductive barriers between them. Genetic drift, gene flow, migration, mutation, and selection are some of the factors that influence species evolution.

5.1 RECOMBINATION

"Recombination is the method of interchanging nucleotide sequences either within or between genomes" (Posada et al., 2002).

It is a process where fragments of DNA are broken and recombined, resulting in the creation of new combinations of alleles. This mechanism of recombination generates genetic diversity at the gene level, showcasing variations in DNA sequences among different organisms. Typically occurring during meiosis in eukaryotic cells and cells with a nucleus and organelles, recombination occurs when gametes (egg and sperm cells) are formed through cell division. In the initial stage of meiosis, homologous pairs of chromosomes from both parents align. Chromosome arms may transiently touch and fuse during this alignment, leading to a crossover event.

5.2 TYPES OF RECOMBINATION

Living organisms have been found to exhibit a minimum of four distinct types of naturally occurring recombination.

5.2.1 HOMOLOGOUS RECOMBINATION

The process of homologous recombination is one where a donor sequence replaces a corresponding region in the acceptor DNA molecule, either homologous (similar) or homologous (similar but not identical) (Lemey and Posada, 2009). In numerous organisms, including yeast, humans, and DSBs, homology recombination (HR) is a tightly controlled biochemical mechanism necessary to maintain genomic integrity. Because it relies on sister chromatids and highly conserved molecular associates for DSB repair and is error-free, HR differs from other techniques such as non-homologous end-joining (NHEJ) and microhomology-mediated end-joining (MMEJ) (Lieber, 2010; Wang and Xu, 2017).

5.2.2 ILLEGITIMATE OR NON-HOMOLOGOUS RECOMBINATION

In regions with limited sequence similarity, illegitimate or non-homologous recombination becomes apparent, such as conversions between further chromosomes or deletions that eliminate multiple the genes found on a chromosome. Nevertheless, upon analyzing, the short regions of similar sequences are seen in the DNA pattern at the boundaries of these events occasionally discovered. Throughout the majority of the cell cycle, non-homologous DNA endjoining (NHEJ) functions as the main rebuilding process for any kind of DNA double-strand break (DSB) and is necessary for the formation of antigen receptors. Ionizing radiation sensitivity and lymphocyte loss are caused by NHEJ deficiencies. All the following steps of NHEJ depend on synapsis, which is the integration of both of the DNA ends of a DSB. Synopsis, like the final processing phase, appears to be done by a variety of mechanisms, according to recent research. The decision between NHEJ and alternative DSB repair pathways is discussed in the opening section of this review. After that, it incorporates current knowledge of the mechanisms behind NHEJ synapsis and provides updates on other NHEJ steps such as DNA end processing (Zhao et al., 2020).

5.2.3 SITE-SPECIFIC RECOMBINATION

Site-specific recombination occurs when definite short patterns, typically around 12 to 24 base pairs in length, are involved in recombination between otherwise dissimilar parental molecules. This type of recombination relies on dedicated enzymatic machinery, with each specific site requiring its own enzyme or enzyme system. Notable examples of site-specific recombination include the integration of certain bacteriophages, a restructuring of antibody genes in vertebrate animals, as well as the introduction of genes, such as lambda and bacterial chromosome.

5.2.4 REPLICATION RECOMBINATION

The third type of recombination is replication recombination, which results in the creation of a fresh copy of a DNA segment. Many transportable elements use replication recombination to create a duplicate of themselves at a new location.

5.3 TYPES OF RNA RECOMBINATION

5.3.1 TYPE I (HOMOLOGOUS) RECOMBINATION

This kind of recombination happens when two RNA molecules with a lot of sequence homology or similarity to one another come together. Joining occurs at flawless locations on the two RNA molecules, producing recombination RNAs with the same pattern and anatomical array of the original RNA molecules. As a result, the term "homologous" denotes crossover events that occur at places that are similar or homologous to the two molecules, in addition to the presence of homology in sequence among the two parental RNAs. Sequence homology is necessary at both surrounding and within the crossing sites, but it need not be present there specifically. When whole viral genomes are involved in RNA recombination, such as in picornavirus recombination, this sort of recombination is frequently seen.

5.3.2 TYPE II (ABERRANT HOMOLOGOUS) RECOMBINATION

Like I-type recombination, II-type recombination occurs when two molecules of RNA with the same pattern come together. Contrary to I-type recombination, II-type recombination crossovers take place at unrelated locations. In this kind of recombination, there is no clear reason for the choice of crossover locations. There is typically no pattern likeness near the joining position of the two molecules of RNA in aberrant homologous reintegration. Only RNA reintegration can take place in this manner. Despite the two RNA molecules in question sharing a sequence, aberrant homologous recombination sometimes serves as the only source of recombination in genetic crosses. When faulty RNAs are used in the recombination process, this sort of recombination occurs more frequently than usual.

5.3.3 TYPE III (NON-HOMOLOGOUS OR ILLEGITIMATE) RECOMBINATION

When there is no pattern likeness between two molecules of RNA, type-III recombination occurs. As a result, it is unclear what mechanism underlies the choice of recombination sites. The joining regions on the two RNAs may have identical subordinate structures. In contrast to homologous recombination, this kind of recombination happens more repeatedly in DNA

recombination than it does in RNA recombination. Gene insertions, deletions, and rearrangements that are seen in RNA viruses may be caused by type-III recombination.

5.4 GENOMIC RECOMBINATION

Genetic recombination, sometimes referred to as genomic reorganization, is the interchange of genomic data across several species, producing offspring that have a distinctive combination of features that are different from the ones of their parents. Meiosis in eukaryotes involves genetic recombination, which can result in the development of a novel genetic structure that can be passed down to the next generations. Generally speaking, there are two types of natural recombination:

5.4.1 INTERCHROMOSOMAL RECOMBINATION

It involves the independent assortment of alleles located on different but homologous chromosomes during meiosis I, and

5.4.2 INTRACHROMOSOMAL RECOMBINATION

Occurs through a process known as crossing over (Daly and Minton, 1995).

5.4.3 GENETIC RECOMBINATION IN EUKARYOTES

Genetic recombination happens when homologous chromosomes link together during meiosis in eukaryotes. This makes it possible for the chromosomes to communicate genetic data. As an alternative, strands of DNA can be severed and then joined to form new DNA molecules.

In eukaryotes, recombination can also take place during mitosis, frequently including both of the sister chromosomes created following genomic repetition. Under this situation, novel alleles no alternatives are made because the twin genomes are frequently similar. Mitosis as well as meiosis involve homologous sequence recombination, which is the mixing together of DNA molecules with comparable structural characteristics. Chromosomes that are not sisters couple up during meiosis, causing recombination among non-sister homologs.

5.5 IMPORTANCE OF RECOMBINATION

Recombination is necessary for maintaining the stability of the genetic material as it passes from one generation to the next and across somatic cell divisions. It must cooperate with other activities taking place on the template for DNA, such as transcription, replication, and the special functions played by chromosomes at the centromeres and telomeres. It is necessary to coordinate the nucleus's spatial and temporal activity appropriately and to time it to the cell cycle stage. Regulatory mechanisms, including posttranslational modifications (PTMs) that directly affect recombination proteins, as well as chromatin changes that impact the DNA substrate, are involved. While the primary goal of recombination is to maintain genomic stability, it can also influence copy-number variations and overall chromosomal structures. Therefore, quality control mechanisms are necessary throughout the recombination process to ensure high fidelity and prevent genomic instability. Recombination has significant implications for human diseases, particularly cancer and potentially neurological disorders, through its regulatory mechanisms (Heyer, 2015).

5.5.1 MECHANISM

Several unique enzymes help to enable the recombination of genetic material. Recombination's strand transfer step is catalyzed by recombinases, a key class of enzymes. RecA, the main reconnecting enzyme in Escherichia coli, is crucial for mending breaks in the double-strand (DSBs). DSB repair is carried out by two recombinases in yeast as well as other eukaryotic species. The RAD51 protein is necessary for both meiotic as well as mitotic reintegration, while DMC1 is solely needed for meiotic recombination. The bacterial RecA protein's archaeal equivalent is called RadA.

5.5.2 BACTERIAL RECOMBINATION

Recombination, which involves the transfer of genetic material, frequently takes place in bacterial systems. However, failed or abortive genetic material transfer, often known as non-productive transfer, can also occur. This refers to the shift of bacterial DNA from the giver cell to the receiver cell where the coming DNA integrates into their genetic makeup but does not result in a successful transfer. Failed transfers have been observed following

transduction and conjugation. Dilution of the transferred fragment often occurs during culture growth (Rieger et al., 2012).

5.5.3 CHROMOSOMAL CROSSOVER

Chromosome crossover plays a crucial role in promoting recombination during meiosis in eukaryotes. This process can occasionally lead to the creation of novel chimeric alleles and results in offspring possessing gene combinations that are distinct from those of their parents. Genetic recombination facilitates the movement of genes, contributing to increased genetic diversity.

Chromosomal crossover occurs when the paired chromosomes inherited from each parent undergo recombination, typically during meiosis. In prophase I at the pachytene stage, the four chromatids align closely, allowing homologous regions of two chromatids to exchange genetic material (Alberts, 2002).

5.6 GENE CONVERSION

- Among the methods of alike recombination, gene conversion makes it easier for genetic material to be transferred unidirectionally from a sequence known as the "donor" to one that is extremely homologous, or the "acceptor."
- Gene conversion can happen during mitosis but typically happens during meiosis.
- Gene conversion processes in mammalian cells often result in small gene-conversion sequences, rarely longer than 1 kb.
- In particular, selection has an impact on how gene reformation affects the multigene family's evolution.
- Over 25,000 recombination geographical areas have been found in the genome of human, and both non-allelic and allelic hotspots exhibit high gene-conversion activity often.
- It is possible to more correctly forecast the exact positions of unstable genomic areas by improving our knowledge of how gene conversion affects the creation or eradication of exchange hotspots in the human genome.
- According to Chen et al. (2007), several human genetic illnesses have gene conversion as one of their contributing factors.

5.7 GENETIC ENGINEERING IN PLANTS

Utilizing genetic engineering to improve plant capacities is essential given the problems posed by the effects of climate change population increase. Farming and molecular farming are both hampered by the labor-intensive nature of present methods and their restriction to a few species. As a result, new methods and equipment are required to increase the variety of species that can be transformed as well as the effectiveness of those transformations. While microbe and animal systems have been transformed by nanotechnology, its use in plants has not yet reached its full potential. However, reports of direct germline editing, plastid and genome of mitochondria modification, as well as nano-mediated delivery for manipulation of genes in plants are beginning to emerge (Squire et al., 2023).

5.8 SAFETY CONSIDERATIONS IN PLANT GENETIC ENGINEERING

In the past, plant breeding depended on the plants' inherent genetic flexibility to produce better types. Early farmers used environmental changes to increase the frequency of desirable qualities in a species by selectively breeding plants with those traits. Expected agronomic impacts are the main foundation for regulatory requirements and safety evaluation studies for transgenic plants. Reliable safety evaluation methods depend on the presence and stable representation of implanted genetic information in subsequent transgenic generations. Humans have substantially altered the genomes of beneficial plants by selective breeding for at over 10,000 years, giving the offspring notable differences from their natural parents. Breeding standards established by the crop’s intended application, the environment in which it may grow, the quantity needed, and the economics were the driving forces behind these adjustments (Käppeli and Aberson, 1998)

5.9 DNA RECOMBINATION

In order to facilitate the transmission of genetic information between chromosomes, repair broken double-strands of (DSBs), and incorporate foreign DNA, DNA recombination activities are essential for meiosis. Repair of DSB and foreign DNA insertion take place in vegetative cells and are thought to be managed by a similar reintegration technology, whereas interchromosomal exchange only occurs in meiotic cells (Mengiste and Paszkowski, 1999; Vergunt and Hooykaas, 1999). For cells to survive, DSBs must be repaired effectively. This can be done either through homologous

recombination (HR), which uses a homologous DNA template for accurate repair, or through non-homologous mechanisms, which ligate DNA ends without requiring homology and result in illegitimate recombination (IR).

Although the enzymes essential to these activities have not changed throughout time, their relative activity changes greatly amongst contrasting individuals. Multicellular eukaryotes generally rely on IR for DSB repair, whereas bacteria and single-celled eukaryotes, such as yeast, mostly use HR (Mengiste and Paszkowski, 1999; Vergunt and Hooykaas, 1999).

As the common DSB reform process also controls the insertion of foreign DNA, this discrepancy has significant consequences for the use of genetics in crop plants. Contrary to IR, which frequently leads to accurate insertion at the identical place of host genetic material and permits gene targeting (GT), HR allows precise absorption at the transgene's random integration site with predictable copy counts. According to Vergunst and Hooykaas (1999; Kumar and Fladung (2001), GT is an important method in functional genomes for decoding unidentified gene functions.

5.9.1 MEIOTIC RECOMBINATION

For homologous chromosomes to be properly segregated during meiosis, crossover recombination is essential. Meiotic recombination is an inherent process started by designed DNA double-strand breaks (DSBs), unlike spontaneous mitotic recombination. The development of a minimum of one crossover per homolog is ensured by meiosis-specific mechanisms, which counterbalance the core recombine machinery's propensity to use sister-chromatid templates and yield noncrossover results (Hunter, 2007)

Chromosome segregation depends on recombination during meiosis because it creates physical links between homologous chromosomes that enable them to align properly on the spindle and separate correctly during the first division (Petronczki et al., 2003).

Recombination failure frequently results in chromosome disjunction failure, which has detrimental effects on gamete development. Meiotic recombination depends on the generation and consequent repair of DSBs, which are caused by the Spo11 protein. DSB generation in Saccharomyces cerevisiae needs the existence of at least nine other proteins, despite Spo11's high degree of conservation. It is yet unclear how exactly these elements play a role. Recombination and the advancement through meiotic prophase are synchronized, ensuring that particular stages in the recombination cycle take place at the correct moment. For example, DSB formation only happens following local DNA replication (Keeney, 2001).

5.10 THE EVOLUTION OF SEX DIMORPHISM IN RECOMBINATION

Male and female meiosis differ in a number of significant ways. Males create four little motile gametes (spermatozoa) while females only make one big gamete from the four meiotic products. Meiosis timing varies greatly between male and female cells, with male meiosis occurring continuously and female meiosis often halting twice. Males and females can experience different levels of genetic recombination after meiosis due to changes in the frequency and location of crossing over.

The first feature, the emergence of heterogamy, has acquired substantial transcendent study. Less consideration has been given to gender-specific recombination differences, particularly in formal models. Recombination dimorphism can happen on the autosomes or the sex chromosomes. Recombination on autosomes may be entirely missing in one sex (achiasmy) or quantitatively different between sexes (heterochiasmy).

5.10.1 ACHIASMY

It is also common, although has gained low observation, to have reintegration dimorphism on the somatic chromosomes, notably achiasmy. One sex in achiasmy appears to be completely devoid of recombination. This is different from the meiosis loss that is frequently seen during parthenogenesis. In species where parthenogenesis is uncommon or unknown, achiasmy develops.

The "Haldane-Huxley rule" declares that in dioecious species, achiasmy predominately takes place in organisms having different sex chromosomes. There are, however, some exceptions, such as specific Musca and Culex species.

Pleiotropy and the no-recombination hypotheses can both be used to explain the origin of achiasmy. Both hypotheses have drawbacks and restrictions on how they might explain the patterns of achiasmy that have been observed.

The pleiotropy hypothesis falls short of explaining the extreme pleiotropic effects, the fact that hermaphrodites only have one gender, and that achiasmy evolved in a group of organisms with a XX/XO sex-determination system.

5.10.2 HETEROCHIASMY

The measurement of heterochiasmy is difficult. Chiasma counts make up the majority of obtained data, which frequently ignore the location of crossing across chromosomes.

Studies employing distance maps between a few points are made more challenging by the difference in chiasma position across sexes. The amount of recombination that appears to occur more frequently in one sex than the other may not actually exist, depending on where markers are located along chromosomes. The analysis of heteroplasmy data has become more challenging as molecular approaches have shown additional variance in recombination frequency between chromosomes and genotypes (Korol and Preigel, 1994).

It is challenging to detect the presence of phylogenetic inertia and establish the number of species or species groups required for hypothesis testing when analyzing heteroplasmy data because of the unidentified value rate of heteroplasmy evolution, for example.

Both Trivers (1988) and Burt et al. (1991) examined chiasma count data and discovered significant gender variations. Although these data need to be further examined, recent map data is likely to confirm their analyses.

Whether they are hermaphrodite or dioecious, about 75% of chiasmata species have recombination rates that differ by more than 5% between the meiosis of the sexes, as determined by chiasma count or map length. In uttermost circumstances, in female meiosis reintegration can be up to three times greater (as in the case of the zebrafish *Danio rerio* and the planarian Schmidtea polychrome) or about 1.5 times lower (as in the case of the monecious species *Pinus taeda*).

According to Trivers (1988), heterogamety does not have a significant impact on heterochiasmy in dioecious species because it tends to demonstrate a tendency toward reduced recombination in male meiosis.

5.11 NEUTRAL HYPOTHESIS

In-depth theoretical research on the evolution of mean recombination rates has been done (as summarized by Barton and Charlesworth, 1991; Otto and Lenormand, 2002), and a few experiments (e.g., Korol and Iliadi, 1994) have shown that it has the ability to evolve. However, the evolution of different recombination rates in males and females has neither theoretical nor empirical basis. Burt et al. (1991) hypothesized that heteroplasmy might be neutral when they failed to discover a relationship between the degree of variation in heteroplasmy and the chance for gender distinctions in selection, which, in their view, must be elevated in bisexual creatures, between in asexual vegetation and relatively small

in hermaphroditic animals. It is crucial to remember that the absence of evidence for a certain hypothesis does not automatically suggest neutrality, especially when that hypothesis lacks a solid theoretical basis and supporting data.

5.12 EVOLUTIONARY EXPLANATION AND SEXUAL SELECTION

Trivers (1988) suggested that sexual selection might lead to heterochiasmy. He proposed that male genes and a combination of genes would, on a typical basis, be better than those handed on by females because of the greater variation in reproductive outcomes among males. According to Trivers, this clarifies that males have lower rates of recombination, with variations being explained by modifications to the system of sexual selection. However, this proposal lacks a theoretical basis for autosomes and is mostly dependent on models of the evolution of sex chromosomes (Nei, 1969). A competing linguistic model has also been put out, which contends that recombination rates should be higher for the sex that has undergone more intensive selection or has a bigger reproductive variation (Burt et al., 1991).

5.13 RECOMBINATION IN VIRUSES

Recombination is a widely observed procedure that generates diversity in the majority of viruses. It involves the fusion of independently emerging variants in a similar viral molecule, opening up novel possibilities for viruses to control selective pressures, adapt to different environments, and adjust to new hosts. As a result, clinical researchers as well as epidemiologists, molecular biologists, and evolutionary biologists are interested in studying viral recombination.

5.13.1 MECHANISM OF RECOMBINATION IN VIRUSES

The molecular procedure of genomic recombination involves crossing over two strands of nucleic acid to create new genomic combinations. We may differentiate between intra-genomic recombination—which involves pieces from the similar genetic unit—and inter-genomic recombination—which involves pieces from other origins—based on the origin of the parental

strands. The regulation of the expression of genes is linked to intra-genomic recombination (Johansonn, 2013). In particular, this is shown in herpesviruses like human Herpesvirus-1 (HSV-1), one of the most well-investigated DNA viruses. To create genetic variety and preserve chromosomal integrity, inter-genomic recombination is an approach. Because it produces new variations with distinct biological characteristics, this type of recombination is particularly pertinent for RNA as well as DNA viruses. This section will concentrate on the process of recombination of inter-genomics.

5.13.2 RECOMBINATION MECHANISMS IN DNA AND RNA VIRUSES

Although just a few have undergone in-depth analysis, many recombination mechanisms have been found in both RNA and DNA viruses. This section will provide comprehensive information on the various methods of recombination, using the HSV-1 virus example of a DNA virus, influenza as an example of an RNA virus, and HIV as an example of a retrovirus, respectively. Since -Herpesviruses are significant models for understanding eukaryotic DNA replication, their example of a DNA virus is particularly well-characterized (Weller and Coen, 2012). The first proof of HSV-1 recombination was discovered by Wildy's research in 1955 utilizing two sensitive-to-temperature HSV-1 mutants. There are two generally recognized recombination methods for RNA viruses: replicative and non-replicative. Replicative and non-replicative recombination are two widely recognized recombination processes in RNA viruses. In contrast to the non-replicative model, which calls for an earlier break in the RNA molecule to enable the development of hybrid genomes, the replicative model entails the exchange of the complex for replication from one RNA template to another (Galli and Bukh, 2014).

5.13.2.1 RECOMBINATION IN DNA VIRUSES

Herpesviruses and bacteriophages both use concatemers, two component recombinases, and proteins in cells in their mechanistic models. This proposes a prehistoric mechanism in which DNA replication and recombination are linked (Weller and Sawitzke, 2014). Additionally, recombination protein and replication of the common replicative complex shared by all Herpesviruses are highly conserved (Rennekamp and Lieberman., 2010). Numerous of these proteins are interchangeable with other Herpesviruses, showing how one virus can replicate a different one (Sarisky and Hayward, 1996). Recombination is essential for DNA damage repair in DNA viruses,

particularly during the latency phase stage. Wilkinson and Weller (2004). However, discrepancies in a repair-initiating sequence of DNA among—as well as—Herpesviruses have been discovered by the investigation of the full genomes of different herpesviruses, revealing potential variances in the recombination processes within these subfamilies (Brown, 2014)

5.13.2.2 RECOMBINATION IN RNA VIRUSES

The main subgroups of RNA viruses have dramatically different recombination rates. While (+) ssRNA viruses frequently have high recombination rates, (–) ssRNA viruses typically have low recombination rates. There are significant outliers to this overall tendency, though. Reassortments are common in Orthomyxoviridae, including the human influenza A virus, which is a member of the (–) ssRNA virus family, while recombination is uncommon in flaviviruses. In contrast to flaviviruses, which have low recombination rates (Taucher et al., 2010), influenza A virus recombination rates in natural populations are ten times greater than thought previously (Marshall et al., 2013).

Recombinant genomes can be produced in RNA viruses via two distinct processes. Recombination stricto sensu happens in nearly all RNA viruses, whereas reassortment only happens in segmented RNA viruses. Recombination particularly happens when two nucleotide sequences interact in a way that allows genetic information to be exchanged between them, creating hybrid RNA sequences. A well-known example of this process is the HIV-1 virus (Galetto and Negroni, 2005). Reassortment happens when at least two viruses infect the same host cell at the same time. This exchange of discrete RNA segments occurs when segments from various origins are packaged into some offspring viruses. Influenza A typical illustration of reassortment is a virus' antigenic change (Nelson et al., 2008).

5.14 EVOLUTION

"Evolution is the process through which inherited traits in biological populations change over successive generations."

Over successive generations, heritable traits undergo evolutionary changes as a consequence of the dynamic forces that shape the prevalence of traits within a population. At every level of biological organization, biodiversity emerges as a result of this process of evolution (Hall and Hallgrímsson, 2008; Voet and Pratt, 2016). The theory of evolution by natural selection was independently developed in the middle of the 19th century by the scientists

Charles Darwin with Alfred Russel Wallace, and Darwin went into great detail about it in the publication of *On the Origin of Species* (Darwin, 2004). Observable facts about living organisms, such as:

1. The production of more offspring than can survive;
2. Variations in characteristics among individuals in terms of morphology, physiology, and behavior;
3. Differential rates of survival and reproduction associated with different traits (differential fitness);
4. The heritability of traits across generations, establish evolution through natural selection (Lewontin, 1970).

Hence, individuals within a population are more prone to being succeeded in future generations by the progeny of parents possessing advantageous traits Alternative theories of evolution like mutationism and orthogenesis were disproved by the development of modern science in the early 20th century. The dependence of evolutionary theory on the Mendelian genetic variation was demonstrated by this synthesis (Futuyma and Kirkpatrick, 2017).

5.14.1 HISTORY OF EVOLUTIONARY

5.14.1.1 CLASSICAL ANTIQUITY

Ancient pre-Socratic Greek thinkers like Anaximander and Empedocles proposed the idea that one species of life may have originated through another (Kirk et al., 1983). In his most important work, "De rerum natura" (On the Natural Order of Things), writer and philosopher Lucretius followed Empedocles in advancing these concepts during the Roman era (Sedley, 2003).

5.14.1.2 MIDDLE AGES

Aristotelianism held the belief that all natural things were actualizations of predetermined forms, representing fixed natural possibilities, contrasting with materialistic perspectives (Torrey, 1937; Hull, 1967). Although Aristotle acknowledged that real organisms might not always exactly match metaphysical forms and gave examples of novel forms of organisms that might appear, variations of this idea spread during the Middle Ages and were established in Christian teachings (Mason, 1962). Ibn Khaldun was one of the notable Arab Muslim philosophers to discuss evolution. Ibn Khaldun suggested that humanity descended of the "universe of the monkeys" by a

process called evolution through which "species became more numerous" in his work "Muqaddimah" from 1377 AD (Kiros, 2001).

5.14.1.3 PRE-DARWINIAN

Instead of depending on established natural categories or a greater cosmic order, the "New Science" of the late 16th century sought to elaborate natural events through universal physical rules. The field of biology, which held onto the idea of fixed natural types, was slow to adopt this new approach. John Ray, although strictly identifying each living thing as a species and defining them based on perpetuating features across generations, continued to use the term "species," previously associated with fixed natural types, for both plants and animals (Mayr, 1982). In 1735, Carl Linnaeus developed a biological system that acknowledged the hierarchical relationships among species, yet he maintained the belief that all species were predetermined according to a divine plan (Waggoner, 2000). During this era, other naturalists speculated about the evolutionary processes of species based on the law of nature. Pierre Louis Maupertuis first explained in 1751 how alterations that happen during reproduction can build up over several generations, leading to the formation of novel species (Bowler, 2003). Erasmus Darwin, who lived from 1731 to 1802, believed that all creatures that were warm-blooded might have descended from a single microbe, but Georges-Louis Leclerc and Comte de Buffon proposed that species might have developed into various organisms.

5.14.1.4 DARWINIAN REVOLUTION

When the scientists Charles Darwin and Alfred Wallace proposed a theory of evolving by the process of natural selection, which explained changes in population over time, the idea of enduring typological categories and types in science experienced a profound alteration. In place of the word "evolution," Darwin adopted the term "descent through alteration" (Darwin & Van, 2002). Limited resources result in many offspring not reaching sexual maturity, which elaborates the coexistence of different plants and animals with distinct lineages while still adhering to the same natural principles applicable to all organisms (Sober, 2009; Mayr, 2002; Bowler, 2003; Sokal and Crovello, 1970).

Darwin started working on his "natural selection" idea in 1838 and was about to finish writing a full book about it at the time Alfred Russel Wallace

provided him with an almost identical notion in 1858. In 1858, they jointly presented their articles at a meeting of the Linnean Association of London (Darwin and Wallace, 1858).

5.14.1.5 PANGENESIS AND HEREDITY

Research into the mechanisms of reproductive heredity and the generation of novel features is still underway. Darwin proposed his tentative idea of pangenesis in his search for solutions (Liu et al., 2009). However, Gregor Mendel's revolutionary discovery in 1865, which showed the predictable inheritance of features by means of the independent assortment & segregation of elements—later known as genes—revolutionized our understanding. Darwin's theory of pangenesis was essentially supplanted by Mendel's laws of heredity (Weiling, 1991).

August Weismann developed the differentiation among somatic cells and germ cells, which gave birth to gametes like egg cells and sperm, in order to prove that inheritance can only occur through the germ line. Darwin's pangenes, according to Hugo de Vries, were concentrated in the cell's nucleus and, after being expressed, could go to the cytoplasmic and affect the shape of cells. De Vries, who popularized Mendel's studies and who believed that heritable alterations transferred through the germline coincided with Mendelian features, advocated this fusion of the Darwinian pangenesis and Weismann's separation of germ cells and somatic cells (Wright, 1984).

5.14.1.6 THE MODERN SYNTHESIS

Between 1920 and 1930, a significant development known as the modern synthesis emerged, integrating Mendelian inheritance-based population genetics with natural selection. This synthesis offered a comprehensive framework applicable to various fields within biology. By examining paleontological fossil transitions, it elucidated patterns observed in populations across different species (Bowler, 1989).

5.15 SPECIES

A biological species is characterized as a group of organisms that possess the capability to naturally interbreed and give rise to offspring that are both viable and fertile.

5.15.1 ORIGIN OF SPECIES

The groundbreaking scientific publication *On the Origin of Species using Natural Selection*, authored by British naturalist Charles Darwin, was published in England on November 24, 1859. In this influential book, Darwin presented his theory of evolution, which posited that organisms undergo gradual changes through the process of "natural selection." According to Darwin's argument, organisms with advantageous genetic variations that are well-suited to their environment have a higher likelihood of producing more offspring, thereby influencing the overall genetic composition of their species.

Darwin's ideas were shaped by the contributions of French naturalist Jean-Baptiste de Lamarck and English economist Thomas Malthus. During his renowned five-year voyage aboard the HMS Beagle in the 1830s, Darwin amassed crucial evidence to support his theory. His explorations of diverse locations, including the Galapagos Islands and New Zealand, provided him with extensive knowledge of various flora, fauna, and geological formations. This wealth of information, combined with subsequent studies on variation and interbreeding conducted in England, played a pivotal role in the development of Darwin's theory of organic evolution.

While the notion of organic evolution had been proposed earlier by individuals such as Darwin's grandfather, Erasmus Darwin, and Lamarck, it was Charles Darwin who provided a practical explanation for this phenomenon. By 1844, Darwin had formulated his theory of natural selection, but he hesitated to make it public due to its contradiction with the biblical account of creation. In 1858, Alfred Russel Wallace, another British naturalist, independently published a paper summarizing ideas similar to Darwin's theory. The two scientists delivered a joint lecture on evolution before the Linnean Society of London in 1858, and Darwin subsequently prepared his book for publication.

Upon its release on November 24, 1859, *Origin of Species* quickly sold out. While most scientists embraced the theory for its ability to solve various biological mysteries, orthodox Christians regarded it as heretical. The controversy surrounding Darwin's ideas intensified with the publication of his work *The Descent of Man, and Selection about Sex* in 1871, which presented evidence for human evolution from apes.

5.15.2 EVIDENCE FOR EVOLUTION

5.15.2.1 ANATOMY AND EMBRYOLOGY

Darwin conceptualized evolution as a gradual process known as "descent with modification," where through time, species undergo modifications that

give way to new species. He suggested that all species share a common ancestor that existed in the distant past and the evolution's past creates an evolving tree with several levels. According to this model, closely related species share more recent common ancestors, and they tend to exhibit shared characteristics inherited from their most recent common ancestor. By retracing the shared traits among species, we can employ this concept to discern their interconnections.

5.15.2.2 HOMOLOGOUS FEATURES

If multiple species share similar physical traits, such as complex bone structures or body plans, it suggests that these traits were inherited from a common ancestor. These shared characteristics, resulting from a shared evolutionary history, are referred to as homologous traits.

A well-known example of homology can be observed in the differences in forelimbs among dogs, birds, and whales.

Homologous structures are most evident during embryonic stages. For instance, in the early stages of development, all vertebrate embryos, including human embryos, possess gill slits and a tail. In some cases, organisms may possess structures that are similar to crucial structures in other animals but no longer serve their original ancestral functions

5.15.2.3 ANALOGOUS FEATURES

Not all resemblances in physical traits can be attributed to shared ancestry, introducing an additional layer of complexity and fascination. Instead, some physical similarities are a result of convergence, where distinct organisms independently evolve similar traits due to similar habitats or shared selective pressures.

Arctic foxes and the bird species ptarmigan, both of which are found in the Arctic region, serve as examples of this process. Despite being distantly related, both species undergo seasonal color changes, transitioning from dark to snowy white. It is unlikely that their common ancestor possessed this ability to change color with the seasons, indicating that this shared characteristic does not stem from a common ancestry. Rather, both species independently developed this trait as a response to similar selective pressures. In the harsh winters of their environment, where predators rely on keen eyesight, the genetically programmed capacity to shift to lighter pigmentation during winter allowed both foxes and ptarmigans to survive and reproduce.

5.15.2.4 MOLECULAR BIOLOGY

Shared evolutionary ancestry can be revealed not only through structural homologous but also through similarities in biological molecules. Fundamentally speaking, all living things have a few things in common:

- They all have DNA as part of their genetic composition.
- Genetic coding that exhibits either identical or highly similar sequences.
- Transcription and translation, are the two essential processes of gene expression.
- Common atomic constituents, such as amino acids.

These similarities show that every living thing descended from a single ancestor who used the genetic code, used DNA as its genetic material, and expressed genes through transcription and translation. As a result, all modern organisms possess these traits.

While features like DNA and gene expression mechanisms are crucial for establishing the common origins of life, they may be less informative when it comes to determining the degree of relatedness between different organisms. Instead, molecular traits such as the nucleotide sequences of genes can be employed to identify the closest relatives within a group of organisms.

5.15.2.5 HOMOLOGOUS GENES

The term "homologous" or "orthologous" genes refers to gene sequences that are similar in two or more species and are frequently used by biologists to determine the evolutionary relationships between them. In general, two species are more distantly related if there are fewer DNA alterations in their homologous genes than between them. For example, the insulin proteins in humans and chimpanzees exhibit a higher degree of similarity (around 98% identical)

5.15.2.6 BIOGEOGRAPHY

The distribution of life on Earth, when examined in light of tectonic plate movements over geological time, aligns best with the principles of evolution. The breakup of the supercontinent Pangaea around 200 million years ago provides insights into these patterns. Species that existed before the split tend to exhibit a global distribution, while those that emerged afterward are typically confined to specific regions.

For instance, different plant and animal species developed in each hemisphere as a result of Pangaea's division into Laurasia and Gondwana in the north and south. This geographic distance significantly influenced the diversity and traits of animals.

5.15.2.7 FOSSIL RECORD

Fossils are the preserved remains or traces of organisms from ancient times. However, it is important to note that the fossil record is incomplete and sporadic due to the rarity of fossilization and the limited discoveries made by humans. Nonetheless, the fossils that have been collected provide valuable insights into the long-term processes of evolution.

By examining the position of fossils within the strata at a particular location, scientists can establish a relative timeline. Furthermore, fossils from different sites can be dated by comparing them to "reference" strata that possess distinctive characteristics. Additionally, scientists utilize radiometric dating techniques, which involve measuring the radioactive decay of specific elements, to estimate the age of fossils with reasonable accuracy

5.16 SPECIATION

The process of speciation, which takes place during evolution, results in the emergence of different species that are unable to reproduce with one another. It entails the creation of isolation for reproduction between populations that had previously interbred. (Burton, 2022).

5.16.1 ECOLOGICAL SPECIATION

The term "ecological speciation" refers to the scenario where divergent selection leads to reproductive isolation. Speciation under uniform selection, polyploid speciation, and speciation by genetic drift is classified as "nonecological" forms of speciation. The main cause of reproductive isolation is shown to be an ecological adaptation, supporting the idea of the "biology for speciation" put forth by Mayr, which includes a variety of mechanisms of speciation (Sobel et al., 2010).

Ecological selection arises from individuals' interactions with their environment while acquiring resources, such as food, attracting pollinators, or

avoiding predators. It can also result from interactions with other organisms during these pursuits, such as competition. Divergent selection occurs when contrasting selection pressures act on two populations, including cases of disruptive selection where extreme phenotypes are favored within a single population, as observed in sympatric speciation.

5.16.2 PLANT SPECIATION

Identical to animal speciation, plant speciation includes the development of barriers to genetic exchange among formerly interbreeding populations. Prezygotic barriers, which prevent interspecies mating or fertilization, are more important in plants than postzygotic barriers, which include selection against hybrid progeny. Numerous reasons, including ecological divergence, regional spread, high fertility, everlasting life history and self-fertilization, or asexual reproduction, may be responsible for this. Last but not least, a variety of biological and geohistorical elements, many of which present ecological potential, are linked to the diversity of plant species (Rieseberg and Willis, 2007).

5.16.3 ECOLOGICAL DIVERGENCE IN PLANTS

Angiosperms, which make up around 90% of all land plant species, are the subject of reviews by Waser and Campbell (2004); Givnish (2010) on the importance of ecology in speciation. It is thought that pollination is essential for ecological diversification in angiosperms. When paired with other factors, changes in floral characteristics and pollination syndromes are thought to be important for reaching reproductive isolation (Kay and Sargent, 2009). Pollination is critical to plant diversification, according to data from macroevolution. Whether among land-based plants (Hernández-Hernández as well as Wiens, 2020) and in particular angiosperm groups (such as figs; Bruun-Lund et al., 2018), studies have demonstrated that lineages using biotic pollinators display greater amounts of diversification than those that do not.

5.16.4 TYPES OF SPECIATION

5.16.4.1 SYMPATRIC SPECIATION

The process of sympatric speciation involves the development of reproductive isolation driven by ecological factors and adaptation to distinct alternative resources or niches. The main driving force for sympatric speciation is the selection against intermediate phenotypes (Nosil, 2012). In contrast,

allopatric speciation occurs when geographic barriers completely prevent gene flow between populations, allowing them to evolve independently and eventually leading to reproductive isolation (Dobzhansky, 1982; Via, 2009). Sympatric speciation, as proposed by Darwin (Darwin, 2004), occurs without geographic barriers, with new species arising from a single ancestral population (Mayr, 1974; Coyne, 1992).

5.16.4.2 ALLOPATRIC SPECIATION

Allopatric speciation occurs when populations are geographically isolated, resulting in no gene flow between them and allowing the isolated populations to evolve independently. This geographic isolation is often caused by various barriers such as mountains, rivers, islands, or other factors. While occasional gene flow may occur in some cases, it remains limited, maintaining the process of speciation. In recent decades, there has been a growing recognition of speciation with gene flow, where allopatry is not strictly necessary for the speciation process to occur (Feder et al., 2012).

An example illustrating allopatric speciation and adaptive radiation is Darwin's finches. Due to geographic isolation, each population underwent independent evolution under the selective pressures imposed by the unique conditions of each island. This is evident in the distinct beak shapes of the finches, which vary according to the available food sources on each island and the feeding habits.

5.17 FACTORS AFFECTING RECOMBINATION AND EVOLUTION

Following evolutionary forces deviate this equilibrium

- Selection (natural and artificial)
- Migration and gene flow
- Mutation
- Genetic drift

5.17.1 SELECTION

5.17.1.1 NATURAL SELECTION

Both a limited and comprehensive view of natural choice may be taken. When seen narrowly, it is unmistakably one of the elegances of defying the Hardy-Weinberg Laws, specifically when viability or fertility depends on the genotype. According to a broad definition, it is the primary force that

drives evolution's inventive and forward-thinking component, adaptation. Ecological processes, which determine the diversity of circumstances and the fitness of phenotypes, developmental processes, which determine the influence of genotype on phenotype, and population genetics, which inform us of changes in genetic makeup in a population while the fitness of the genotypes are known, could all be combined to form a complete theory of evolution, one that no longer exists.

- Alleles that are favored by selection increase frequency and vice versa.

The consequences of selection on variety are:

- Directivity, so variety is reduced.
- Balanced for diversity. Heterozygotes have the highest suitability such that selection favors maintenance of multiple alleles.

When goats were domesticated, artificial selection for desirable features was found, which tended to result in some alleles wanting specific properties. This shaped genetic variation among populations. Due to this, numerous breeds that were generated and raised for a variety of purposes were established and given birth. (Manunza et al., 2023)

5.17.1.2 ARTIFICIAL SELECTION

Artificial selection is the steps of modifying individuals in a population accidentally or on purpose by human intervention, resulting in the phenotypic evolution of both animals and plants. Urbanization of food crops derived from populations of hectic ancestors and advancement via the breeding of crops were important changes for plants in the rise of human civilization. As a result, crop varieties underwent intense selection at the genes governing key agronomic properties. As a result, it is reasonable to support that genes identified as the objects of artificial selection are crucial genetic regulators of agronomic features (Vigouroux et al., 2002; Yamasaki et al., 2005).

Over the past 50 years, dairy animal breeds have supported various artificial selections to improve features related to milk production (Flori et al., 2009).

Goldfish breeding for aesthetic reasons can be traced back to Chinese writings from the Song dynasty, making it the most ancient fish species to be subject to artificial selection. During the Ming dynasty, many breeds emerged that are morphologically divergent, indicating that artificial selection has had a significant impact on the development of goldfish morphology. Goldfish are a wonderful model for studying the evolution of fish shape because of their extensive diversity and obvious history of artificial selection (Ota and Abe, 2016).

It is unknown if evolutionary adaptation will organize sharply enough to ensure the persistence of many species in the current atmosphere of global warming. In particular, we lack the understanding regarding the capacity for adaptation of the various populations about the many limitations that can impede specific adaptations. There is evidence that plants in temperate zones often benefited from early flowering as an adaptive mechanism. Therefore, the objective of this study was to assess *Lupinus angustifolius L.*s capacity to adapt in order to accelerate the commencement of blooming (Fabaceae). A public garden in Spain was used to grow the seeds from four populations that were obtained from two different latitudes. By choosing twenty-five percent of the individuals who flowered sooner in the first generation, three distinct early blooming selection lines were created across three generations utilizing both self-crosses and outcrosses. The artificial selection lines in the populations' furthest north considerably accelerated their flowering relative to the baseline line, but not in the populations' furthest south (Sacristan-Bajo et al., 2023).

5.17.2 MIGRATION

- It is the individual movement or introduction of genes within different populations.
- Migration is becoming more diverse, with rates because it is large, and the frequency changes significantly.
- The change in gene frequency is the frequency difference between the beneficiary population and the average donor population.

Many insect species, especially pests of herbivorous crops, frequently migrate. Spodoptera exigua, a polyphagous armyworm, migrates over great distances, although little is known about its underlying environmental factors. In this study, we used population genetics to identify the movement patterns of *S. exigua* across several Asian countries (Wang et al., 2022),

Immigration is the transfer of people into one population from another permanently.

Emigration is the transfer of people from one population to another. The size of the gene pool is diminished.

It is typical for migration to act as an evolutionary pressure, causing groups to become more genetically similar. Under certain situations, herbal choice will be working in the contrary direction, and it is miles genuinely of hobby to peer to what extent one pressure will be triumphant over the other.

From a genetic point of view, migration does not just mean individual migration however, this move may lead to new alleles (gene flow). Movement

causes changes in gene frequency because more copies of an existing allele are introduced, or this is because new alleles are added.

Several factors affect the migration of crop species:

- Breeding system
- Compassion for wild and/or weedy relatives
- Pollinators
- Seed dispersal

A direct effect of migration increases the population's genetic diversity thus helping to increase the resilience of this population to tolerate environmental changes. Migration also helps mix and prevent population deviation.

Consider a population where the native's frequency is 1–*m* and there are m new immigrants every generation. Let *q*0 stand for the frequency of the gene in the native population and *qm* for the frequency of the gene in the immigrant population. In the mixed population, the frequency will then be:

$$q1 = qm+(1-m)\ q0$$

$$q1 = m(qm-q0) + q0$$

Migration causes a change in gene frequency that can be measured by alleles the frequency of alleles before and after migration.

$$\Delta q = q1-q0$$

$$\Delta q = m(qm-q0)$$

As a result, both the velocity of migration and the differential in allele frequency of allele among the immigrant and native populations affect the change caused by migration. A population may acquire new alleles at a rate and a greater number of loci than would be anticipated from mutation due to migration or gene flow (Merrick et al., 2023)

The genome-wide frequency of allele data is increasingly being utilized to calculate the estimated amount of migration of processes, or edges (*m*), on population trees using the software Treemix. The number of corners that should be included is not obvious. The second degree of variation in probability (m) over subsequent values of m can be used to determine the *m* value that is optimal. When utilized for the purpose it was intended for, which was to evaluate the total amount of population groups (*K*) represented by the software's structure, simulation populations that m perform exactly in addition to the current Treemix recommendations (Fitak, 2021).

5.17.3 MUTATION

It is crucial to recognize that Monroe et al. (2022) made several false claims concerning the mutation and selection of the population. For instance, they claimed that "the widespread fact that genetic variants are discovered less frequently in functionally limited parts of the genome is assumed to be owing exclusively to selection after random mutation." It may lead to the occurrence of new alleles, so it changes the gene pool of a population.

The concept that mutations occur randomly regarding their impact has dominated evolutionary theory since the first half of the 20th century. Here, we utilize massive *de novo* mutation surveys Arabidopsis thaliana plant to test this presumption. In parts of the genome that are functionally constrained, we discover that the frequency of mutations is reduced by two-thirds in important genes and by half inside gene bodies, in contrast to expectations. Using various genomic mutation datasets, including those from the biggest Arabidopsis mutation accumulation experiment, they show that epigenomic and physical variables explain for over 90% of the diversity in the genome-arrangement of mutation bias surrounding genes.

Researching the reaction of mutation rate to population environments aids in our understanding population of destiny and dynamics in novel contexts since mutation is the basis of evolutionary material and because organisms constantly meet environmental changes. Mutation rates can change and adapt. For instance, it has been discovered that several stressful situations, including extreme hunger, antibiotic exposure, and high population densities, increase the plastically enhanced mutation rates of bacteria. Moreover, hypermutator alleles that boost spontaneous mutation rates—can become fixed in microbial populations that have evolved in challenging conditions (Galhardo et al., 2007; Liu and Zang, 2019; Long et al., 2016; Summaa et al., 2006; Weilgoss et al., 2013). For example, at a faster mutation rate during about 2400 generations in a glucose-limited medium (Good et al., 2017).

Our understanding of a long-lasting genome is that changes in genomes are malleable and adaptable to environmental modifications. A growing body of research demonstrates that a range of environmental pressures cause genetic instability in human cancer cells, yeast, and bacteria, leading to the sporadic appearance of mutants that are fitter and possibly hastening adaptive evolution (Wielgoss et al., 2013).

A distinct purpose for mutation exists. Since it causes arbitrary changes to the genetic code, it is in a way a harmful force. Such alterations are overwhelmingly likely to be harmful in any specially engineered organism.

Making arbitrary alterations to a well-crafted watch or making arbitrary changes are the standard analogies we use in such situations. While random changes occasionally improve the accuracy of the watch or the impact of the poem, given more chances, one is more likely to make things worse. Comparably, migration can also cause organisms to be relocated into settings that are not suitable for them.

5.17.4 GENETIC DRIFT

Genetic drift is the modification in population frequency of a pre-existing gene variant carried on by random chance. Gene divergences may disappear due to genetic drift, hence decreasing genetic variation. Also, it might show the substantially greater frequency and maybe fixation of previously rare alleles. Founder effect and bottleneck are two terms referring to various processes by which a small population develops reproductive isolation, the two basic types of genetic drift.

The most popular breed of dog both internationally and in Brazil is the German Shepherd. Understanding the history of the Brazilian German Shepherd dog breed needs a knowledge of the population structure through pedigree records. The Brazilian German Shepherd dog breed's genetic variety and population network existed evaluated research. A total of 77,938 animals born between 1970 and 2014 were featured in the pedigree. In this population, there were 3.91 generations on average each year. About 2183 founders were revealed when the reference population was factored in. During 2010 and 2014, the native population lost nearly 3% of its genetic diversity, mainly on account of genetic drift (Bignardi and Santana Júnior, 2023).

In the breeding of plants and animals, it is important to predict the genetic gain that will arise from the artificial selection of a trait. Lush's classical breeder's equation (BE), which could also be exploited to evolve under natural selection, is usually used for this purpose. Breeders may be able to detect both the additive and non-additive genetic impacts at the individual gene level owing to the recent use of high-throughput sequencing techniques (He et al., 2023).

According to research, the majority of species that live in human-impacted landscapes are losing their ability to adapt due to the magnitude and velocity at which this is now occurring. Long-term evolution is thought to benefit from the genetic relevance of population subdivision. Here, we present a unique as well as with our understanding, the first real-time examination of

this process to show how to assess mitigating efforts like specie crossings effectively. They were able to do this by utilizing a sizable genetic dataset gathered from a group of koalas that had been split apart while a linear transportation system was being developed. First, we found that the effective number of individuals as well as allelic richness both declined over time. This research also demonstrated that, after just 10 generations of separation, the two populations might have a genetic diversity decreased between 12 and 69% (Frère et al., 2023).

KEYWORDS

- **recombination**
- **types of recombination**
- **types of RNA recombination**
- **genomic recombination**
- **genetic recombination in eukaryotes**
- **importance of recombination**
- **gene conversion**

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CHAPTER 6

Mutations, Mutagens, and Types

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ABSTRACT

Since mutations are the random and heritable changes in any organism's genetic architecture, they play a pivotal role in the process of evolution and diversification of species. Mutants that are more suited to their environment—natural or artificial—have a selective advantage. Mutation can be of autonomous or induced and wild species has been greatly aided by mutation in their domestication. Induced mutations using different kinds of mutagens resulted in development of thousands of new mutant varieties in several crop plants which are being used as food, feed, fodder, and fiber. Now, with the aid of modern biological techniques, it is possible to induce site-directed mutagenesis that ultimately reduced the time-consuming process of induction of random mutations, identification, and screening. *In vitro* culture techniques, next-generation sequencing techniques, and phenotypic screening methods enhanced the accuracy of generation of desired mutants with specific characteristics. This chapter looks into the current state of mutation breeding for crop enhancement and evaluates its potential future paths.

6.1 INTRODUCTION

The viability of global agriculture is imperiled by rising human populations, dwindling arable land, and shifting climatic trends. In order to solve these

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issues and quickly produce unique germplasm, plant mutant breeding is a key strategy. Three key components make up plant mutation breeding: (1) the selection and application of mutagens, (2) the growth and progress of appropriate mutant lines, and (3) the documentation and collection of specific mutants. Because of the randomness of the procedure and the paucity of desirable mutants with lower mutational background loads, checking for preferred mutants has been a significant problem for induced mutagenesis in plants. For screening of the mutants responsive against abiotic stress and with enhanced nutritional features, new phenotypic and genotypic approaches have been devised.

By 2050, the population of the world is expected to touch 9 billion people. In order to boost crop production and meet the growing demand for food, either more farmed land must be planted or better crop plant varieties with higher yields must be used. Abiotic, biotic, and recently shifting climatic conditions all pose a danger to the dilemma of food security. Acidification, drought, temperature fluctuations (heat and cold), nutritional shortages, and mineral deficiencies are examples of abiotic stress factors. They seem to be the main factors limiting crop plant growth, which has an effect on yield (Roy et al., 2011). The proportion of farmed land and plant growth and productivity both are significantly impacted with varying climatic conditions, which leads to a loss in total food output with a variety of agricultural produce (Islam et al., 2012). As a result, the sustainability of global agriculture is threatened by rising human population and declining arable land availability.

Using hybridization, crossover, and recurrent selection processes, traditional breeding integrates advantageous new combinations of genes into superior genotypes in order to strengthen crop plants. In order to deal with food safety and sustainability in agriculture, extra methods are needed, such as mutant breeding and genetic engineering, since conventional breeding frequently fails to produce the desired outcomes. Genetic diversity within a species is necessary for this to happen. With less genetic diversity, however, there are fewer cultivated or improved cultivars available. Genetic vulnerability increases as a result of breeding gene pools losing genetic diversity (Duvick, 1984).

Plant breeding program, now, includes the use of both mutant detection and mutant induction method, and the achievement has been documented in numerous crop species today. Worldwide adoption of mutation breeding has resulted in the introduction of over 3,200 mutant cultivars in more than 200 different crop species. The efficiency of inducing mutations and the effectiveness of selecting desirable mutants, however, are the two main constraints in plant mutation breeding.

A quick method of creating novel and useful genetic material for crop development is provided by plant mutation breeding. It does have some limits, though. The usage of radioactive gamma rays is currently subject to strict laws, which makes the development of new physical mutagens necessary. The creation of uncommon mutations in a genetic background that is largely unaltered is necessary for physical mutagenesis to succeed. Significant mutant populations must be maintained, and proper screening must be established to detect significant mutant plants. The creation of mutations by using X-ray as a substitute for gamma rays, new techniques for creating acceptable mutant populations, and new techniques for high-throughput screening are the possible measures to ease the process of physical mutagenesis.

Among the various strategies in plant biotechnology, mutation breeding/induced mutagenesis is one of them which supports crop improvement by producing new varieties or cultivars of numbers of crop plants. New gene alleles can be created through a process called mutation induction, and crucially, this can be done in superior modern breeding stock. Induction of mutation in plants is therefore a type of plant breeding with extra pace and advantage (Forster et al., 2015).

Induction of mutation has been used in plants since 1921 with the goals of increasing plant production, including yield, resistance to biotic and abiotic stressors, and nutritive value for agricultural plants. The genes implicated in several systems, including response to stress, proteomic, and metabolomic adaptations, have recently been found using mutation genetics. The identification of gene sequences has made it possible to analyze the genetic variability of natural germplasm and to overcome the "phenotype gap," or the divergence of genotype and phenotype. Various mutants in different crop plants have been developed for use in feed, food, fiber, industrial products, and pharmaceuticals. Induction of mutations has been shown to be potent in increasing hereditary diversity in different species.

The initial definition of "mutation" was given by de Vries (1901, 1903, 1905) and was encapsulated as unanticipated transmissible variations in an organism's hereditary make-up irrespective of recombination or segregation (Forster and Shu, 2012). Mutations can increase genetic diversity in living things and can be either spontaneous (natural) or purposefully generated. Numerous species of plants have been domesticated in crops thanks to spontaneous mutant features, with profound ramifications for humanity. Several major crops are produced under conditions that are away from the place of their progenitors' centers of origin originated because mutations provide the genetic variety that enabled plants adaptability to new surroundings.

Significant agricultural crop species can therefore grow in a broad variety of latitudes, longitudes, and altitudes, all of which have unique physical (light, climate, water, and soil) and biological (diseases and pests) characteristics. Crop plants underwent adaptive mutant trait selection during their development, but because effective natural mutants only exist at extremely low occurrences, improvement became sluggish (Maluszynski et al., 1995).

6.2 MUTATION AND ITS TYPE

Although there are many reasons for mutation, they can generally be categorized as spontaneous (natural) or manmade (induced).

6.2.1 SPONTANEOUS MUTATIONS

Spontaneous mutations are those ones that occur automatically in nature without any anthropogenic activity. Their causes or origins are frequently obscure. Plant propagation methods (alterations to meiotic or mitotic molecular processes), atmospheric physical factors (daylight, solar radiation, UV irradiation, environmental radioactivity), as well as chemical (particularly alkalyting agents) and biological materials can all cause mutations. The wide range of diversity found in wild life on the earth is the result of spontaneously inherited mutations. Defects in replication and repair of DNA, deamination, and the migration of mobile genetic components are some of the processes that fix the mutations in the genome sequences of the plants (van Harten, 1998; Ellegren, 2004). Realistic figures of the rate of natural automatic mutations in plants in sexually reproduced species are now possible due to genomic technologies, with nuclear genomes of multicellular organisms showing a rate of autonomous mutations in the range of 10^{-6} (Lynch, 2010).

6.2.2 INDUCED MUTATIONS

Even though, they have been successful in producing disparity in a number of characters of a species or variety, autonomous mutations are sternly constrained due to the truncated occurrence and uncommon rate in pertinent genotype. By causing mutations at a rate far higher than what can be seen in nature, Muller (1930, 1927), respectively, offered method to overcome this restriction. According to recent studies, using a mutagen may result in 103 times as many mutants being produced as by naturally occurring mutations, dramatically increasing the mutation frequency (van Harten, 1998).

In *Arabidopsis thaliana*, the frequency of spontaneous mutations for 30 successive generation was 7×10^{-9}, however, after treatment with EMS, mostly A:T<G:C conversion alterations were found after each 300 kbs in the another group (Greene et al., 2003; Ossowski et al., 2010).

Plant tissues are subjected to chemical or physical treatments, such as sodium azide, ethylmethane sulphonate, diethyl sulfate, gamma rays, ion beam, and fast neutrons, in order to induce mutations. Stadler (1929) performed X-ray irradiation on barley and maize to create the first effective purposeful mutation for genetic improvement (Stadler, 1928a, 1928b). DNA was not recognized as the genetic substance until the late 1920s. This would happen many years later. The process of diverse physical and chemical agents' ability to induce mutation was therefore not known for several years.

Since whole genome analysis methods have just recently been developed, most research on induction of mutations in plants has concentrated on visible features. Thus, the phenotypic of mutant plants served as their initial classification. The three main classifications provided by Lundqvist et al. (2012), which are briefly detailed below, are genome, chromosomal, and gene mutations. The effectiveness of mutagenic agents in generating genetic alterations in the treated population of various plant species is now well documented such as *Trigonella foenum-graecum* (Jabee et al., 2007), *Helianthus annuus* (Khurseed et al., 2009), *Capsicum annuum* (Abdul Salam et al., 2010; Sri Devi and Mullainathan, 2011; Gulfishan et al., 2010, 2011, 2012, 2013, 2015), Chickpea (Laskar et al., 2015; Raina et al., 2017), Lentil (Laskar et al., 2018), *Hordeum vulgare* (Jafri et al., 2012, 2012, 2013), *Solanum melongena* (Shahab et al., 2018), *Vigna mungo* (Goyal et al., 2019), *Vigna unguiculata*, and cowpea (Raina et al., 2018, 2020).

6.2.2.1 GENOME MUTATIONS

Ploidy (the genomes number) and aneuploidy (the addition or deletion of chromosomes within such a genome) are both altered by these alterations. By creating haploid embryos, irradiated pollen has been used to decrease the number of genomes (Murovec and Bohanec, 2012; Gustafson and Ekberg, 1995). The use of haploids in plant breeding is beneficial (Forster and Thomas, 2005). It is interesting to note that the TILLING-induced mutant in *Arabidopsis* promoted haploid formation by preventing chromosomal attachment of spindle during mitotic division (Ravi and Chan, 2010).

The physical growth of the nucleus, which in turn increases the size of the plant cell and tissues and increases crop output, is one benefit of

polyploidy. Another benefit is the richness of gene variety (new genes are added). Soybean, maize, rubber, and rice are examples of historical allopolyploid crops; these plants essentially behave as diploids with bivalent production at meiosis. Chromosome duplications, deletion, translocations, and mutations can be generated as well as spontaneous in nature which can frequently be involved in the process of evolution of species.

6.2.2.2 CHROMOSOME MUTATIONS

Chromosome deficits, extra chromosomes, chromosome replacements, and chromosome translocations are all examples of aneuploids. They can be created by breeding, particularly in combinations when the parents contributed different quantities of genomes or chromosomes. The random loss of chromosomes caused by radiation can result in aneuploidy. Aneuploids of wheat are widely employed in crop improvement research (Law and Worland, 1987; Shimelis and Spies, 2011). Chromosomal breakage and subsequent chromosomal aberrations lead to chromosome rearrangements. Although chemical mutagens and physical mutagens both are with the potential to cause these changes, ionizing radiation is the most common cause. Duplications, deletions, translocations, and inversions can result from errors in repair systems. 90% or more of the deletion mutations caused by ionizing radiation are fatal. A small number could be practical and helpful.

6.2.2.3 GENE MUTATIONS

Single-nucleotide modifications or tiny indels are examples of the minor changes that frequently occur in gene mutations. Such changes could produce a new functional allele. Plant breeding is intrigued by these mutations because they introduce new useful variety. In an agronomically significant semi-dwarf mutant of rice, the GA20 oxidase gene (GA20ox-2) was found to have single-nucleotide deletions (Ashikari et al., 2002). More nucleotide changes have led to increased disease and insect resistance, improved abiotic stress tolerance, nutritional quality, and height of plants like wheat, rice, soybean, and barley (Jehan and Lakhanpaul, 2006).

Gene function is unaffected by mutations that take place in nongenic areas. These are frequently described as silent mutations and are harmless. Typically, they involve single-base alterations (point mutations) which did not affect transcription (Lee et al., 2012). Chemical mutagens like alkylating compounds frequently cause silent mutations. Intergenic alterations may be

silent because regulatory elements are not impacted by them. Though, base insertion or deletion might lead to the change in gene's coding sequence in the form of a frame-shift. This could lead to untimely termination codon with a flawed peptide.

6.3 INDUCTION OF MUTATION

A detailed specification of the kind, amount, and tissue to be mutagenized is necessary in situations where inducing new mutations is desired. These choices directly affect the project's success as they alter the frequency and range of induced mutations.

6.3.1 PLANT MATERIALS

Any plant tissue can generally undergo a mutation. The primary restriction is the survivability of the plant cells utilized to create the mutant population. The type of crop (seeds or vegetatively propagated) and the facilities available are the main factors in the selection of the plant material to be mutagenized. The whole plants, stems, buds, seeds, tubers, bulbs, pollen, embryos, microspores, *in vitro* plantlets, callus, and other propagules are examples of materials that have been frequently utilized in genetics and breeding programs. Among them seed is the best choice material because of ease in handling.

With accessibility to a mutagenic source, any breeder can easily perform mutation breeding on crops that are grown from seeds. Effective techniques have been established for mutagenesis of facultative and obligatory vegetatively propagated plants (van Harten, 1998; Mba et al., 2010).

6.3.2 MUTAGENS

The plant's genetic makeup has been altered using a variety of mutagens. Mutagens are generally classified into physical, chemical, and biological. The two basic types of physical mutagens are: 1) ionizing radiation, which is composed of elementary particles such as protons, electrons, neutrons, ά and β particles, and deuterons (Sparrow and Singleton, 1953; Mba et al., 2012), where ion beams and fast neutron are frequently utilized for induction of mutations in crop plant (Burtscher and Casta, 1967; Tanaka et al., 2010); 2) electromagnetic radiation that is ionizing, like X-rays, gamma, and cosmic rays (Luxiang and Qicheng, 1997; Liu et al., 2009). Employing

X-rays on *Zea maize* and *Hordeum vulgare* L., Stadler (1928a, 1928b) for the first time demonstrated induction of mutations in crop plants. The first usage of chemical in the form of a mutagen, particularly mustard gas, was described by Auerbach and Robson in 1944. They also compared the effects of ionizing radiation and mustard gas for mutations induction. Today, a large variety of chemical mutagens are documented; however, the majority are classified as alkylating agents, such as diethyl sulphate (DES), ethyleneimine (EI), ethyl methane sulfonate (EMS), ethyl nitroso urethane, ethyl nitroso urea, and methyl nitroso urea (Heslot, 1977). In addition to these, some antibiotics such as streptozotocin, mitomycin C; nitric oxide, nitrous acid, base analogues, and related substances, as well as inter-calating agents and topoisomerase, are non-alkylating mutagens. Leitão (2012) has reported their mutagenic properties and use in plant mutation breeding. The most popular non-alkylating mutagen employed in plant mutagenesis is sodium azide, which is major industrial nitrogen gas generator, bactericide, and pesticide (Kleinhofs et al., 1974; Gruszka et al., 2012).

6.3.3 RECOMPENCES AND SHORTCOMINGS OF MUTAGENS

The various chemical and physical mutagens used in mutation induction have several drawbacks in addition to their high efficiency in generating genetic diversity. Gamma rays, for instance, have the benefit of being utilized on a variety of plant materials, but they come up with certain drawbacks when only tiny dosages are needed (it is particularly difficult with new, very energetic isotope sources). Furthermore, the accessibility of gamma irradiators is a crucial factor in gamma irradiation. There are rigorous guidelines on the shipment of sources for gamma rays, like ^{60}Co and ^{137}Cs, due to their radioactivity. As a result, gamma service centers have been established in countries all over the world. These include Indonesia, Malaysia, Pakistan, the Joint FAO/IAEA Laboratories in Austria, and other recent investors in gamma irradiation facilities. For some nations, these limits may be prohibitive. For the other side, there are not many restrictions on X-ray irradiators. In order to ensure even irradiation, X-ray irradiators must be fitted with sample rotating devices because irradiating big samples with X-rays has a difficulty with homogeneity. Ion beams are hampered by time-consuming sample processing and significant material degradation. It is well known that fast neutron irradiation results in a very low transient radiation in the treated materials, which can provide a wellbeing issue for handling after irradiation and calculating absorbed dosage. Plant breeders are highly interested

in chemical mutagens since they are known to be efficient at inducing gene mutation. However, the chemicals used are dangerous, and the high intensity of genetic alterations that can be generated may not be what is needed.

6.4 MUTATION BREEDING AND CROP IMPROVEMENT

6.4.1 INDUCTION OF GENETIC VARIABILITY

Without any preference for a particular gene or allele across the entire genome, mutation is randomly induced by chemical or physical mutagens. This is one of the most important traits of mutagenesis since it gives the opportunity to produce mutations for any chosen gene or genes as well as permits the generation of many mutations for any particular gene in a prognostic approach. These various mutant alleles provide the genetic diversity for focused gene study and plant breeding. Without affecting the species' wild form or wild relative, mutations—the alteration of genetic material—provided the essential raw material for species evolution. The rate of autonomous mutations in nature that create the traits or characters with economic significance is quite low, but the breeding program's success was enabled by utilizing the very few advantageous natural mutations that occur in agricultural plants. One of the best instances of this method was the emergence of wheat and rice semi-dwarf mutants during the "Green Revolution." The Norin-10 and Dee-geo-woo-gen varieties of wheat and rice, respectively, produced the genes that cause dwarfism in those plants. When given the right nutrients, these two mutant plants produce a high grain yield and an improved mechanical strength due to their short height and stiff stem. A remarkable increase in yield was observed, resolving the global food crisis at that time. In many crop plants with limited genetic diversity, the process of induced mutagenesis has greatly evolved since its discovery and increased genetic diversity without altering the wild gene pool. Additionally, it enhances the current variety in certain aspects that are economically significant while supplementing the wild material. In several crop plants, several researchers used induced mutagenesis such as *Vicia faba* L. (Bhat et al., 2005, 2006, 2007, 2015, 2017, 2019, 2021; Gulfishan 2010, Khursheed et al., 2018, 2019), Cowpea (Raina and Khan 2020), Lentil (Wani et al., 2021, Lashkar 218,), *Vigna mungo* (Goyal et al., 2019, 2021), *Trigonella* (Hasan et al., 2018), *Nigella* (Raina et al., 2019) etc. and upsurge the genetic diversity along with their wild genotypes.

6.4.2 ISOLATIONS OF DESIRED MUTANTS

Three steps make up the induced mutagenesis procedure: (1) inducing the mutation, (2) identifying potential or putative mutants, and (3) testing the mutants before releasing them. The testing and formal release of mutants are country-specific and independent of efforts to increase efficiency through research and development (Jankowicz-Cieslak et al., 2012). Generally, the selection of the mutagen employed in mutant breeding programs has been based on the prior success reports of the specific mutagen for a certain agricultural plant species in addition to the accessibility of mutagens, costs, and infrastructure facilities (Bado et al., 2015; Mba, 2013; Gulfishan, 2015). Ionizing radiation, or gamma rays, revealed to be one of the most potent mutagens in terms of mutant formation in several crop plants (Table 6.1), while EMS demonstrated to be the most effective physical mutagen with the maximum number of mutants (Table 6.2). (MVD, 2023). In total, 341 mutants of various crop plants have been made available in India. Tables 6.3 and 6.4 provide the mutants with improved characteristic (general and specific). The information on MVD mutant varieties is primarily divided into five groups: quality and nutrition, agronomic and botanical trait, resistance to biotic stress, tolerance to abiotic stress, yield and contribution. Among the 3,364 formally recognized mutant types, improvement is seen in multiple characters, e.g., a mutant with enhanced yield also showed resistance to biotic stress (Fig. 6.1).

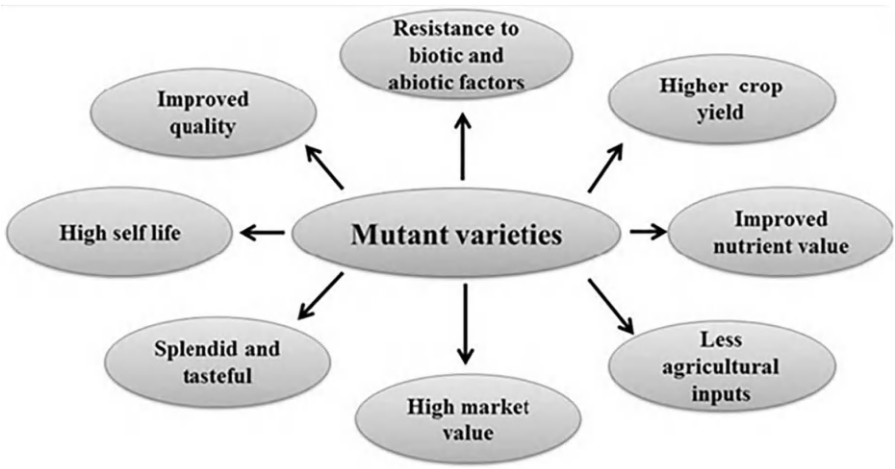


FIGURE 6.1 Characteristics of mutants. ⏎

Source: Reprinted from Gulfishan et al., 2015

TABLE 6.1 Mutants Developed by Various Physical Mutagens in Crop Plant (MVD, 2023). ⏎

S. No.	Name of mutagens	Numbers of mutants
1.	Gamma rays	1703
2.	x-rays	561
3.	Aerospace	46
4.	Laser	26
5.	Neutrons	24
6.	Ion beams	20
7.	Beta rays	16
8.	Electron	10
9.	Ultrasound	01
10.	UV light	01

TABLE 6.2 Mutants Developed by Various Chemical Mutagens in Crop Plant (MVD, 2023). ⏎

S.No.	Name of mutagens	Numbers of mutants
1.	EMS	108
2.	NEU	57
3.	Calcium	47
4.	E1	36
5.	DMS	19
6.	DES	14
7.	Tissue culture induced	06
8.	NMU+NEU	02
9.	SA	02
10.	ENH	01

TABLE 6.3 Mutants with Improved Character (General) (MVD, 2023). ⏎

S.No.	Improved character	No. of mutants
1	Agronomic and Botanic Traits	2987
2	Quality and Nutrition	1174
3	Yield and Contributors	1029
4	Resistant to Biotic Stress	558
5	Tolerance to Abiotic Stress	248

TABLE 6.4 Mutants with Improved Character (Specific) (MVD 2023). ⏎

S. No	Improved character	No. of mutants
1	Drought Tolerant	103
2	Disease Resistance	34
3	Salinity Tolerance	32
4	Early Maturity	31
5	Fiber Quality	02

6.4.3 IMPACT OF MUTANTS

For enhancing productivity, biomass accumulation, crop stature, insect, pest and disease resistance, plant's physiological features, nutritive quality, mutants' sexual reproduction (autogamy or allogamy) has been successfully induced by mutation breeding (Konzak et al., 1984). Induction of mutation in asexually propagated crops like potatoes, sweet potatoes, horticultural crops, fruiting trees, and other plants was evaluated in 1989 by van Harten and Broertjes. Officially, around the world, 3364 mutant varieties of 214 species of different plants have been released, which can be used to gauge the impact breeding via induction of mutations. Since the 1950s, there have been more direct and indirect mutant cultivars made available each decade. Several mutants were overlooked because of a variety of reasons, including the following: (1) manufacturers and producers may want to conceal the source of their materials; (2) breeders may not be particularly interested in the desired trait or may not be aware that it is due to mutation; (3) a large number of mutant types are the result from traditional breeding with a mutant line rather than being unambiguous mutants; (4) plant breeders are not required to make genetic modification and pedigree information public; and (5) induced mutagenesis is already a common practice in many nations around the world. Mutant cultivars are essential components of global crop production, providing food, fuel, feed, fiber, and recreational opportunities. They provide the building resources for industrial operations and give farmers extra revenue worth billions of dollars. Mutants, like other cultivars, become available as they usually have an edge in terms of output and quality, can be more resilient to biotic and abiotic stresses, efficient usage of nutrients, have higher nutritional value, and possess other improved characteristics.

6.5 CONCLUSION AND FUTURE AHEAD

Mutation breeding program became successful and provided food for the entire planet with 3364 mutant types created in various crop plants around the world. Induced mutagenesis will unquestionably be one of the leading ways to encounter with the food requirement by the tremendous population expansion to reach nine billion by 2050. Nowadays mutation breeding is going a long way with modern biological techniques like *in vitro* culture technique where micropropagation, somatic embryogenesis, and double haploidy. The recovery and screening of recessive mutations are made more successful by double haploidy. DNA-based technologies like next-generation sequencing

help in identification and mapping of mutations induced in the genome of organism. Numerous next-generation sequencing technologies, including chromatin studies, RNA sequencing, and various condensed input libraries for genome sequencing of plants with large genomes, can be used to evaluate mutant plants. High-throughput phenotyping techniques offer a useful technique for selection and screening of mutant of desired features associated with abiotic and biotic stresses. Today, phenomics offers an incredibly broad range of innovative and effective strategies and tools to improve the efficacy of mutant breeding. These methods will help plant breeders create germplasm with desired and long-lasting features.

KEYWORDS

- **mutation**
- **mutagen**
- **mutagenesis**
- **crop improvement**
- **gamma rays**
- **EMS**

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CHAPTER 7

Genetic Engineering and Agricultural Science

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ABSTRACT

Genetic engineering has become a very important part of modern agricultural biotechnology approaches. In the past few years, many transgenic crops have been modified to fulfill the two fundamental necessities of agriculture. Healthy food and durable crops that can survive under limiting and stressful conditions. Plants have been genetically altered to enhance their ability to tolerate greater biological and environmental stresses or improve their physiological traits, thereby increasing their yield potential. Furthermore, the scientific community has invested significant time and effort in identifying genes and quantitative trait loci (QTL) that can enhance plant characteristics. Economically important crops have been modified via overexpression or downregulation of target genes and QTL. This chapter offers an outlook on recent advances in agricultural science and practices driven by genetic

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engineering, as well as examines the effect of transgenic crops on the diversity of flora and fauna along with human health. Additionally, the attitudes and opinions of farmers regarding GM crops are also part of the discussion.

7.1 INTRODUCTION

The ever-increasing world population requires food for sustenance and proper growth, especially in the case of undernourished developing children, lack of certain micronutrients and macronutrients can result in permanent physical and mental damage. Currently, 11% of the world's total population lacks food intake to fulfill their minimum energy requirement. Furthermore, 22% of children lack proper growth and development due to a lack of adequate nourishment and repeated infections. Micronutrient deficiencies, including zinc, iron, calcium, and vitamin deficiencies, are relatively more widespread. For instance, a lack of iron causes anemia whereas a lack of vitamin A causes night blindness (Hazra, 2009; Ritchie and Roser, 2017). These issues can only be resolved by providing nutritious food, but the resources utilized by agricultural practices are finite, such as water and land; hence, the number of edible crops annually produced is also bound to these limitations. Moreover, certain abiotic and biotic conditions cause the loss of planted crops. Therefore, it is requisite to increase the plant's nutritional values and survival under limiting conditions.

Genetic engineering can effectively mitigate crop losses caused by stress, and it can also be utilized for biofortification of crops. Plants can be targeted for the improvements that can aid their ability to withstand higher biological and environmental stress or they can be targeted to improve their physiological characteristics that can increase the yield. Among the properties that can improve the net yield are root architecture, plant height, stem length, number of seeds per plantlet, etc. Marker-aided selection (MAS) methodology has successfully provided DNA markers within plants that can be targeted for successful transgenic plant creation. In addition, various quantitative trait loci (QTL) are recognized in plants that can significantly enhance plant characteristics. These QTL can be overexpressed via increasing their copy number or downregulated via small interfering RNA (siRNA) treatments or knockout mutations. Biolistic and agrobacterium-mediated gene transfer methods are the most widely utilized methods. In recent years, CRISPR, transcription activator-like effector nuclease (TALEN), and zinc finger nuclease (ZFN)-mediated transformations have emerged as effective and accurate methods (Kole et al., 2015).

7.2 AGRICULTURAL DEMANDS

Over the years, a number of genes and QLT have been identified within plants that can aid in biofortification and stress management in plants. Some successful modifications have been implemented in plants, resulting in success.

7.2.1 BIOFORTIFICATION

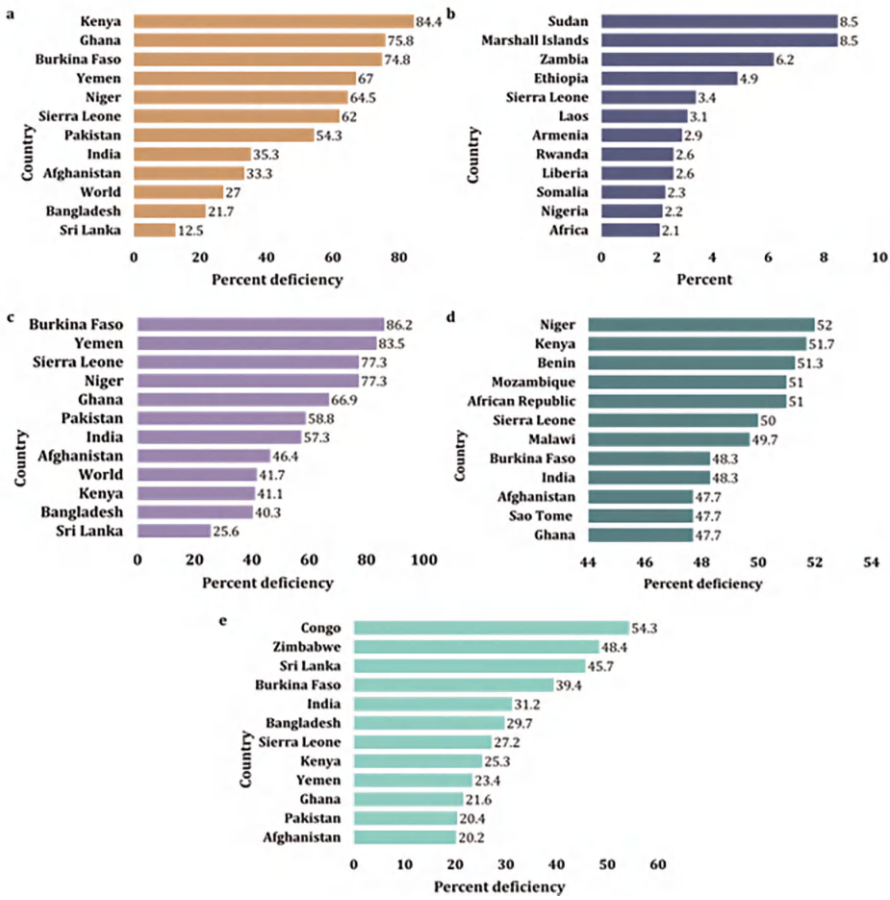


FIGURE 7.1 Statistics related to the occurrence of micronutrient deficiencies in children and related physiological conditions. (a) Prevalence of night blindness in children (2005) children under the age of 5, (b) Vitamin-A deficiency in toddlers and preschoolers noted in 2017, (c) Vitamin-B12 deficiency in toddlers and preschoolers was noted in 2017, (d) The frequency of malnutrition in preschool children, and (e) zinc deficiency in men, women, and children in 2005.

Source: Adapted from Ritchie and Roser, 2017

Biofortification refers to the methods of increasing the concentration of micronutrients in plants using various techniques such as breeding programs or genetic engineering-based modifications. Existing approaches to overcome nutritional deficiencies, such as postprocessing food modifications and distribution of pills with micronutrients, are much more expensive than genetic engineering and breeding (Simkin, 2019). Over the years, attempts for the biofortification of vitamins A, E, C, B_{12}, and Zn have been made in various plants.

Vitamin A, also known as retinol, is essential for the synthesis of rhodopsin, which is critical for functioning eyesight under low-light conditions. Moreover, vitamin A is also obligatory for the proper functioning of the immune system, and lack of it results in death and miscarriages in pregnant women (Ritchie and Roser, 2017). β-carotene, β-cryptoxanthin, and α-carotene are three major precursors of vitamin A. β-Carotene is most frequently occurring in vegetables such as lettuce, carrot, and peach. Nonetheless, such foods are not commonly consumed, whereas widely ingested staple foods (wheat, potato, and rice) have meager vitamin A precursors. Over the course of time, a variety of transgenic crops of tomato, potato, wheat, maize, and rice have been created to express a variety of genes that can increase the amount of vitamin A. Among those genes *crtY*, *crtL*: lycopene beta-cyclase; and *ctrI*, *Psy*, *crtB*: phytoene synthase have been most effective (Simkin, 2019). Tetrahydrofolate and its derivatives are essential for various metabolic functions such as purine, thymidylate, methionine, serine biosynthesis, and histidine degradation. Lack of folates can result in cancers, megaloblastic anemia, and cardiovascular disease. Moreover, unlike microbes and plants, mammals cannot synthesize *de novo* folates; hence, they must rely on external sources such as staple foods. *GmGCHI* (GTP cyclohydrolase I) and *GmADCS* (aminodeoxychorismate synthase) are two genes associated with soybean folate biosynthesis. These genes have been successfully introduced into the maize and wheat to increase their folate levels (Liang et al., 2019). Proteins linked with histone and DNA demethylation often use vitamin C (L-ascorbic acid) as a cofactor. Furthermore, it has been recognized as a molecule necessary for the synchronization of gene expression patterns and environmental changes (Young et al., 2015). Various citrus and berry fruits are sources of vitamin C that have been genetically altered to overexpress the genes coding for the enzymes GDP-L-Gal phosphorylase, dehydroascorbate reductase, D-glucuronate reductase, myoinositol oxygenase, and dehydroascorbate reductase (Simkin, 2019).

Moreover, efforts have been undertaken to develop genetically modified (GM) plants capable of producing an abundance of various vitamins. In South African corn, increased production of vitamin C, folate, and provitamin A has been achieved via the upregulation of genes coding for carotene desaturase and phytoene synthase (Naqvi et al., 2009). Vitamin B_{12} (Cobalamin) aids the functioning of fat and protein metabolism. Furthermore, the process of neurotransmitter production also requires it as a cofactor. Hence, deficiencies of B_{12} can lead to neurological disorders such as memory loss, psychosis, and depression with anemia, which causes weakness. People with a vegan diet often suffer from cobalamin deficiency as all primary sources are animal products such as meat, milk, eggs, etc. Due to the complex synthesis process, the construction of transgenic plants that can produce and accumulate a significant amount of cobalamin needs more extensive research (Watanabe, 2007). Also, in some cases, improvement in production and accumulation of certain elements results in a decreased concentration of other metabolites. For instance, in *Brassica napus* gene *crtB* was upregulated, resulting in increased carotenoid production with reduced chlorophyll and tocopherol production. In another study conducted on potatoes, increased carotenoid production was accompanied by reduced starch content (Shewmaker et al., 1999; Beyene et al., 2018).

7.2.2 BIOTIC STRESS MANAGEMENT

Fungi, bacteria, viruses, insects, pests, and nematodes are causative agents of the biotic stress in plants. In India, ~35,877 million dollars' worth of crops are lost to insects only. Statistical data regarding the losses of 5 significant crops, rice, maize, wheat, potato, and soybean has been given in Table 7.1. To create crops that can sustain against these stress-causing agents, a variety of antifungal, antibacterial, antiviral, and anti-insecticidal molecules-producing genes have been incorporated into the wild varieties. Various genes and QTL have been identified in various plants that can protect against fungal, bacterial, nematode, and viral pathogens. These loci can be specific for organisms or be broad in their action. There is also the possibility that these QTL are simply dampening the damages caused by pathogens rather than actively diminishing their growth.

TABLE 7.1 Losses in Crop Yields Resulting from Biotic Stress Factors.

Sr. No.	Crop	Disease	Causative agents	Yield loss (%)
1.	Wheat	Tan spot	*Pyrenophora tritici-repentis*	11.09
		Fusarium head blight (FHB)/scab	*Fusarium graminearum*	11.95
		Wheat blast	*Magnaporthe oryzae*	3.52
		Spot blotch	*Cochliobolus sativus*	7.29
		Stem rust	*Puccinia graminis* f.sp. *tritici*	8.89
		Total loss		42.74
2.	Rice	Sheath blight	*Rhizoctonia solani*	15.81
		Stem borers	*Scirpophaga incertulas*	8.75
		Blast	*M. oryzae*	5.89
		Brown spot	*Cochliobolus miyabeanus*	5.86
		Bacterial blight	*Xanthomonas oryzae*	8.51
		Yellow mottle	Rice yellow mottle virus	4.33
		Total loss		49.15
3.	Maize	Stalk rots	*Erwinia dissolvens*; *F. graminearum*	14.53
		Armyworm	*Mythimna separata*	14.93
		Southern rust	*Puccinia polysora*	7.87
		African corn borer	*Busseola fusca*	4.01
		Total loss		41.34
4.	Potato	Late blight	*Phytophthora infestans*	20.4
		Cyst nematode	*Globodera* spp.	3.13
		Brown rot	*Ralstonia solanacearum*	11.27
		Apical leaf curl	Potato apical leaf curl virus	3.65
		Total loss		38.45
5.	Soybean	Cyst nematode	*Heterodera glycines*	14.55
		White mold	*Sclerotinia sclerotiorum*	4.11
		Rust	*Phakopsora pachyrhizi*	6.65
		Total loss		25.31

Data represents the maximum cumulative losses of five major regions: United States Midwest and Canada (USM & C), South Brazil, Paraguay, Uruguay, and Argentina (SB&A), China, Indo–Gangetic Plain (IGP), sub-Saharan Africa (SSA) derived from Savary et al., 2019.

Cecropin B is a broad-spectrum antibacterial peptide that kills bacteria via the formation of ion channels in the cell membrane; moreover, this antibacterial peptide does not react with eukaryotic cells. To counteract,

the bacterial pathogen *X. oryzae* pv. *oryzae*, antibacterial peptide cecropin B encoding gene, *cecB*, present in *Bobyx mori* has been successfully introduced in rice. After the introduction, transgenic plants were found to be accumulating a significant amount of cecropin B in their apoplast. Also, the spread of pathogens after exposure was found to be restricted to the area of inoculation (Sharma et al., 2000). In another study, *cecB* gene, along with s*hiva A*, has been utilized to increase the immunity of Jincheng and Newhall navel orange (*Citrus sinensis* Osbeck) against *X. axonopodis* pv. *citri*, the causative agent of citrus canker. Extended studies in greenhouses and farms have confirmed the steady expression of both transgene with significant resistant toward pathogen (He et al., 2011). *TaHRC* is a putative histidine-rich calcium-binding protein-encoding gene that makes wheat more susceptible to FHB disease. Reduced expression of this gene can aid in developing FHB-resistant wheat (Su et al., 2019). Furthermore, the *Pm3* gene encoded nucleotide-binding leucine-rich repeat (NLR) immune receptor from wheat variant W150, has been utilized to attenuate the pathogen *Blumeria graminis* f.sp. *tritici* which causes powdery mildew (Koller et al., 2019).

Verticillium dahlia, *Verticillium albo-atrum*, and *Fusarium* are the causative agents of wilt in more than 400 plant species that extend from annual plant species, such as potato and tomato, to perennial species, such as maple. Efforts to locate the markers that can enhance the resistance against these fungal pathogens have resulted in the identification of *Ve1* and *Ve2* genes in tomato that are effective against *Verticillium* species (Arens et al., 2010). *Fusarium* is a devastating plant pathogen that affects critical agricultural plants by causing various foliar diseases. In cereals, *F. graminearum* causes scab or FHB, wilt, crown rot, etc. To create transgenic wheat plants that can sustain and compete against FHB, QTL linked to FHB resistance has been identified. Their activities have been verified via single-floret inoculation studies under standard greenhouse conditions. Seven QTL located on chromosome arms 2DS, 3AS, 3BS, 3BC, 4DL, 5AS, and 6BS are necessary to resist the establishment of FHB infection at the initial stages furthermore, three of these QTL present on chromosomes 2DS, 3BS, and 6BS, along with 7BL, play an essential role in the resistance of infection spread (Yang et al., 2005). Moreover, six QTL present on chromosomes 1DL, 2DS, 3BS, 3BC, 4DL, and 6BS have been identified as necessary for kernel infection resistance (Yang et al., 2005). Among various species of *Fusarium*, *Fusarium oxysporum* is the most widely occurring and targets multiple fruits and vegetables. Transgenic tomato plants resistant to *F. oxysporum* have been created via the integration of QTL *I* and *I2*

located on the chromosomal arm 11 of *Solanum pimpinellifolium* (Arens et al., 2010). Six loci necessary for resistance against *F. oxysporum* have been recognized in *Arabidopsis*, on chromosome 1 (*RFO1*; *RFO2*), 3 (*RFO3*), 4 (*RFO4*), and 5 (*RFO5*; *RFO6*) (Diener and Ausubel, 2005). Three other QLT, namely, *RPW10*, *RPW11*, and *RPW12*, which are active against infection of *Podosphaera xanthii*—a causative agent of powdery mildew—are recognized in *Arabidopsis thaliana* (Schiff et al., 2001). Other than the aforementioned pathogens, *Puccinia striiformis*, *Puccinia triticina*, and *P. graminis* are phytopathogens of economic importance as they are the causative agents of yellow rust, leaf rust, and stem rust, respectively, in various essential agricultural commodities. In wheat plants, resistance against leaf rust is conducted by loci *QLr.fcu-3BL, 3AL, 6BL, 4DL*, and *5BL*, (Chu et al., 2009). A study carried out on wheat suggests the existence of antirust QTL on chromosomes 2B, 5B, 6A, 6B, 6D, 7B, and 7D where on chromosome 2B interval *wPt5556–wPt6278* contains gene *Lr13*. Loci *wPt1325–wPt3045* and *barc352–gwm111*, respectively, located on chromosome 6B and 7D, contain gene *Lr3bg* and *Lr34*. Other loci *wPt2633–psp3152*, *barc4–wPt0103*, *ksm25m50b–wPt8721*, and *wmc323–gwm537* located on chromosome 6A, 5B, 6B, and 7B, respectively, were also found to be essential but genes encoded by these regions are unknown. For stem rust resistance 2 QTL intervals *wPt7181–psp3152*, and *barc183–wPt3879* encoding *Sr26* and *Sr5* are necessary (Prins et al., 2011).

Burkholderia plantarii and *Burkholderia glumae* are the causative agents of grain rot where they are mostly transmitted via seeds. The thionin gene *Asthi1* from *Avena sativa* cv. z*ensin* was used to create transgenic rice plants capable of resisting these pathogens. The resulting plants could resist the disease spread even when infected with a population load of 10^6 CFU/ml, resulting in the death of wild-type plants (Iwai et al. 2002). Furthermore, rice plants are known targets of the bacterial pathogen *X. oryzae* that causes blight. To create cultivars resistant to this bacteria, gene *Rxo1* from maize has been utilized (Zhao et al., 2005). In the population of *Populus tremula*, *Agrobacterium tumefacuience*, and *Xanthomonas populi* are the most frequently occurring pathogens that cause crown gall and bacterial canker, respectively. Transgenic *P. tremula* plants containing a gene for antimicrobial peptide D4E1 have been created that show resistance to these pathogens (Mentag et al., 2003). In GM rice plants, heavy expression of *NH1ox* gene, an ortholog of *NPR1* gene, confers resistance to *X. oryzae* pv. o*ryzae*. Along with this, the growth of *NH1ox*-containing plants in a growth chamber under low light shows a significantly increased

concentration of salicylic acid compared to wild-type plants (Chern et al., 2005). In grapefruit, the overexpression of *NPR1* gene (*AtNPR1*) from *Arabidopsis*, a cardinal positive regulator of systemic acquired resistance (SAR), shows resistance against *Xanthomonas citri* subsp. *citri* the causative agent of citrus canker, where up to 10 times drop in pathogen growth was observed (Zhang et al., 2010). Also, *Xanthomonas hortorum* and *Xanthomonas campestris* have been recognized as agents responsible for bacterial blight and bacterial spot, respectively, in carrot and tomato. In both species, *AtNPR1* is recognized as an effective way to resist pathogen infections (Lin et al., 2004; Wally et al., 2009). *Agrobacterium tumefaciens* use overexpression of oncogenes to induce the formation of crown gall. *IaaM* and *ipt* are such oncogenes; their silencing in *A. thaliana* and *Lycopersicon esculentum* using the RNAi silencing method resulted in a reduction of crown gall formation (Escobar et al., 2001). Also, in a separate study conducted on *A. thaliana*, a siRNA molecule capable of downregulating *AtRAP* gene has been identified, as the absence of *AtRAP* encourages resistance against pathogens. Overexpression of this siRNA confers resistance against *Pseudomonas syringae* that carries gene *avrRpt2*, an encoder of effector protein (Katiyar-Agarwal et al., 2007).

Genes *Tm1* and *Tm2* provide resilience against "tomato mosaic virus." These genes were originally identified in *Solanum habrochaites* (*Tm1*) and *Solanum peruvianum* (*Tm2*) on chromosomes 2 and 9, respectively (Arens et al., 2010). In peanuts, the "tomato-spotted wilt virus" (TSWV) causes heavy yield losses where thrips act as their carriers. *qF5TPS1*, *qF2TPS1*, and *qF2TPS2* are three major QLT located in peanuts that are active against thrips whereas, for TSWV, 24 QTL were located, out of which 6 QTL, namely, *qF2TSWV3*, *qF2TSWV8*, *qF2TSWV4*, *qF2TSWV6*, *qF5TSWV4*, and *qF5TSWV7* were found to be consistent in different environments while rest were having irregular expression (Wang et al., 2013). Apart from the resistance toward specific pathogens, it has aspired to create plants with resistance to a broad range of pathogens. For instance, phytocystatins are known inhibitors of digestive functions in insect pests which ultimately causes their death. However, the antibacterial and antifungal properties of phytocystatins are not consistent, they differ from plant to plant. In wheat, *TaMDC1* is the gene that encodes multidomain cystatin that has been reported to be active against insects, along with bacterial and fungal pathogens. Moreover, this gene was utilized to create a transgenic tomato plant that exhibited resistance toward *P. syringae*, *Alternaria alternata*, *Botrytis cinerea*, and *Leptinotarsa decem-lineata* (Christova et al., 2018).

7.2.3 ABIOTIC STRESS MANAGEMENT

Apart from biotic stresses constantly changing environment raises another concern for agriculture. Altered rain patterns, drought, heat, cold, salinity, flood, and submergence may lead to decreased plant survival by increasing the susceptibility to pathogens and other competing organisms present in the niche. These constantly changing environmental parameters pose certain dangers to crops and the current food supply, one assessment shows that global warming, along with weather change, will cause production losses for major staple crops such as maize (9%), rice (11%), wheat (14%) in upcoming years (Solari and Hisas, 2011). Resistance to frosty temperatures is the main part of winter endurance, yet additionally of impressive significance is the ability to withstand blends of stresses caused by drying up, wind, ice-encasement, hurling, low light, snow cover, psychrophilic microbes, and fluctuating temperatures. Protection from drying up through the maintenance of the cell layer and cell water is fundamental, and it is obvious that a similar hereditary reaction to the beginning of frosty temperatures is frequently seen with dry spells or salt stress (Yue et al., 2006). Flooding is another significant issue for cereals around the world. Hereditary variety in waterlogging resilience has been accounted for in different cereals, including wheat, maize, and oats, where assorted components are related to phenology and morphology, anabolism, catabolism, anaerobic metabolism and anoxia resilience, and post-anoxia harm and recuperation (Setter and Waters, 2003). Hence, generating more robust plant varieties using these native genes from respective species and genes from other species that have adapted to these stresses has become important. Genetic engineering has greatly aided these endeavors.

Heat stress, ultraviolet light, and most stresses are known to increase the amount of reactive oxygen species (ROS) in plants, where this ROS can cause membrane damage via lipid peroxidation, which leads to tissue desiccation. Using ascorbate peroxidase (*cAPX*) from pea-transgenic tomato plants has been formed that can survive temperatures as high as 40 °C (Wang et al., 2006). *GASA4* (*Arabidopsis*) and *TTO6* (*Zea mays*) are known to have cardinal participation in surviving heat-related stresses in respective plants. Furthermore, knockout studies for gene *GASA4* in *Arabidopsis* have confirmed the role of heat stress tolerance. Whereas gene *TTO6* is reportedly expressed in yeast cells to make them more resilient to higher temperatures (Ko et al., 2007). Maize (*Z. mays*) split seed explants transformed with *Arabidopsis* transcriptional factor *CBF3* heavily influenced the cold, dry season, and salt resilience; furthermore, this transcription factor has been used in

Arabidopsis, tobacco, and wheat to produce similar results (Al-Abed et al., 2007). The C-repeat binding factor (CBF) is a known modulator of COR (cold-regulated genes), where they recognize the cis-acting components in the regulatory sections of COR genes (Stockinger et al., 1997). Chromosome 5H, known for *Fr-H2* cold tolerance loci, harbors the 11 CBF genes in barley (Skinner et al., 2006; Francia et al., 2007). In wheat chromosome 5H holds this region in the proximity of *Fr-Am2* loci. In cereals, the number of CBF genes varies with winter forms; crops with higher stability in cold environments tend to have a higher copy number of these genes (Knox et al., 2010). On the contrary, 2B, 7B, and 7D are recognized as key genomic regions that play an important role in heat stress tolerance in wheat plants. In these regions, QTL of chromosomes 2B, 7B, and 7D was identified between markers *Xgwm935–Xgwm1273*, *Xgwm1025–Xgwm745*, and *Xgwm3062–Xgwm4335*, respectively. Based on this information similar locus might be observed in orthologous regions of other plants (Paliwal et al., 2012). Flooding often leads to hypoxia and root development impairment, which can significantly halt the germination of seeds. Developing plant embryos requires a higher amount of energy; under hypoxia, various oxygen-mediated pathways are inhibited, which halts the conversion of complex starch reserves into simple sugars, and the growth of the embryo is interrupted (Septiningsih et al., 2013). Plants capable of utilizing starch under hypoxia via anaerobic pathways can generate energy to sustain embryo growth. In rice, chromosomes 9 (*qAG-9-2*) and 7 (*qAG-7-1*) contain the two major QTLs associated with stress tolerance created by waterlogging. Furthermore, *SUB1* QTL present on chromosome 9 contains the gene *SUB1A-1*, known to produce ethylene response factor (ERF), which is cloned in rice to create flood tolerant variety (Xu et al., 2006; Angaji et al., 2010; Septiningsih et al., 2013). Flooding sometimes leads to high salt concentration accumulation; under such conditions, plant's ability to discriminate between K^+ and Na^+ can be of great importance. In *Triticum turgidum,* chromosome 2A contains genes *Nax1* and *Nax2.* These genes have been associated with K^+ and Na^+ differentiation in plants where their presence increases the preference for K^+ over Na^+ (James et al., 2006). *HvNax3* identified in barley (*Hordeum vulgare*) on chromosome 7H can reduce the Na^+ accumulation in the shoot region by up to 25% (Shavrukov et al., 2010). In rice, the QTL *satol* on chromosome 1 associated with sodium/potassium homeostasis has been identified to participate in salinity survival (Thomson et al., 2010). The use of herbicides is a very common practice among farmers, but often, these herbicides damage the crops and target weeds. Hence, herbicide tolerance is one of the sought-after

properties in cultivars. To create glyphosate herbicide tolerant plant cultivars, enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) encoding cDNA has been obtained from glyphosate-tolerant *Petunia hybrida* that are capable of overproducing this enzyme (Shah et al., 1986). The *bar* gene has been utilized in transgenic pineapple plants that can tolerate bialaphos. Also, the ability of this transgenic plant to tolerate herbicide was further evaluated using Basta herbicide at the dosage of 1600 mL/rai (Sripaoraya et al., 2006).

7.3 METHODOLOGIES FOR MODIFICATION

Targeted mutagenesis revolutionized genetic engineering in agricultural plants; it incorporates the application of emerging technologies such as TALENs, ZFNs, and CRISPR/Cas (Cas, CRISPR-associated) system. 20 nucleotide short synthetic gRNA that binds target sequence of DNA effectively and Cas9 nuclease enzyme that systematically cleaves 3–4 bases after protospacer adjacent motif are the key requirements for the CRISPR technology (Jinek et al., 2012; Jaganathan et al., 2018). CRISPR technology involves the following steps: identification of PAM sequence in targeted gene series, sgRNA synthesis, sgRNS cloned into an acceptable binary vector, incorporation into targeted cell line or host species, screening, and finally, its validation (Jaganathan et al., 2018). Basic designing, flexibility, cost-effectiveness, higher performance, and specification are some of the advantages of CRISPR (Jinek et al., 2012).

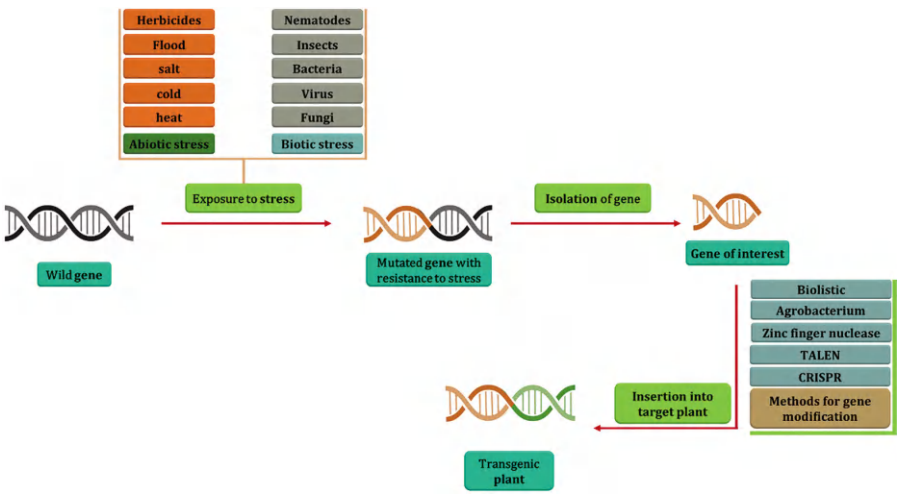


FIGURE 7.2 Generalized steps for the creation of transgenic plants. ⏎

The ease of implementation is among the key factors that have extensively promoted the utilization of CRISPR technology for genome modification in various plants, outperforming other approaches from the past few decades. In contrast, TALEN technology, which includes modifying transcription activator-like effector (TALE) domain repeats for targeted sequence identification and subsequent fusion with FokI nucleases, is also an efficient genome editing method. Due to its longer length, TALEN offers greater target specificity than ZFN. However, some challenges associated with adopting TALEN include the requirement for a thymine base at the starting point, its extensive length, and its repetitive nature (Jaganathan et al., 2018). ZFNs are custom-made proteins designed to cut targeted genome sequences and emerge as significant tools in gene editing. ZFN promoted the pairing of the FokI endonuclease component, nonspecific cleavage domain (N), with zinc finger proteins (ZFPs), causing the formation of double-strand breaks within the DNA (Durai et al., 2005). Preserving the temporal and tissue-specific gene expression is the promising advantage of ZFN-based genome modification compared to other conventional methodologies. The success of the application of the following three gene editing technologies in the targeted modification of desirable agricultural crops is mentioned. Whereas successful transformations via Agrobacterium and biolistic mediated methods have been enlisted in Table 7.2. The generalized flow of transgenic plant creation has been depicted in Figure 7.2.

7.4 SUCCESSES

7.4.1 CRISPR

Due to its high effectiveness, CRISPR/Cas9 is systematically studied across a variety of organisms, offering various useful outcomes, including mutation and regulation of gene expression via either repression or activation and epigenome editing (Song et al., 2016). CRISPR/Cas9 technology efficiently adopted to modify multiple traits, including knockout for yield improvement, resistance against biological and environmental stress, and resistance against plant pathogens in varieties of agricultural crops (Jaganathan et al., 2018).

7.4.1.1 SOYBEAN

Flowering time is a vital agronomic trait in plants, as it directly influences their adaptability within diverse cultivation systems. A comprehensive understanding of the soybean photoperiod flowering pathway primarily relies on

TABLE 7.2 Transgenic Plants and Sources for Transgene. ⏎

No	Plant	Gene/ gene locus	Source of gene	Method of transformation	Altered characters	Reference
	Wheat	*Rht8, Rht5, Rht13*	Chuan-Mai 18; Marfed Dwarf	Near isogenic line development	Early vigor development for better weed competitiveness	(Aharon et al., 2020)
		Pm21	*Haynaldia villosa*	Biolistics	Resistance to *Blumeria graminis* f.sp. *Tritici*	(Xing et al., 2018)
		ZmDof1	*Z. mays*	Agrobacterium	Improved biomass and yield	(Peña et al., 2017)
		CspA, *CspB*	*Escherichia coli*	Agrobacterium	Salt and drought tolerance, grain yield increase	(Yu et al., 2017)
		Pepc, *ppdk*	*Z. mays*	Biolistic	Improved photosynthetic rates	(Zhang et al., 2014)
		AtNHX1	*A. thaliana*	Agrobacterium	High tolerance to salt stress	(Moghaieb et al., 2014)
	Rice	*pfe*	*Phaseolus vulgaris*	Agrobacterium	Increased iron content	(Lucca et al., 2001)
		phyA	*Aspergillus fumigatus*	Agrobacterium	Increased iron content	(Lucca et al., 2001)
		Psy and β-lcy	*Narcissus pseudonarcissus*	Agrobacterium	β-Carotene synthesis	(Beyer et al., 2002)
		crtI	*Erwinia uredovora*	Agrobacterium	β-Carotene synthesis	(Beyer et al., 2002)
		AlaAT	*H. vulgare*	Agrobacterium	Enhanced nitrogen utilization efficiency	(Shrawat et al., 2008)
	Maize	*CBF3*	*A. thaliana*	Biolistic	Stress resistance	(Al-Abed et al., 2007)
		DdmC	*Pseudomonas maltophilia*	Agrobacterium	Herbicide tolerance	(Cao et al., 2011)
		AtGIF	*A. thaliana*	Agrobacterium	Grain weight and size increased	(Li et al., 2013)
		OsGIF	*Oryza sativa*	Agrobacterium	Grain yield improvement	(Li et al., 2013)
		zmm28	*Z. mays*	Agrobacterium	Grain yield improvement	(Wu et al., 2019)
	Barley	*ALMT1*	*Triticum aestivum*	Agrobacterium	Aluminum tolerance	(Delhaize et al., 2004)
	Sugarcane	*bar*	*Saccharum ocinarum* NCo 310	Agrobacterium	Herbicide resistance	(Enríquez-Obregón et al., 1998)
		CP	Sugarcane mosaic potyvirus (SCMV)	Biolistic	Resistance to SCMV	(Joyce et al., 1998)

TABLE 7.2 *(Continued)*

No	Plant	Gene/ gene locus	Source of gene	Method of transformation	Altered characters	Reference
		CP4	*Agrobacterium* sp. strain *CP4*	-	Herbicide resistance	(Watt et al., 2010)
	Potato	*At-HPT*, *At-γ-TMT*	*A. thaliana*	Agrobacterium	Improved α-tocopherol content and abiotic stress tolerance	(Upadhyaya et al., 2020)
		atDGAT1; atWRI1	*A. thaliana*	Agrobacterium	Increased oil content	(Liu et al., 2017)
		siOLEOSIN	*Sesamum indicum*	Agrobacterium	Increased oil content	(Liu et al., 2017)
	Cotton	*GAFP*	*Gastrodia elata*	-	Verticillium wilt resistance	(Wang et al., 2016c)
		SOD	*Gossypium hirsutum*	Agrobacterium	Increased antioxidant activity and abiotic stress tolerance	(Payton et al., 2001)
		APX	*Pisum sativum*	Agrobacterium	Increased antioxidant activity and abiotic stress tolerance	(Payton et al., 2001)
		GR	*A. thaliana*	Agrobacterium	Increased antioxidant activity and abiotic stress tolerance	(Payton et al., 2001)
	Tomato	*codA*	*Arthrobacter globiformis*	Agrobacterium	Resistance toward chilling stress	(Park et al., 2004)
		rs-afp2	*Raphanus sativus*	Agrobacterium	Increased fungal resistance	(Parashina et al., 2000)
		V1	Tomato yellow leaf curl virus (TYLCV)	Agrobacterium	Resistance against TYLCV	(Kunik et al., 1994)
	Peanut	*DREB1A*	*A. thaliana*	Agrobacterium	Better drought tolerance	(Bhatnagar-Mathur et al., 2007)
		BjD	*Brassica* sp.	Agrobacterium	Resistance to fungal pathogens	(Swathi Anuradha et al., 2008)
		SbpAPX	*Salicornia brachiata*	Agrobacterium	Resistance to salt stress	(Singh et al., 2014)
	Chickpea	*P5CSF129A*	*Vigna aconitifolia*	Agrobacterium	Drought tolerance	(Bhatnagar-Mathur et al., 2009)

the effective detection of key genes and QTL associated with flowering time. A reproductive cross involving the late-flowering soybean cultivar "Zigongdongdou (ZGDD)" and the early flowering cultivar "Heihe27 (HH27)" results in 308 recombinant inbred lines (RILs). Pseudo-response regulator protein, GmPRR37, detected from QTL mapping along with subsequent candidate gene analysis reveals it is accountable for major QTL, qFT12-2. Early flowering under normal, long-term (NLD) conditions is demonstrated by Gmprr37-ZGDD soybean mutants created using CRISPR/Cas9 (Wang et al., 2020a). The mutagenesis induced by CRISPR in the GmSPL gene in soybeans promoted the quantity of roots, leaves, and nodes and thereby affected the plant architecture (Bao et al., 2019). Many mid- and high-latitude growing soybean varieties mature and blossom early at lower latitudes; this normally contributes to poor crop yield. *GmFT2a* and *GmFT5a* function as a major flowering activator and integrators among 10 *FT* homologs identified in soybean. Under short-day conditions, delay flowering by approximately 31.3 days is exhibited by the *ft2aft5a* double mutants and also significantly increases the number of nods, pods, and seeds per plant in comparison with wild varieties. Thus, mutants can increase soybean breeding and regional adaptability (Cai et al., 2020).

7.4.1.2 RICE

The newly identified CRISPR-Cas mechanism is CRISPR-Cpf1 which is introduced as a molecular method for selective genome modification. In rice plants, both targeted genes *OsPDS* and *OsBEL* are successfully mutated using CRISPR-Cpf1 (Xu et al., 2017). sgRNA:Cas9 induced gene knockout in the rice plant with a mutation rate of 9.4% in the rice phytoene desaturase gene *OsPDS*-SP1 and 7.1% in gene *OsBADH2* (Shan et al., 2013). Use of CRISPR-Cas9 for intron-induced site-specific gene replacement and insertion via the NHEJ pathway is highly appreciated. Using this approach, the substitution of rice endogenous gene, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), with TIPS amino acid leads to glyphosate-resistant plants (Li et al., 2016a). In rice cultivar Zhonghua 11, genes responsible as regulators of grain numbers (*Gn1a*), panicle architecture (DEP_1), size of grain (GS_3), and plant architecture (IPA_1) are mutated using CRISPR-Cas 9 technology. Targeted gene editing in the first generation of transformed plants shows highly efficacious results of 42.5% in *Gn1a*, 67.5% in DEP_1, 57.5% in GS_3, and 27.5 in IPA_1. Thus, the modification of key genes using CRISPR

facilitates high-yielding potential in plants (Li et al., 2016b). CRISPR-Cas 9 mediated mutagenesis in *SBEI* and *SBEIIb* demonstrated 26.7–40% mutation frequency in the T0 generation. An increase of 25% in amylose content (AC) and 9.8% in resistant starch (RS) and a higher fraction of long chains in amylopectin is shown by *sbeII* mutant. Thus, the improved nutritional importance of starch in rice would offer probable benefit to health (Sun et al., 2017). CRISPR-Cas method is a powerful strategy to boost the resistance against blast disease in rice plants using *OsERF922* gene as a target (Wang et al., 2016a).

7.4.1.3 MAIZE

A novel negative regulator variant of ethylene, *AGROS8*, is generated with CRISPR-Cas technology, elevates the level of *AGROS8* transcript in the *AGROS8* variant, and eventually augments the net maize grain quantity by 176 kg per acre under the environmental conditions that promote the flowering stress, furthermore, no reduction in net quantity is reported when water is adequately provided (Shi et al., 2017). CRISPR-Cas genome modification in the *waxy* allele of maize plant leads to an agronomically superior hybrid with a higher yield of 5.5 bushels per acre. Therefore, this method was reported as way quicker and more productive compared to the products obtained using modern trait introgression methods (Gao et al., 2020). CRISPR-Cas approach is successfully utilized in developing herbicide-resistant maize by potentially targeting ALS genes (*ALS1* and *ALS2*). The plant modified using these strategies confers modified endogenous genes and is significantly transferred to the next generation (Svitashev et al., 2015).

7.4.1.4 CRISPR FOR NITROGEN FIXATION

In recent years, new genome alteration technologies such as ZFNs, TALENs, and CRISPR-Cas have been developed and deployed to utilize on leguminous plants where major advancements have been made. As for legumes, the first use of CRISPR/Cas9 was for the alteration of the soya bean genome using hairy root transformation conducted by *Rhizobium rhizogenes* (Wang et al., 2017b). AZC_2928 gene of *Azorhizobium caulinodans* ORS571 regulates chemotaxis and biofilm formation and is efficiently knocked out using CRISPR/Cas9. The knockout of these genes achieves an editing efficiency

of 34% and also enhances the attachment, interaction, and biofilm formation ability of nitrogen-fixing bacteria and their host plant (Wang et al., 2020b). Effective downregulation of symbiosis receptor-like kinase (*SYMRK*), a symbiotic nitrogen fixation gene, has been reported in *Lotus japonicus* (Wang et al., 2016b).

7.4.2 TALEN

Numerous articles have been published in the last 5 years evidencing the successful precise modifications in the rice genome using TALENs. In rice, the major factor that influences the viability and longevity of seeds is lipoxygenases (LOXs), responsible for converting polyunsaturated fatty acids to hydroperoxide via dioxygenation. TALEN-mediated knockout of the endogenous *LOX3* gene results in the advancement in grain quality and storage durability (Ma et al., 2015). In rice, the *badh2* allele produces betaine aldehyde dehydrogenase (BADH2), which influences the synthesis of 2-acetyl-1-pyrroline (2AP), a major fragrant element of rice. It is efficiently knocked out using the TALEN method, which results in increasing the amount of fragrance compound in rice and also in the creation of fragrant rice from nonfragrant varieties (Shan et al., 2015). TALEN-mediated double point mutation in acetolactate synthase gene (*OsALS*) in rice successfully develops herbicide-resistant rice. The efficiency of 1.4–6.3% is obtained in 9 homologous recombination events with distinct *OsALS* genotypes in the T0 generation. The herbicide resistance can be effectively transferred into subsequent generations *(*Li et al., *2016c)*. In rice, *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) and *X. oryzae* pv. *oryzae* (*Xoo*) are leading pathogens that cause bacterial leaf streak and leaf blight, respectively. The identification of *tal7* resulted from the individual gene transfer from the hypervirulent strain (*Xoc* RS105) to the hypovirulent strain (*Xoc* YNB0-17). *Tal7* gene binds with promoter genes *Os09g29100* and *Os12g42970* in rice which encodes Cyclin-D4-1 and GATA zinc finger family protein, respectively. *Tal7* gene downregulates *avrXa7-Xa7* defense in rice with upregulation of *Os09g29100*. Thus, the TALEN-mediated modification of *Tal7* interaction sites in *Os09g29100* promoter leads to the reduction in the binding of *Tal7* and reduced severity of the disease. Thus, this method depicts enormous potential in engineering rice plants, which pose less susceptibility to disease-causing pathogens (Cai et al., 2017).

7.4.3 GENE EDITING IN POLYPLOID CROPS

7.4.3.1 WHEAT

Globally, the genetic advancement in wheat is less than 1% which is inadequate to satisfy the potentially rising demands. Thus, genome editing approaches can boost growth by incorporating accurate and specific modifications at the genomic level. Relatively less scientific data is available on the use of the CRISPR/Cas system in wheat as compared to other plants (Kumar et al., 2019). Wang et al. (2014) reported the application of the CRISPR and TALEN approach to introduce mutation at the targeted MLO (Mildew Resistance Locus) loci. Using these methods, the knockout of three *TaMLO* (*TaMLO-A1, TaMLO-B1,* and *TaMLO-D1*) confers reduces the chances of powdery mildew infection establishment in bread wheat. CRISPR has also been used for precise genome modification in wheat protoplasts to specifically target ethylene-responsive factor 3 (TaERF3) and wheat dehydration-responsive element binding protein 2 (TaDREB2) transcription factor genes that are associated with environmental stress regulation (Kim et al., 2018). The biolistic method of CRISPR/Cas9 ribonucleoproteins (RNPs) comes forward as an efficient and productive genome editing method (Liang et al., 2017).

7.4.3.2 CAMELINA

Camelina seed oil is the healthier option for cooking and industrial oil. knockout of the target *FAD2* gene (fatty acid desaturase 2) in *Camelina sativa* is successfully achieved using CRISPR/Cas9 technology. In total fatty acid composition, the concentration of oleic acid increases to 50% from 16%. Along with this, the decrease in undesirable fatty acids, such as linoleic and linolenic acids, is reduced approximately by a factor of 4 (Jiang et al., 2017). A similar result is obtained from (Morineau et al., 2017) on the use of CRISPR/Cas technology in the targeted knockout of three delta-12-desaturase (*FAD2*) genes from *C. sativa*, resulting in the enhancement of oleic acid concentration ranging from 10 to 62% in oil.

7.4.3.3 POTATO

Globally, potatoes are among the most consumed crops, which makes it imperative to adapt to the changing climatic conditions. However, very limited studies have been done on the implementation of CRISPR/Cas technology on potatoes. The second exon of the *StIAA2* gene is mutated via the CRISPR/Cas approach. The targeted mutation is efficiently transferred into

the T1 generation of the plant (Wang et al., 2015). One of the two single-guided RNAs (sgRNAs) targeting *Acetolactate synthase1* (*StALS1*) gene in potato is expressed through CRISPR/Cas system and tested for the induction of mutation in callus and steady events of agrobacterium-mediated diploid and tetraploid potatoes with either conventional T-DNA or a modified geminivirus T-DNA is also evaluated (Butler et al., 2015). Complete removal of the granule-bound starch synthase (*GBSS*) gene in potatoes is mediated by the CRISPR/Cas9 system (Andersson et al., 2017). The *St16DOX* gene, which produces 16α-hydroxylase involved in the formation of steroidal glycoalkaloids (SGA), is removed using CRISPR/Cas9 technology. The accumulation of SGA imparts bitterness and toxicity to different organisms. New CRISPR/Cas9 vector pMgP237-2A-GFP is used for *Agrobacterium rhizogenes*-driven transformation and development of two SGA-free *St16DOX*-disrupted hairy root lines of potato (Nakayasu et al., 2018).

7.4.3.4 COTTON AND PEANUT

In cotton, mutation in three distinct genes *GhPDS*, *GhCLA1*, and *GhEF1* is induced using CRISPR/Cas system. Multiple gene targeting with concurrent expression of sgRNA in cotton is demonstrated. Moreover, the albino phenotype is generated due to mutation in *GhCLA1* (Gao et al., 2017). CRISPR/Cas9-mediated genome modification of the *Gh14-3-3d* gene shows resistance to *V. dahlia.* The resulting plant shows higher resistance compared to the wild type; furthermore, careful breeding has the potential to yield disease-resistant cotton (Zhang et al., 2018). In cotton, a selective mutation in *Cloroplastos alterados 1* (*GhCLA1*) and *vacuolar H^+-pyrophosphatase* (*GhVP*) results in the generation of transgenic cotton cultivars which exhibit 47.6–81.8% mutation efficiency (Chen et al., 2017).

Peanut (*Arachis hypogaea* L.) is an allotetraploid crop grown worldwide and is significantly important due to high oil production. CRISPR/Cas has been used for inducing mutation in *ahFAD2A* and *ahFAD2B*, responsible for producing fatty acid desaturases that participate in the formation of linoleic acid from oleic acid. Thus, the mutation in *ahFAD2A* induces higher oleic acid content and improves overall oil quality (Yuan et al., 2019).

7.4.3.5 CITRUS

For the first time, Lachnospiraceae bacterium ND2006 derived CRISPR-LbCas12a (LbCpf1) was effectively employed to edit the genome of citrus.

The *CsPDS* gene of *Ducan grapefruit* is edited by LbCas12a via Xcc-facilitated agroinfiltration, where LbCas12a edits the genome of citrus (Jia et al., 2019). In the citrus plant, the most severe disease, that is, citrus cranker, results from infection of *X. citri* subsp. *Citri* (Xcc). Homozygous and biallelic citrus cranker-resistant citrus plant is obtained in T0 generation by mutating LATERAL ORGAN BOUNDARIES 1 (LOB1) by CRISPR SpCas9 (Jia and Wang, 2020). *Arabidopsis* YAO promoter-regulated CRISPR/Cas9 system is capable of efficiently editing the genome of citrus by using the PDS gene as a target (Zhang et al., 2017). The development resistance against citrus canker via targeting the promoter of the susceptibility gene *CsLOB1* present in Wanjincheng orange (*C. sinensis* Osbeck) is documented using the CRISPR/Cas9 system. A modification of 42% is evident in PthA4 EBE in mutant citrus plants. Thus, the editing of gene *CsLOB1* using CRISPR technology imparts heavy resistance to citrus canker (Peng et al., 2017).

7.4.3.6 TOMATO

Due to its features of basic diploid genetics, ease of transition, successful proliferation, and short life cycle, Tomato is an ideal model for the production and maturation of fleshy fruit. Deletion of the *lncRNA1459* gene using the *CRISPR/Cas9* method causes a reduction in the accumulation of ethylene and lycopene, thus repressing the repining of tomato in the *lncRNA1459* mutant plant as compared to wild type (Li et al., 2018). In tomato, the *RIN* gene is responsible for the production of MADS-BOX transcription factor, which is the key player in the ripening process. Mutation in this gene produces an RIN protein defective mutant, which shows incomplete ripening by producing a lower amount of red pigment in fruit, whereas wild type shows complete red pigmentation, thus, this explains the importance of RIN protein in the ripening of tomato (Ito et al., 2015). Mitogen-activated protein kinases (MAPKs) are crucial proteins produced as a response to drought stress. CRISPR-mediated knockout of the *SlMAPK3* gene results in the *SlMAPK3* mutant, which under drought conditions displays a serious wilting effect, higher level of hydrogen peroxide content, decreased production of antioxidant enzyme, and more membrane damage as compared to wild type. Therefore, *SlMAPK3* is among the most important product of drought response in tomato plants which operates by curbing the oxidative damage caused to the cell membranes along with further regulating the expression genes associated with stress response (Wang et al., 2017a). CRISPR-mediated somatic

mutation in a parthenocarpic gene, *SLIAA*$_9$, produces a mutant plant with a 100% mutation rate, which generates seedless fruit and morphologically distinct leaf shape (Ueta et al., 2017).

7.5 CHALLENGES

Although the success of genetic engineering in the creation of plants with good durability and production yield is obvious, there are some concerns and challenges that need proper focus. For instance, interactions and gene flow between transgenic plants and natural biodiversity have piqued the interest of the scientific community. On the contrary, consumers and farmers around the globe have mixed attitudes toward GM crops; hence, understanding the current opinions of the population toward GM crops can greatly aid in the development of future strategies. Furthermore, products originating from GM crops raise certain concerns related to safety among consumers that need to be dealt with before moving ahead.

7.5.1 GM CROPS AND BIODIVERSITY

In agriculture, transgenic crops resistant to herbicides and pests are most widely utilized; hence, the effects of such crops on their target and nontarget species are of utmost concern. So far, there are no major reports of damage or unwanted impacts on nontarget species and organisms. On the contrary, herbicide and pest-resistant crops have created high selection pressure on their target organisms, which has caused a reduction in their population. However, the survivors of these target species are resistant to such pressure, creating GM crops. Currently, EPSPS and *Bacillus thuringiensis* (Bt) protein-expressing plants are widely utilized, which has resulted in the emergence of glyphosate-resistant weeds and Bt-resistant pests (Baxter et al., 2011; Gassmann et al., 2011; Shaner et al., 2012). Another persistent concern with transgenic crops is hybridization and gene transfer in their wild relatives, nonrelative plants, and microbes, which can result in the permanent introgression of transgenes into unwanted and unintended species. Such unwanted transgenic species pose the danger of further added selective pressure on pathogens and pests even in the absence of any transgenic crop, encouraging them to develop resistance toward the gene in question. For instance, transgenic *B. napus* with glyphosate resistance and glufosinate resistance have been utilized for a while, and studies have confirmed the

escape of these genes in wild species as well. Most of the wild *B. napus* plants have resistance to at least one of the aforementioned herbicides, and some have resistance to both (Schafer et al., 2011). Although the occurrence of such gene transfers and appearances of resistance in populations is a natural phenomenon that exists even without transgenic crops, nevertheless, it is important to reduce the velocity and freedom with which such transfers take place among transgenic and wild species. To implement such restrictions, certain precautions, such as male sterility, delayed flowering etc., need to be explored (Kwit et al., 2011; Nicolia et al., 2014).

Contamination of non-genetically modified (non-GM) products with GM products has been an issue that mainly arises via three major phenomena: (i) pollen transfer between GM and non-GM plants, (ii) during harvest seed left behind in the field, (iii) mixture of GM, and non-GM agricultural product during transportation and storage. Pollen-mediated gene transfer (PMGT) study carried out on wheat plants suggests that such transfers are most likely to happen within the field; hence, additional distance and isolation of GM wheat is sufficient to limit such PMGT (Foetzki et al., 2012). Soil contains a heavy load of microorganisms that can receive the transgene from GM crops. Moreover, the frequent occurrence of horizontal gene transfer among bacteria, along with their ability to develop symbiotic and mutualistic relationships with plants makes them prime candidates to receive such transgene from plants. To avoid such transfers, the utilization of antibacterial resistance genes as a selectable marker gene (SMG) has been proposed, but due to concerns related to the spread of such genes in bacterial communities, alternatives to these methods are needed (Rosellini, 2012). To reduce such unwanted transfers, native plant genes as an alternative to SMGs have been recommended, along with the complete elimination of all transgenes from final products (EFSA, 2011).

7.5.2 GM CROPS AS FOOD

On average humans consume around 1.0 g of DNA per day from different food sources that are not genetically altered. Safety concerns related to transgenic DNA and RNA ingestion are still a matter of ongoing debate among researchers, but so far, studies have proven that these transgenic matters are safe to ingest and pose no health hazard (Parrott et al., 2010). Moreover, general food preparation practices, such as baking, frying, etc., reduce the amount of intact transgenic DNA and RNA present in food. Concerns for

the transformation of intestinal microflora via horizontal gene transfer (HGT) have been expressed for a while, but the occurrence of such events is extremely rare (Rizzi et al., 2012). Whereas for humans, the transfer of transgene from food to the gastrointestinal (GI) tract is possible only when the gene is present at a high copy number and such an event has been noted for rabbit meat (Forsman et al., 2003). To further ensure the safety of consumers, GM crops are tested for their nutritional and chemical composition. Any deviation in the profile from non-GM plants is further assessed for human safety before the product is allowed for selling (EFSA, 2011). For instance, cauliflower mosaic virus (CaMV) promoter *P35S* is extensively utilized in GM crops for stable gene expression, but this promoter has an overlapping region with viral gene *VI*; hence, it is recommended that overlapping regions are removed before utilization (Podevin and Du Jardin,, 2012).

7.5.3 GM CROPS AND FARMERS

USA, Brazil, and Argentina are the current leading producers of GM crops. Farmers from these countries acknowledge the profits of using GM crops, such as lower expenses for weed and pest management, which ultimately reduces the costs of plant protection. However, on the contrary, the profitability of such crops is debatable because, in some instances, farmers have failed to get higher profits due to royalties associated with these crops (Massarani et al., 2013). In some cases, associated with GM soy, higher profits were initially reported that were reduced later (Van Scharrel and Van Der Sluis, 2004). Recently, a study was conducted to assess the attitude of Georgian farmers toward GM crops. Survey data of 611 farmers revealed that farmers have a low level of knowledge of GM crops, and up to 91% of farmers are not ready to accept such crops. This negative attitude is the fruit of the thought process that natural seeds are sufficient for production rather than a lack of information about GM crops. Also, it was found in the study that younger and educated farmers were more welcoming of GM crops (Nugzar and Teona , 2017). A similar study was carried out for the farmers of Florida who are associated with citrus production. Citrus industries and famers of Florida have suffered great losses due to bacterial disease spread. Hence, farmers were surveyed for their attitudes toward acceptance of GM citrus. Farmers of the region had a decent knowledge of GM crops. Moreover, they were ready to accept GM crops, but regardless of the losses they suffered, money was not their primary concern. One of the biggest queries

raised by the majority of farmers was the effects of GM citrus on consumers and the environment. Hence, it is evident that educational, economic, and cultural aspects play very crucial roles in the spread of GM crops in different communities.

7.6 FUTURE PROSPECTS

Biofortification and stress management to increase the amount of yield produced using existing resources remains a matter of priority. In biofortification, pathways, and genes associated with cobalamin production, multivitamin regulation, and manipulation need to be targeted for the creation of cultivars with the ability to provide multiple vitamins in a single intake. Moreover, complex internal regulation of vitamin synthesis pathways needs more exploration to create cultivars with stable nutrient increases without reducing the formation of nontargeted nutrients. A better understanding of the synergy between biotic and abiotic stress is crucial to dampen their negative impacts effectively. QTL that can target simultaneously more than one stress-causing agent needs to be identified in various plants of agricultural importance. Extended studies to better understand the spread of transgene among wild varieties and unintended organisms are required. Also, effective strategies for transgene escape from GM plants are the need of the hour. Although the products of transgene plants are safe for human consumption, rare events such as dsRNA and DNA absorption by the GI tract are still concerning and require further exploration. At last, the opinions of farmers and consumers about GM plants need to be heard and understood for better, safer, and unanimous utilization of transgene cultivars.

KEYWORDS

- **gene editing**
- **abiotic stress**
- **biotic stress**
- **CRISPR**
- **agriculture**

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CHAPTER 8

Protein Synthesis

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ABSTRACT

Three-base sequence "words" used to "read" DNA are utilized to direct the production of certain amino acids. These three-base triplets, also known as codons, are arranged along the DNA in an orderly manner. A gene is a section of DNA that is linear and codes for a specific protein. The collection of all the genes found in the human population is known as the human genome. Even though just a small quantity of DNA may be observed in the mitochondria, and while the cytoplasm is where almost all protein synthesis occurs, the nucleus of the cell houses the majority of the DNA. The process of making proteins from polypeptides is the process of creating chains of amino acids. It is the second component of the essential genetics dogma. It takes place in the cytoplasm's ribosomes. The process of translation involves decoding the genetic code from mRNA to produce proteins. Initiation, elongation, and termination are the three stages that make up the overall translation process. Protein synthesis is followed by proteolysis and protein folding. After translation and the creation of peptide bonds, a polypeptide chain is exposed to an enzymatic procedure called posttranslational modification. To provide the best cellular localization and performance, the side chains and terminals of the polypeptide might be altered. Polypeptide chains fold into secondary and tertiary structures during protein folding.

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8.1 INTRODUCTION

Amino acid polymers make up proteins. One protein is synthesized by hundreds of amino acids linked together. As building blocks or monomers, amino acids come in 20 different types, and they can be combined in an endless number of ways. It may include hundreds or thousands of monomers. Polypeptide chains are the name for these lengthy chains. The constituent components of proteins are known as amino acids. These can be brought into being as a pool of amino acids in the cytoplasmic matrix. Protein synthesis is made up of two processes: transcription and translation. Polypeptide chains completed by amino acids are hundreds to thousands in number and are used to make up the enormous molecules known as proteins. Twenty different types of amino acids combine to make these polypeptide chains. A basic amino group ($–NH_2$) and an acidic carboxyl group (–COOH) combine to form an amino acid. The arrangement of the amino acids in the polypeptide chain gives each protein a unique structure. Protoplasmic proteins are essential components and the building blocks of cells. They participate in the cell's structural and functional organization. Functional proteins, such as enzymes and hormones, regulate the cell's metabolism, biosynthesis, creation of energy, control of growth, and sensory and reproductive functions. Most biological reactions that include enzymes use them as catalysts. Enzymes even control the expression of genes. The proteinaceous enzymes regulate the transcription of RNA and the replication of DNA (Troyanskaya et al., 2002).

8.2 COMPONENTS OF PROTEIN SYNTHESIS

The building blocks of protein synthesis are as follows: (Hershey and Merrick, 2000)

8.2.1 DNA

The DNA molecule contains the genetic data for the variety of amino acids in a polypeptide chain. Protein synthesis is governed and managed by DNA's structure and characteristics. The cytoplasm, where eukaryotic proteins are made, receives information from the nucleus in the form of messenger RNA (mRNA). The mRNA conveys information about the amino acid order of the polypeptide chain that has to be synthesized. A genetic code serves

as the vehicle for this message or data. The language used to build amino acids into polypeptides is specified in this genetic code. By interpreting the genetic code, it is converted into a string of amino acids. Proteins that are identical to those already present in a cell must be produced for it to keep its unique properties. To make the desired polypeptides, the provided quantities and types of amino acids must be coupled in a certain order, which requires specificity control in protein synthesis. through the DNA double helix's three consecutive nitrogenous bases, mRNA sequences collectively form the genetic code or DNA, which regulates specificity. Each base triplet represents the encoding of a particular amino acid. The proteins made by a cell are similar to the proteins that already exist since DNA is often stable.

8.2.2 THREE TYPES OF RNAS

8.2.2.1 MRNA

The instructions for producing proteins are found in the mRNA. It has been found that in eukaryotes, the amino acid order of the synthesized polypeptide chain and the nucleotide sequence of mRNA are colinear. It is created inside the nucleus and has a 5′ cap, 5′ untranslated region (5'UTR) region, coding region, 3'UTR region, and poly(A) tail. The copies required for gene expression are found in the coding region of a DNA segment. Start and stop codons are located, respectively, at the 5' end and the 3′ end (Shatsky et al., 1991).

8.2.2.2 TRNA

The necessary amino acid is transported to the ribosome by transfer RNA (tRNA), also known as tRNA, where it is joined to the increasing chain of amino acids. The acceptor stem and the anticodon arm are the two key locations. The mRNA codon's corresponding base pair anticodon is found in the anticodon arm. A particular amino acid is connected to the acceptor stem at this location, resulting in the term "aminoacyl-tRNA" for the tRNA that has the attached amino acid. The expanding polypeptide chain is held in place by a peptidyl-tRNA. There is a complementary anticodon for every of the amino acid codons that each tRNA carries. As an illustration, the tRNA anticodon UUC will temporarily attach to the codon AAG since It is the anticodon for the AAG-transcribed amino acid lysine. When the tRNA is joined to mRNA, the amino acid within is released. rRNA then helps in the

creation of linkages between the amino acids as they are given one at a time to the ribosomes, resulting in the construction of a polypeptide chain. When the polypeptide chain reaches its longest length, a stop codon is achieved (RajBhandary, 1994).

8.2.2.3 RRNA

It functions as a ribosome component. Known for acting as a location for protein synthesis, Prokaryotic and eukaryotic cells both contain the cytoplasmic element known as the ribosome. Eukaryotes and Prokaryotes can be distinguished by their ribosomes. Eukaryotes have 80S ribosomes, whereas prokaryotes have 70S ribosomes. The ribozyme, which is present in the most significant portion, promotes the creation of peptide bonds among amino acids, two subunits of differing sizes. The largest part acts as the ribozyme, which stimulates the development of peptide bonds among amino acids. One of the three binding sites for rRNA, the aminoacyl-tRNA docks at the aminoacyl-tRNA site, the peptidyl-tRNA attaches at the P site, and the tRNA departs the ribosome at the E site.

8.2.3 RIBOSOMES

Since ribosomal RNA (rRNA) has an enzymatic activity and proteins make up ribosomes, they are also referred to as ribozymes. The big subunit of the ribosomes has three active sites (E, P, and A), which are essential for the ribosomes' catalytic activity. The ribosomes also have two protein and rRNA subunits (Green and Noller, 1997). A macromolecular structure called a ribosome control how proteins are made. A nucleoprotein particle with two subunits is called a ribosome. Together, these two unique subunits form a polypeptide chain. In *Escherichia coli*, the ribosome is a 70S particle with the 30S and 50S subunits. The concentration of magnesium affects both their connection and dissociation (Nissen et al., 2001). The decoding center of the ribosome's small subunit is where charged tRNAs decode mRNA codons. A newly synthesized peptide chain's succeeding amino acids form peptide bonds with one another thanks to the big subunit's peptidyl transferase core. Proteins and rRNA make up both the 30S and 50S subunits (Schluenzen et al., 2000). To form peptide bonds between the following amino acids in a newly synthesized polypeptide chain, the ribosome is primarily responsible. There are two channels inside the ribosome. The decoding core is located in

the channel through which the linear mRNA enters and exits. The charged tRNAs can reach this channel. Through the other pathway, the freshly created polypeptide chain eludes detection (Ogle et al., 2003).

8.2.4 Enzymes

The peptidyl transferase enzyme is the main one used in translation. It is present in ribosomes and functions as an enzyme that aids in the creation of covalent peptide bonds between neighboring amino acids. The enzyme's function during translation is to use tRNAs to create peptide connections between nearby amino acids. One of the two substrates contains both the amino acid being added to the chain and the growing peptide chain that the enzyme uses to carry out its job. Peptidyl transferase, which is a component of the primary ribosomal subunit, is primarily responsible for catalyzing the addition of amino acid subunits to polypeptide chains so that they can lengthen. The function of the peptidyl transferase enzyme is mediated by rRNA, a ribozyme comprised of ribonucleotides. Prokaryotic cells include the 23S subunit, whereas eukaryotic cells contain the 28S subunit, which has a peptidyl transferase between the tRNA's A and O sites.

8.3 STEPS INVOLVED IN PROTEIN SYNTHESIS

Main steps of protein synthesis:

- Transcription
- Translation
- Posttranslation

8.3.1 TRANSCRIPTION

A mRNA template is translated from DNA to act as a template for protein translation with the help of the enzyme RNA polymerase. This template includes the protein's amino acid sequence in a trinucleotide code. Transcription is, therefore, viewed as the beginning of gene expression. Similar to DNA replication, transcription moves in a 5′-3′ orientation. Contrary to DNA replication, transcription does not require a primer to begin, and uracil pairs with adenine in place of thymine. Prokaryotic TATA box 35 base pairs or so upstream of the starting point 5'-TGTT-GACA-3'; a different sequence known as TATA Box or Pribnow box is located approximately 10 base pairs upstream. Goldbeg Hogness box, Mammals' slightly different TATA boxes

are referred to as gold beg Hogness boxes. It is situated at position 25–30. Another sequence known as the CAAT Box is located further upstream between 70 and 80 bp. Transcriptional elongation, promoter escape, initiation, and termination are the steps in the process.

Step 1: Initiation

With the help of particular transcription factors (TFs), initiation takes place when the RNA polymerase binds to the DNA promoter. This results in the opening (unwinding) of the DNA's promoter region, which creates a transcription bubble. The RNA polymerase attaches to a transcription start site in the transcription bubble. Short mRNA transcripts (between 2 and 15 nucleotides) are released as a result of a phase of failed cycles of synthesis.

Step 2: Promoter escape

To move on to the elongation step, the RNA polymerase must first exit the promoter.

Step 3: Elongation

Base pairs are made with the nucleotides on them during elongation as RNA polymerase proceeds down the template (noncoding) strand of DNA. As a result, an mRNA transcript contains a copy of the coding strand of DNA, with the exception of the thymine's, which is converted to uracils. RNA polymerase aids in the formation of the sugar-phosphate backbone.

Step 4: Termination

Termination is the final phase. The mRNA transcript undergoes further processing in eukaryotes. It passes through splicing, capping, and polyadenylation.

8.3.1.1 POSTTRANSCRIPTIONAL MODIFICATIONS

There are three critical phases in posttranscriptional changes:

1. Adding a 5' cap on the pre-mRNA molecule's 5' end.
2. The 3' end of the pre-mRNA molecule receives a 3' poly(A) tail.
3. Intron deletion by splicing of RNA.

The pre-mRNA molecule's 5' end is connected to the 5' cap, which is created by the methylation of the guanine nucleotide. The 5' cap differentiates

mRNA from other RNAs in the cell, aids ribosome binding to mature mRNA to start translation, and prevents mature mRNA molecules from being degraded before translation. Comparatively, the 3' poly(A) tail, which is made up of 100–200 adenine bases and is connected to the mRNA molecule 3' end. The cell can determine whether the 5' cap and 3' tail denote the entire mRNA, thanks to these particular mRNA changes. The altered pre-mRNA molecule is then subjected to RNA splicing. Exons are nucleotide sequences that directly result in the production of a protein, whereas introns do not. Due to the presence of introns and exons in both the precursor mRNA molecule and the underlying DNA sequence. A mature mRNA molecule that encodes a protein must undergo splicing. During splicing, a multiprotein complex called a spliceosome, consisting of about 150 proteins and RNA, is in charge of removing the intervening introns from the pre-mRNA molecule. Through nuclear pores, the mature mRNA molecule is then transferred from the nucleus to the cytoplasm (Mangus et al., 2003).

8.3.2 TRANSCRIPTION IN PROKARYOTES

8.3.2.1 RNA POLYMERASE IN E. COLI

All three different forms of *E. coli* RNA molecules—messenger, transfer, and ribosomal—are synthesized by a single species of enzyme. The discovery in eukaryotic cells, where a variety of RNA polymerases have been identified, is in contrast to this. Five protein subunits make up the entire *E. coli* RNA polymerase: two α subunits, one β subunit, and one β′ subunit, and a sigma subunit. Only DNA sequence recognition at a promoter location necessitates the component. Initiation, elongation, and termination are the three stages of the transcription process (Troyanskaya et al., 2002).

8.3.2.2 INITIATION

An exact location just in front (upstream) of the gene where the RNA polymerase enzyme binds to a DNA molecule to begin transcription. Promoter is the term for these areas of attachment. An RNA polymerase recognizes a promoter as a site to attach to DNA to start transcription. A promoter is a brief nucleotide sequence. Although each promoter has a unique DNA sequence, all promoters share a consensus sequence that, for *E. coli*, consists of two distinct parts: a –35 box and a –10 box. The exact DNA sequence

at a promoter site differs from gene to gene, but all promoters have this consensus pattern. As the other subunits work together to catalyze RNA synthesis during transcription, the σ subunit binds to the promoter sequence after being recognized. After about eight ribonucleotides have been polymerized into RNA, the subunit separates from the rest of the enzyme, allowing the core enzyme to carry out the remainder of transcription. The RNA polymerase complex initially has an extended structure and can cover up to 80bp of DNA; as it moves down the DNA, the core enzyme only covers around 30bp. At a temperature of 37°C, the ideal rate of polymerization is thought to be around 40 nucleotides per second (Hosid and Bolshoy, 2004).

8.3.2.3 ELONGATION

In the transcription process, the RNA polymerase moves along the DNA's length elongation step, reading the template data in the 5′-to-3′ direction. As a result, an RNA–DNA hybrid develops in the unwound areas. It should be emphasized that maintaining antiparallel nature is a prerequisite for all double-stranded nucleic acid structures. The freshly synthesized RNA strand is pushed aside as the RNA polymerase advances down the DNA template, and the DNA duplex recovers behind the transcriptional complex (Altmann et al., 1994).

Three things should be emphasized:

1. The transcript is shorter than the gene in one respect: Position +1 is not the beginning of the gene, but rather the point at which the production of the RNA transcript actually starts. Before the RNA polymerase enzyme reaches the gene, it almost always transcribes a leader segment. Every gene has a different leader sequence, which varies in length. Similar to this, when the enzyme reaches the gene's terminus, it continues to transcribe a trailer segment before termination takes place (Arndt and Chamberlin, 1990).
2. The double helix is only partially unfolded at any given time. Once the process of elongation has begun, the polymerized portion of the RNA molecule can gradually separate from the template strand, allowing the double helix to revert to its initial configuration. There are 12–17 RNA–DNA base pairs in the open region or transcription bubble. This is crucial since there is not much molecular room for error when unwinding a section of the double helix because it requires over-winding the nearby regions (Bardeleben et al., 1994).

3. The rate of elongation fluctuates. It has been noticed that the enzyme would occasionally slow down, stop, and then resume its forward motion, or even go so far as to reverse for a little period of time, removing ribonucleotides from the end of the freshly synthesized transcript before continuing (Burova et al., 1995).

8.3.2.4 TERMINATION

Although the termination signals for *E. coli* genes differ greatly, they all share complimentary palindromes as a common trait. By allowing a stem-loop to develop in the expanding RNA molecule, the complementary palindromes have an impact on transcription. When a stem-loop terminator is followed with a run of 5–10 A's in the DNA template, the transcript will remain connected to the template by a string of flimsy A–U base pairs after being translated into U's. It is believed that shortly after the stem-loop formation, RNA polymerase pauses and that the A–U base pairs break, causing the transcript to separate from the template (Brendel et al., 1986). There is no run of A's among other terminators. Rho-dependent terminators are the words used for them. Despite being a big protein, Rho is not a real RNA polymerase subunit. When the polymerase pauses at the stem-loop structure, Rho binds to the developing RNA molecule and prevents the DNA template and RNA transcript from joining up properly. The RNA polymerase enzyme separates from the DNA when transcription is stopped, releasing the RNA transcript (Henkin and Yanofsky, 2002).

8.3.3 TRANSCRIPTION IN EUKARYOTES

8.3.3.1 EUKARYOTIC RNA POLYMERASES

Each of the three RNA polymerases found in eukaryotic cells serves a specific functional purpose. By using the rDNA gene as a template, RNA polymerase I produces rRNA. The synthesis of mRNA is catalyzed by RNA polymerase II, and poly(A) polymerase is responsible for adding poly(A) tails afterward. RNA polymerase III synthesizes tRNA along with a number of different shorter nuclear and cytoplasmic RNAs. The fact that there are three distinct eukaryotic RNA polymerases suggests that there are three distinct promoter types that can each be controlled separately. Several proteins that make up

eukaryotic RNA polymerases interact with DNA at various places as well as with one another.

8.3.3.2 INITIATION

In eukaryotes, for transcription to begin, RNA polymerase II must recognize and bind to a promoter region. There is other nucleotide sequences involved in the transcription of transcripts besides this attachment site. The binding will also involve other potential sequences that span several hundred base pairs. A few TFs are additionally implicated. The RNA polymerase II attachment location is why the eukaryotic core promoter element is known as a "TATA box." The consensus sequence at the attachment site is as follows: 5-TATAAAT-3′.

The TATA box The TATA box is also known as the "-25 box" because it is often found approximately 25 nucleotides upstream of the site at which transcription begins. The attachment of RNA polymerase is mediated by a group of proteins called TFs to the TATA box. To distinguish them from the TFI and TFIII TFs, which function with RNA polymerase I and III, respectively, these TFs have been given the designations TFIIA, TFIIB, and so forth. The TFIID binds to the TATA box, which is most likely mediated by TFIIA, which is the initial step in this attachment. As soon as TFIID binds to the DNA template, the transcription complex begins to take shape. The following TFs to bind are TFIIB, RNA polymerase II, and both TFIIE and TFIIF TFs. The transcription complex is now prepared to start producing RNA (Hänggi et al., 1986).

8.3.3.3 TERMINATION

Because the 3′ ends of the transcripts are cut off right away after synthesis, it has been challenging to identify termination signals for RNA polymerase. However, it is evident that the trailer sections, which extend 1000–2000 bp downstream of the gene, may be quite long. As a result, it was hypothesized that genes transcribed by RNA polymerase II do not actually have predetermined termination locations. It is possible that one of the TFs breaks free from the complex near the end of the gene, destabilizing it and causing it to separate from the template later. It is discussed in the following Figure 8.1.

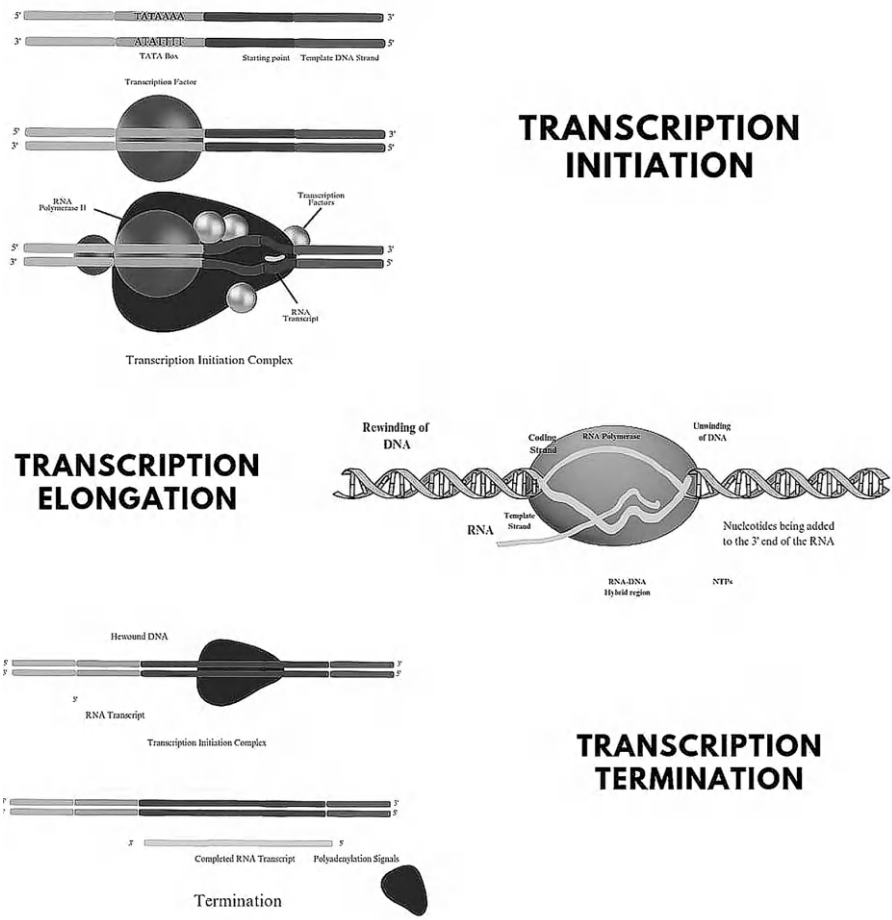


FIGURE 8.1 Transcription process.

8.3.3.4 GENETIC CODE

The nucleotide and amino acid sequences are represented by the genetic code. Words in dictionaries are represented by codons, which are triplets of nucleotides; there are 64 codons overall, three of which are nonsensical, corresponding to 61 codons for 20 amino acids. The nucleotides that make up DNA are shown by the letters A, T, G, and C. Codons serve to organize them. Genetic code is the term used to describe the group of codons. Codons are supposed to correspond to 20 amino acids. To provide each amino acid specificity, each codon needs three nucleotides (Altmann et al., 1994).

For a particular codon:

- A nucleotide can be combined in four different ways.
- Sixteen different ways.
- Sixty-four different ways (most suitable for 20 amino acids).

Three crucial components make up the genetic code:

1. The code is degenerate because all amino acids—aside from methionine and tryptophan—have more than one codon, giving meaning to every possible triplet. The majority of synonymous codons are categorized into families; for instance, GGA, GGU, GGG, and GGC all code for glycine (Henkin and Yanofsky, 2002).
2. The punctuation codons in the code are: When present in the center of a hetero-polymer, the three codons UAA, UGA, and UAG, which code for amino acids, block the production of proteins. One of these three termination codons is always present at the end of a gene, where translation must cease. Similar to this, AUG almost always appears at the beginning of a gene and designates the place where translation should start. Initiation codon (AUG), which codes for methionine. Thus, even if it might be added later, this amino acid will typically be at the amino terminus of newly synthesized polypeptides. by the protein's posttranslational modification.
3. The genetic code's universality: When the genetic code was finished in 1966, it was expected that all species would utilize it. However, there is a lot of evidence to suggest that the genetic code is fundamentally universal. However, there is some variance in how different organisms favor various codons. For instance, the eukaryotic AUA codon codes for the amino acid isoleucine, while *E. coli* uses the AUU and AUC codons instead. Another somewhat different code is used by mitochondrial genes, where tryptophan is encoded by the stop codon UGA. While generally coding for arginine and methionine, respectively, in the mitochondrial genome, AGG and AGA are stop codons, but isoleucine is often coded for by these codons. Other uncommon variations have also been discovered.

8.3.4 TRANSLATION

Amino acids are united together during translation in a definite order in accord through the genetic code. The ribosomes are present in the cytoplasm where protein syntheses take place. There are four stages (Kozak, 1999):

1. Activation, in which the amino acid forms a covalent link with the tRNA;
2. Initiation, in which the small ribosomal subunit joins to the 5′ end of the mRNA with the assistance of initiation factors;
3. Elongation, in which an elongation factor and the following amino-acyl-tRNA in line bind to the ribosome;
4. Termination (a stop codon is located at the ribosome's A-site).

8.3.4.1 ACTIVATION OF AMINO ACID

Messenger RNA (mRNA) leaves the nucleus and travels into the cytoplasm. An enzyme called aminoacyl tRNA synthetase is located in the cytoplasm and uses the mRNA strand as a template for protein production. Two binding sites are present on the macromolecule of the enzyme. the adenosine triphosphate (ATP) molecule binds, and the pyrophosphate is released, activating the amino acid methionine. The activated amino acid and the tRNA then attach to one another. This aminoacylated tRNA, also referred to as met tRNA, is released from the enzyme. The initial stage of protein synthesis has now started, as shown in Figure 8.2 (Davuluri et al., 2000).

8.3.4.2 TRANSLATION INITIATION

mRNA, ribosomes, and tRNA are only a few of the initiation factors that trigger the start of protein synthesis. (IF1, IF2, and IF3).

- At the beginning of the mRNA, the small subunit binds to the upstream 5′ end. At the start codon (AUG, GUG, or UUG), the ribosome starts to read mRNA in the 5′ to 3′ orientation. The initiator fMet-tRNA (*N*-formylMet-tRNA) is able to distinguish between both of these start codons. when they are present. The methionine (Met) carried by this initiation factor attaches to the ribosome's P site (Kennell and Riezman, 1977).

N-Formylmethionine, the first amino acid polypeptide, is produced in this process. Because the initiator fMet-tRNA has a normal methionine anticodon, the *N*-formylmethionine is inserted. This shows that the chain's initial amino acid addition and appearance is the methionine.

- The initial stages of translation typically involve three processes;
- The beginning of mRNA binding to the 30S small ribosome subunit, which causes IF3 to become active. As a result, the ribosomal subunits are divided into two;

- At the P-site of the ribosomes, the initiator factor IF2 then binds to both the initiator fMet-tRNA and the guanine-triphosphate (GTP).
- By splitting the GTP attached to IF2 with a ribosomal protein, the two ribosomal subunits are assembled more quickly. IF3 and IF2 are made available (Petrelli et al., 2001).

8.3.4.3 TRANSLATION ELONGATION

Three protein factors, EF-Tu, EF-G, and EF-Ts, help to prolong protein synthesis (Kozak, 2005). The well-known ribosomal function, which moves a codon at a time and catalyzes the processes occurring at its three locations, moves one codon at a time.

The new amino acid is inserted after the ribosomal complex is added by a charged tRNA every time a polypeptide is expanded by one, while an uncharged tRNA exits. Prokaryotes translate 200 polypeptide amino acids in less than 10 s, or at least one amino acid every 0.05 s. The source of the link established among each amino acid is GTP, which is very similar to ATP. The ribosome itself interacts with each type of RNA, and the three translational sites (A, P, and E) all have a part to play in the process. Translation therefore involves the following three separate steps:

The addition of amino-acyl-tRNAs into the A-site is mediated by elongation factor-Tu (EF-Tu). EF-Tu must connect to GTP to activate the EF-Tu-GTP complex to bind to tRNA. A phosphate molecule that supplies energy is produced after the hydrolysis of the GTP into GDP, which promotes aminoacyl-tRNA binding to the A-site. The tRNA is now in the A-site after the EF-Tu has been released. The EF-Tu-GTP is then formed, and the ribosomes discharge the EF-Tu-GDP complex by the elongation factor EF-Ts. The polypeptide chain is moved during this translocation process. Peptidyl transferase catalyzes the transition on the A-site from the peptidyl-tRNA to the aminoacyl-tRNA. The elongation factor EF-G then directs the ribosomes to advance one codon along the mRNA in the 5′-to-3′ direction. The conversion of GTP to GDP serves as the catalyst for this step. Peptidyl-tRNA that has just been synthesized is transported by the release of uncharged tRNA from the P-site to the A-site (Rodnina et al., 2000).

8.3.4.4 TRANSLATION TERMINATION

If any stop codons (UAA, UGA, and UAG) come into contact with one another, translation will halt. Release factor 1 (RF1) and RF2, which are

proteins located in the ribosomes, are what really recognize these triplets stop codons instead of the tRNA (Schlax and Worhunsky, 2003).

- While RF2 recognizes UAA and UGA, RF1 only recognizes the triplet UAA and UAG. RF3, a third component that aids in catalyzing the termination process, is another such component. The stop codon's RF attaches to the P site upon the arrival of the peptidyl-tRNA from the elongation stage. Releasing the polypeptide from the P site, these enable the ribosomes to split into two subunits with the help of the energy from GTP, leaving the mRNA behind.
- The mRNA is destroyed once many ribosomes have finished translating the message, allowing the nucleotides to be utilized in subsequent transcription events.

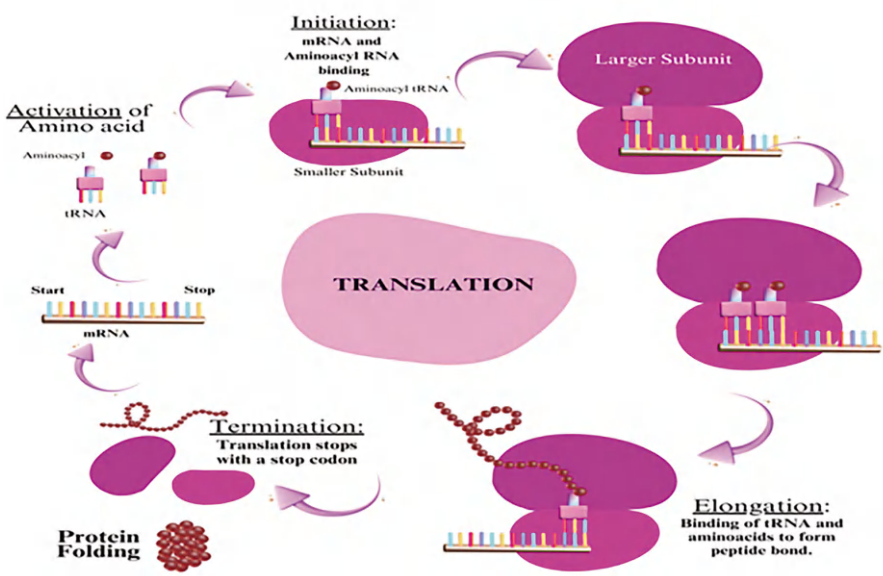


FIGURE 8.2 Translation process. ⏎

8.3.5 POSTTRANSLATIONAL MODIFICATIONS

The process of a protein maturing does not necessarily terminate when protein folding is complete because the newly created polypeptide may not be biologically functional. This is why it goes through multiple folding and processing steps known as posttranslation modification. Even after folding,

a protein can still undergo posttranslational modifications to be used in other processes. A protein's function, capability to interact with further proteins, and intracellular location—such as in the cytoplasm or nucleus can all be altered by these modifications.

There are steps of posttranslational modification:

8.3.6 CLEAVAGE

Protease enzymes can cleave proteins, which is an irreversible posttranslational change. These proteases frequently break only a few peptide bonds inside the target protein due to their exceptional specificity. This modification leads to a shorter protein with a modified polypeptide chain that has at the start and end of the chain, there are diverse amino acids at the beginning and end of the chain. The cleavage could either inactivate or activate the protein as a result of this posttranslational alteration, which frequently modifies the protein's function and can lead the protein to exhibit novel biological activities.

8.3.7 ADDITION OF CHEMICAL GROUPS

After translation, amino acids may have small chemical groups added to them in the finished protein structure. Adding chemical groups to the protein can be done through various processes, including acetylation, phosphorylation, and methylation.

Methyltransferase enzymes catalyze the reversible adding up of a methyl group to an amino acid, which is known as methylation. Lysine and arginine are the two amino acids most frequently impacted, and nine out of the twenty amino acids are methylated.

A histone is one example of a protein that frequently undergoes methylation. Histones are proteins that are present in the cell's nucleus. Histones are firmly wrapped around by DNA, which is maintained in place by interactions between the DNA's negative charges and the + charges on the histone protein and other proteins.

What regions of the DNA are tightly wound and incapable of transcription, and what regions are loosely wound and capable of transcription, are determined by a very definite pattern of amino acid methylation on the histone proteins. Acetylation has an effect on how histones regulate DNA transcription. The enzyme acetyltransferase adds an acetyl group to a lysine

amino acid in a reversible covalent reaction. An acetyl coenzyme called donor molecule A loses its acetyl group before being delivered to the target protein. Histone acetyltransferase are enzymes that acetylate the lysine residues in histones. A greater number of DNA genes are accessible for transcription as a result of acetylation, which weakens the charge connections between the histone and DNA.

Phosphorylation is the final and most frequent posttranslational chemical group change. Serine, threonine, and tyrosine are three specific amino acids inside the protein that get phosphate-added to in a reversible, covalent process known as phosphorylation. Adenosine diphosphate is produced as a byproduct of a protein kinase's removal of the phosphate group from ATP molecule and transfer of it to the target amino acid hydroxyl group. The enzyme protein phosphatase has the ability to reverse this action and remove the phosphate group. The phosphorylated protein may develop a binding site by phosphorylation, allowing it to interact with other proteins and form sizable, multiprotein complexes. As an alternative, phosphorylation can affect a protein's capacity to bind its substrate, which changes the level of protein activity.

8.3.8 ADDITION OF COMPLEX MOLECULES

Larger, more complicated molecules can be incorporated into the folded protein structure through posttranslational modifications. One frequent illustration of this is glycosylation, which is sometimes regarded as the most prevalent posttranslational modification and involves the insertion of a polysaccharide molecule.

In the process of glycosylation, enzymes in the Golgi apparatus and endoplasmic reticulum covalently add a polysaccharide molecule (sometimes referred to as a glycan) to the target protein. The target protein's final, folded 3D structure can be greatly influenced by glycosylation. In some circumstances, glycosylation is required for proper folding. By making proteins more soluble, *N*-linked glycosylation facilitates protein binding to protein chaperones and promotes protein folding. Chaperone proteins are responsible for assisting other proteins in folding and maintaining their structural integrity.

N-Linked glycosylation and *O*-linked glycosylation are the two main forms of glycosylation. The endoplasmic reticulum is where *N*-linked glycosylation begins with the insertion of a pioneer glycan. The amino

acid asparagine nitrogen is covalently bonded to the complex glycan that develops as a result of the Golgi apparatus' alteration of the pioneer glycan. Contrarily, *O*-linked glycosylation refers to the sequential covalent attachment of particular sugars to the oxygen in the amino acids' serine and threonine inside the final protein structure.

8.3.9 FORMATION OF COVALENT BONDS

To perform their functions as extracellular proteins, many proteins made inside the cell are released external to the cell. A multitude of environmental factors can affect extracellular proteins. The 3D protein structure is stabilized by the formation of covalent bonds, either inside the protein itself or between many polypeptide chains that make up the protein's quaternary structure. The most frequent form is disulfide bonds, often known as disulfide bridges. Using the side chains of two cysteine amino acids' thiol functional groups, which include sulfur atoms, a disulfide bond is created among the two residues of cysteine amino acids. The protein's current structure is stabilized by disulfide links. To produce disulfide bonds, which occur when two thiol groups oxidize one another, an oxidizing situation is required. As a result, in the oxidizing situation of the endoplasmic reticulum, protein disulfide isomerases frequently catalyze the production of disulfide bonds. The cytoplasm is a reducing environment; thus, disulfide bonds rarely form there (Mann and Jensen, 2003).

8.3.10 PROTEIN FOLDING

After the polypeptide chain's synthesis ends up, it folds to take on a certain structure that allows the protein to perform its intended tasks. The essential structure of a protein is a polypeptide chain, which is a collection of amino acids that are covalently bonded together. A gene encodes the fundamental structural details of a protein. The basic structure of the protein, as well as all successive levels of protein structure, may, therefore, be altered by any changes to the gene's sequence, which would ultimately change the protein's overall structure and function. A protein's polypeptide chain, which is the primary structural component of the protein, is folded or coiled to create the secondary protein structure. The small structures known as an α-helix or β-sheet, which are produced when hydrogen bonds form within the polypeptide chain, are the most prevalent types of secondary structures. The folding

of this secondary structure results in the formation of the protein's tertiary structure. The overall 3D structure of the protein is produced by the tertiary structure of the protein, which is composed of multiple secondary structures fusing together. The tertiary structure folds and creates crucial protein properties, such as the active site, which is necessary for the protein to perform function. Finally, some proteins may adopt a complicated quaternary structure. The majority of proteins are constructed of a single polypeptide chain, but certain proteins are composed of several polypeptide chains (also known as subunits), which fold and interact to form the quaternary structure. A number of folded polypeptide chain subunits, including hemoglobin, make up the protein, which is a multisubunit complex. The proteins that the cell has created must be delivered to the nucleus or other target organelles. The signal sequence is a polypeptide with 13–36 amino acids that is present in newly synthesized polypeptides. The leader sequence is the term used to describe it. The transfer takes place when proteins are produced on free ribosomes after translation. When a transfer occurs at the same time as translation during protein synthesis on ribosomes connected to the rough ER, this circumstance is known as co-translational transfer. The proteins that make it to the Golgi apparatus and the rough ER lumen may also enter into lysosomes. By means of protease enzymes, the signal sequence is destroyed. Once all of these proteins are put together in their right positions, they offer the necessary biochemical machinery to keep the cell nourished, moving, dividing, and alive (Dobson, 2003).

8.3.11 POLYPEPTIDE CHAIN ELONGATION

Some elongation factors are necessary for polypeptide chain elongation. EF-Tu brings AA2-tRNA and GTP into a complex and deposits it at the ribosome's "A" site. Once the AA2-tRNA has been introduced into the "A" site, the EF-Tu is released from the ribosome, and the GTP is hydrolyzed to GDP.

8.3.12 TRANSLOCATION

The two amino acids that were carried at the "A" site of the peptidyl tRNA have now shifted to the "P" site. The term for this movement is translocation. Elongation factor translocation is regulated by EF-G. The name translocase is given to this G factor. The energy required for the transport and release of deacylated (amino acid-free) tRNA is provided by the hydrolysis of GTP.

Additionally, during translocation, the ribosome moves one codon from the first codon to the second codon along the 3′ end of the mRNA. The two amino acids carrying dipeptidyl tRNA are moved from the "A" site to the "P" site during this process. On the 50 S ribosome, in addition to these two sites P and A, a third site known as "E" (exit site) is also present. With the dipeptidyl chain, which has two amino acids on the P site, A peptide bond is created by the third amino acid at the "A" location. The "P" location is subsequently given the three amino acid chains. Three amino acids are now present in the polypeptide chain. This process of elongation never ends. The polypeptide chain is extended by a new amino acid at each step. The ribosome extends by one codon in the 5′-to-3′ direction after each elongation.

8.3.13 CHAIN TERMINATION

On the mRNA, termination codons or stop codons indicate the conclusion of the polypeptide chain. UAA, UAG, and UGA are the stop codons. To these codons, no tRNA can bind. Chain termination is aided by three RFs seen in prokaryotes. They go under the names RF1, RF2, and RF3.

8.4 PROTEIN SYNTHESIS IN EUKARYOTES

With a few exceptions, eukaryotic protein synthesis is largely comparable to that of prokaryotes (Kozak, 1999). Eukaryotes have 80S ribosomes with 40S and 60S subunits. Methionine, not f-methionine as in prokaryotes, is the beginning amino acid in eukaryotes. Methionine is bound to the start codon AUG by a unique tRNA. The name of this tRNA is tRNAiMet. No other internal locations in the polypeptide are bound by this RNA to the amino acid methionine, in contrast to tRNAMet. The Shine–Dalgarno region, which acts as a ribosome binding site (RBS), is absent from eukaryotic mRNA. Between the 5′ end and the AUG codon, the mRNA contains the base sequence known as the cap. Up until it comes across the 5′-AUG-3′ codon, the ribosomal subunit, which is small, examines the mRNA in the 5′-to-3′ direction. The poly(A) tail of the mRNA's 3′ end forms a strong connection with initiation factors. Circularizing mRNA via its poly(A) tail is done by initiation factors. In this manner, the poly(A) tail also aids in mRNA translation. Eukaryotic mRNAs contain a single open reading frame because they are mono-cistronic and only encode a single polypeptide.

In eukaryotes, there are ten initiation factors eIF2, eIF3, eIF5 eIF4A, eIF4C, eIF4B, eIF4D, eIF4F, and eIF6 (also known as eukaryotic initiating factors, or elF) (Jackson, 2005). Like prokaryotes, eukaryotes have two elongation factors. They are called eEFl (like EF-Tu) and eEF2 (like EF-G). The eRF, the sole RF found in eukaryotes, is required for the end of protein synthesis brought on by GTP. It can recognize all three of the stop codons. mRNA is produced, processed, and altered in the nucleus of eukaryotes before being transported by nucleopores into the cytoplasm. Within the cytoplasm, protein synthesis occurs. The mRNA life span in prokaryotes is incredibly brief, lasting a few minutes. The prolonged half-life of eukaryotic mRNA, which can last for several days, makes it relatively stable.

8.5 PROTEIN SORTING OR PROTEIN TRAFFICKING OR PROTEIN TARGETING

The cell's protein production must be transported to the nucleus or other target organelles. Signal sequences, which are polypeptides and are found in newly synthesized polypeptides, range in length from 13–36 amino acids. The leader sequence is the term for it. The membranes of the target organelle include receptors that can recognize this signal sequence (Thommen et al., 2017).

After translation, the transfer happens when proteins are made on free ribosomes. Co-translational transfer, which occurs when a transfer occurs at the same time as translation when protein synthesis occurs on ribosomes connected to the rough ER, is used to describe this situation. The proteins that make it to the Golgi apparatus, where they may later enter the lysosomes, may also penetrate the rough ER lumen. Proteases break down the signal sequence (Nilsson et al., 2015).

Once all of these proteins have been put together correctly, they supply the necessary biochemical machinery to maintain the cell growing, moving, and reproducing (Chang et al., 2007).

8.6 INHIBITORS OF PROTEIN SYNTHESIS IN PROKARYOTES

Many substances, both man-made and those derived from other sources, such as fungi, connect to the translation machinery's components and stop the translation process. The vast majority of them are antibiotics or antibacterial substances that solely combat bacteria, making them effective weapons in the hands of man to combat a variety of infectious disorders. Translational

machinery inhibitors make up the majority of antibiotics (Zarivach et al., 2002).

1. **Puromycin** This antibiotic functions as a terminal aminoacyl-adenosine portion of the aminoacyl-tRNA analogue. By releasing bacterial polypeptide chains prior to their full synthesis, this antibiotic prevents the synthesis of proteins. By connecting its amino group to the developing polypeptide chain's carbonyl group on the A-site, which is how it achieves its mechanism. Additionally, puromycin includes an amino group similar to that on aminoacyl-tRNA that joins up with the carboxyl group of the forming peptide with puromycin residues to produce a covalent peptide bond that helps the ribosomes separate (Novac et al., 2004).
2. **Streptomycin** This trisaccharide influences the ribosomal binding of formyl methionyl-tRNA. As a result, protein synthesis cannot properly start.
3. **Aminoglycoside** Antibiotics that disrupt the 16s rRNA of the small subunit's decoding site, including gentamycin, kanamycin, and neomycin.
4. **Chloramphenicol** The action of peptidyl transferase is inhibited.
5. **Erythromycin** Inhibits translocation by interacting with the 50S subunit.
6. **Cycloheximide** is used as a laboratory technique to prevent eukaryotic cells from synthesizing protein and to inhibit peptidyl transferase in eukaryotic ribosomes.

Inhibitors in Eukaryotes:

Corynebacterium diphtheriae produces the toxin known as diphtheria toxin. As a result, the eukaryotic elongation factor changes.

8.7 PROKARYOTIC VS. EUKARYOTIC PROTEIN SYNTHESIS

Proteins, a fundamental class of macromolecules, are essential for the existence of all living things. Prokaryotes and eukaryotes both manufacture proteins for a range of purposes and processes. Some proteins serve as biological catalysts, whereas others have structural processes (Ramachandiran et al., 2000).

8.7.1 PROKARYOTES AND EUKARYOTES CREATE PROTEINS IN VARIOUS WAYS

In prokaryotes in the cytoplasm, proteins are made. Even before mRNA transcription is finished, translation starts. Bacterial mRNA synthesis does not involve the insertion of a cap and a poly(A) tail, with the exception of archaebacteria. At the codon AUG, translation starts. The polycistronic nature of the mRNA used for translation allows it to code for several polypeptide genes. Translation and transcription can be combined. No modifications are made to the mRNA prior to translation. A unique initiator PIF-1, PIF-2, or PIF-3 tRNA Met-tRNAf or Met-tRNA is utilized, correspondingly. *N*-Formylmethionine serves as the first amino acid. They have numerous places for both initiation and termination. The Shine–Dalgarno sequence, which comes 10 nucleotides before the initiation codon, is the RBS on mRNA. EF-Tu, EF-Ts, and EP-G are the three chain elongation factors. To identify termination codons, three release factors (RF-2, RF-1, and RF-3) are provided. Every prokaryotic organism shares the same genetic code (Laursen et al., 2005).

In eukaryotes in the nucleus, the first step (transcription) takes place. Transcript (mRNA) is produced and then moves to the cytoplasm, where ribosomes are located. Here, the mRNA is used to produce an amino acid chain. The poly(A) tail and 5′ cap is attached to the 3′ end of the mRNA transcript as part of the mRNA. By connecting the mRNA to the ribosomal unit at the main AUG codon via the 5′ cap, translation can start. The starting factors are the eIF1-6, eIF4B, eIF4C, eIF4D, and eIF4F proteins. The two chain elongation factors are EF-1 and EF-2. The monocistronic translation mRNA only codes for one gene of polypeptides. Cellular RNA is created by one of the three forms of RNA polymerase. It involves both the 40S and 60S subunits of the ribosomes. Since transcription and translation happen independently, there is no overlap. Before they are translated, mRNAs or pre-mRNAs are modified. They do have a unique tRNA initiator complex. It starts with methionine, an amino acid. The start codon forms the center of the Kozak sequence, which is the ribosomal binding site. They only have one location for both initiation and termination. For the identification of the three termination codons (UAA, UGA, and UAG), there is a single eukaryotic release factor (eRF). The genetic makeup of the mitochondria and chloroplasts may be different. Prokaryotes and eukaryotes protein synthesis is compared in the following table.

TABLE 8.1 Difference Between Prokaryotes and Eukaryotes.

Eukaryotes	Prokaryotes
In monocistronic translation, mRNA only codes for one polypeptide gene.	Since the mRNA is polycistronic, its codes for many polypeptide chains.
The three RNA polymerases are used for the making of cellular RNA.	The production of different types of RNA molecules is regulated by one RNA polymerase.
It has two subunits of the ribosomes, i.e, the 40S and 60S subunits.	70S ribosomes are involved.
Since transcription and translation happen independently, there is no overlap.	Translation and transcription can be combined.
Before they are translated, mRNAs or pre-mRNAs are modified.	No modifications are made to the mRNA prior to translation.
They do possess a unique tRNA complex initiator.	A unique tRNA initiator uses Met-tRNA.
It starts with methionine.	An amino acid. *N*-Formylmethionine is the first amino acid.
They only have one location for both initiation and termination.	They have numerous places for both initiation and termination.
The start codon forms the center of the Kozak sequence, which is the ribosomal binding site.	The RBS on mRNA is the Shine–Dalgarno sequence, which is situated 10 nucleotides before the initiation codon.
The synthesis of the polypetide chain is started by a number of initiation factors, including eIF-2, eIF-a2eIF-2al, and eIF-a.	It involves IF-1, IF-3, and IF-2, as three initiation factors.
EF-1 and EF-2 elongation factors.	EP-G, EF-Tu, and EF-Ts, are the three chain elongation factors.
For the detection of the three termination codons (UAG, UAA, and UGA), only one release factor (eRF).	For the identification of termination codons, three factors (RF-1, RF-2, and RF-3) are available
The genetic makeup of chloroplast and mitochondria may be different.	Every prokaryotic organism has the same genetic makeup.
The 3′ end of the mRNA transcript is where a poly(A) tail and 5′ cap are added as part of the mRNA synthesis process.	With the exception of Archaebacteria, bacterial mRNA synthesis does not involve the incorporation of a cap and a poly(A) tail.
Translation happens after transcription.	Even before the end of mRNA transcription, translation takes place.

8.8 ROLE OF PROTEIN SYNTHESIS IN DISEASE

Because the relationship between the nucleotide sequence and amino acid sequence of the protein is direct, many diseases are brought on by genetic

alterations. A protein's main structure can change, which can cause the protein to be misfolded or act improperly. Sickle cell disease is one of several single-gene disorders, which are conditions brought on by changes to a single gene. Translation initiation in eukaryotic cells can occur in two different ways: cap-independent and cap-dependent or internal ribosome entry site (IRES)-driven. Either pathway's deregulation of protein synthesis can promote oncogenesis.

8.8.1 SICKLE CELL DISEASE

A constituent of the oxygen-transporting protein hemoglobin, which originate in red blood cells, is mutable, and this results in a group of conditions known as sickle cell disease. The most severe sickle cell disease is sickle cell anemia. The most prevalent homozygous recessive particular gene illness is sickle cell anemia, and to be ill, a person must have equal copies of the defective gene (inherited from both parents) mutated. Hemoglobin is made up of four polypeptide subunits and has a complicated quaternary structure—two subunits of A and two subunits of B (Habara and Steinberg, 2016). Missense or substitution mutations in the gene that codes for the polypeptide chain are present in sickle cell anemia patients that make up hemoglobin B. When a nucleotide alters the triplet of codons overall in such a manner that the new codon is connected to a different amino acid, this is known as a missense mutation. The most common missense mutation in the hemoglobin B component gene's particular nucleotide change from thymine to adenine causes sickle cell anemia (Mangla et al., 2021). As a result, codon 6 now codes for valine instead of the amino acid glutamic acid. This alteration in the hemoglobin B subunit's polypeptide chain's main structure has an impact on how the complex's multiple subunits function in low-oxygen environments. The mutant hemoglobin protein begins to adhere to one another and create a semi-solid structure when the body's tissues obtain oxygen from red blood cells. Red blood cells become less flexible as a result of this and lose their typical "sickle" form. These fragile, malformed red blood cells can collect in blood arteries and obstruct flow. The obstruction prevents blood from reaching tissues, which could lead to tissue death and cause the person great discomfort (Ilesanmi, 2010).

8.8.2 DYSREGULATED EXPRESSION OF EIFS

The production of specific proteins is upregulated when the intracellular accessibility of essential translation machinery constituents is enhanced.

These proteins are generally engaged in the processes of cell propagation and cell survival and are encoded via "weak mRNAs" (Davuluri et al., 2000).

eIF4E was the first initiating factor to be demonstrated to be connected to cell transformation (Lazaris-Karatzas and Sonenberg, 1992). The overexpression of eIF4E in a variation of cancers, with the breast, lung, bladder, colon, and prostate, has been demonstrated (Graff et al., 2008, Silvera et al., 2009). Other members of the eIF4F complex are linked to malignant transformation (Keiper et al., 1999). Both eIF4A and eIF4E inhibitors are being researched as potential cancer therapies (Cencic et al., 2009).

Oncogenesis has also been linked to other aspects of the translational machinery. For instance, it has been demonstrated that malignant transformation is brought on by the overexpression of five out of the 13 subunits of increased amounts of eIF3h and eIF3i, leading to a rise in protein synthesis, whereas overexpression of eIF3a and eIF3c raises the level of eIF3 overall, without increasing eIF3 levels (Zhang et al., 2006). It has been demonstrated that variations in the phosphorylation of eIF3h are significant in this regard. According to these findings, eIF3h is found in high concentrations in various tumors (Nupponen et al., 1999), and it has been established that eIF3h causes colon cancer (Tenesa et al., 2008).

8.8.3 DYSREGULATION OF IRES-MEDIATED TRANSLATION

Components in these "weak mRNAs" can help to boost the production of their respective proteins by contributing to the overexpression of these mRNAs (Braunstein et al., 2007). In particular, it has been demonstrated that numerous mRNAs with IRESs, such as those encoding c-Myc and Apaf-1, encode various growth-promoting and cell persistence proteins (Spriggs et al., 2005) and that IRES-mediated translation can occur on these mRNAs during situations of cell stress (Stoneley et al., 2000).

Tumor progression requires the development of tumor vasculature, which often happens as a result of an angiogenic switch activated by tumor hypoxia (Bergers and Benjamin, 2003). 4EBP-1 and eIF4G have been found to be significantly overexpressed in breast cancers as a result of this hypoxia-activated transition (Braunstein et al., 2007). Because eIF4E is sequestered by 4EBP-1 and allows translation to switch from cap-dependent to cap-independent. Similar to this, it has been demonstrated that increased expression of e-cadherin by eIF4G in inflammatory breast cancer (Silvera et al., 2009).

In general, ITAFs are necessary for cellular IRESs to operate. The majority of cellular IRESs interact with PTB-1 (Mitchell et al., 2005), and a group of substances, including PSF, p54/nrb, GRSF, and YB1, that fix to the Myc family of IRESs were recently characterized (Cobbold et al., 2008) in addition to the hnRNPK that has already been found (Evans et al., 2003). YB1 Upregulation also promotes metastasis by inducing the epithelial-mesenchymal transition (EMT). An amount of mRNAs with SNAI1 are translated by IRES, which causes EMT (Evdokimova et al., 2009).

Neoplasia has been linked to mutations in cellular IRESs, according to research (Chappell et al., 2000). Due to its increased propensity for binding to the c-Myc ITAF hnRNPK, this alteration was found to promote the appearance of c-Myc (Evans et al., 2003). Nevertheless, it was discovered that other, as of yet undiscovered proteins, too, have improved affinity to the altered IRES (Paulin et al., 1996).

IRES-mediated translation has been demonstrated to be impacted by changes in ribosome modification. Patients with this condition are more likely to develop cancer, and it has been hypothesized that part of the reason for this component of the dyskeratosis congenita (DKC) phenotype is due to the lower expression of p27 (Yoon et al., 2006).

8.9 REGULATION OF TERNARY COMPLEX FORMATION AND ONCOGENIC TRANSFORMATION

It is recognized that a diversity of stressors, such as the phosphorylation of eIF2, start the physiological prosurvival response identified as the integrated stress response (ISR) (Harding et al., 2003). This reaction, nevertheless, can be manipulated to support the survival of cancer cells. The lack of oxygen and nutrients in the tumor's microenvironment activates eIF2 kinases such as PERK, which broadly suppress translation (Blais et al., 2006). However, because the TF ATF4 has an uORF in its 5′ UTR, this promotes the translation of ATF4. The stress recovery genes are then transcriptionally upregulated by ATF4. The hypoxic zones of apoptosis in tumors made from transformed cells are larger and more intense than those in tumors made from tumors with an entire ISR (Bi et al., 2005). The drop in ternary complex levels is probably associated with a more favorable regulation of the translation of new mRNAs that include uORFs. It has been discovered that some antiapoptotic proteins and proto-oncogenes, including Her-2 (ERBB2), have uORFs that prevent translation under typical conditions (Mehta et al., 2006). It would

be expected that this method, when used in hypoxic settings, would increase the translation of these proteins, promoting the survival and proliferation of cancer cells (Pickering and Willis, 2005).

Alterations in the ternary complex's levels are linked to oncogenesis in numerous other studies. Moreover, it has been demonstrated that protein synthesis, cell proliferation, and morphological change can all be induced by increased tRNAiMet expression (Marshall et al., 2008). Additionally, elevated eIF2B levels have been observed in several breast tumors (Braunstein et al., 2007). All of these findings imply that ISR has been a promising target for cutting-edge antitumor treatments (Fels and Koumenis, 2006).

8.10 MICRO-RNAS IN CANCER

Known for the crucial function micro-RNAs (miRs) have in determining tissue type and development, as shown by an early study in *C. elegans* (Lau et al., 2001), it is only logical that they also have an impact on the expansion and progression of cancer. In many experimental systems, modifications to their typical expression not only directly contribute to the cancer phenotype but also correlate with malignancy.

Numerous investigations have produced profiles of miR expression in both healthy and cancerous tissues. Similar to mRNA, specific miR expression signatures have been linked to various tissues and the cancers that arise from them (Volinia et al., 2006). In fact, miR expression profiles perform better than mRNA at identifying cancers with weak tumor differentiation (Lu et al., 2005). MiR expression profiles were created for breast cancer that replicates the molecular subtype identified by mRNA profiling. In a different data set, these characteristics were able to discriminate between subtypes (Blenkiron et al., 2007). In a group of 27 human sarcomas, miR profiling produced signals that precisely identified and, more crucially, demonstrated the early misdiagnosis of two of the unusual tumors (Subramanian et al., 2008). This makes it possible to use miRs to diagnose these difficult-to-diagnose organisms.

Several miRs function as either oncogenes or tumor suppressors (referred to as "oncomirs") have now been established. implicated in the development of cancer via their under- or overexpression, respectively. However, it is still unclear how a miR's effects are exerted, even when it has been demonstrated that it is causally connected to the malignant phenotype. being overexpressed in a variety of malignancies and hemophilia's, containing

glioblastoma breast cancer, compared to healthy tissue (Chan et al., 2005) and colon cancer (Schetter et al., 2008), miR-21 (MIR21) may be regarded as the prototypical oncomir. In mouse models of cardiac hypertrophy, it is also upregulated (Van Rooij et al., 2006), suggesting a broader function in proliferative conditions. Growth, invasiveness, and decreased apoptosis are only a few of the characteristics that have been linked to miR-21 overexpression (Zhu et al., 2007). The translation and transformation inhibitor PDCD4 is one of the miR-21 targets that has been experimentally supported (Lu et al., 2008), by the tumor suppressor TPM1 (Zhu et al., 2007) and the tumor-suppressing enzymes phosphatidylinositol-3,4,5-trisphosphatase (PTEN) (Meng et al., 2007).

The miR-10 family is overexpressed in a variety of cancers, such as B-CLL, glioblastoma, and colon cancer (Ciafre et al., 2005). In cell culture, miR-10a overexpression promotes transformation (Ørom et al., 2008), and in a mouse xenograft model, miR-10b enhances cancer invasion and metastasis (Tenesa et al., 2008). These targets include a wide variety of mRNAs that are typically upregulated in the tumors themselves and encode the translational apparatus (Hill et al., 2009). It is yet unknown whether miR-10's cancer-causing properties result from this particular role. A combination of the two methods, more traditional mRNA targeting (as certain mRNAs expressing homeobox genes have demonstrated), or both (Garzon et al., 2006).

Twelve (12) members make up the miRs in humans; in contrast, they are present throughout various epithelial tissues as a hallmark of terminal differentiation. In the early stages of embryogenesis, they are downregulated (Schulman et al., 2005). Furthermore, stem-like subpopulations of cultivated cells were found (Ibarra et al., 2007). When carcinomas, such as lung cancer, are developing, let-7 expression is lost early (Takamizawa et al., 2004) and breast cancer (Sempere et al., 2007). Moreover, ectopic overexpression of let-7 slows the development of cancer cells. (Lee and Dutta, 2007). According to (Peter, 2009), EMT and cellular dedifferentiation are linked to let-7 depletion. Let-7 has a number of known targets, as well as the conventional oncogene genes producing "oncofetal" proteins (Boyerinas et al., 2008). In oral squamous cell carcinomas, a change in the KRAS 3′ UTR let-7 binding area is associated with a worse survival rate (Christensen et al., 2009).

It is also believed that miR-200 family members prevent EMT (Gregory et al., 2008). MiR-205 (MIR205) has been found to suppress invasion in the context of basal breast epithelium (Sempere et al., 2007), basal skin, and urothelium (Sempere et al., 2007). However (Iorio et al., 2009), it is often not expressed in breast tumors (Wang et al., 2008). There is still a connection

between the basal marker CK14 and miR-205 expression in breast cancer (Sempere et al., 2007). Even in tumors that are CK14-positive, miR-205 is downregulated in comparison to healthy tissue, demonstrating its ability to prevent cancer growth. HER-3 (ERBB3), a component of the HER-2, and VEGF-A are examples of known mRNA targets (Wu et al., 2009).

MiR-34 is a significant p53 downstream effector. When expressed ectopically, it is capable of recapitulating several p53 functions and is directly triggered by p53 (Chang et al., 2007). It has been demonstrated that by targeting SIRT1, it can modify apoptosis (Yamakuchi et al., 2008). It is unclear how miR expression is regulated and dysregulated. It is apparent that transcriptional regulation is important because miR gene loci have statistically enriched regions of copy number variation in cancer (Zhang et al., 2006), suggesting that genomic gain or loss contributes to the dysregulation of these loci. It also controls how miRs are processed. For instance, Lin28, particularly, inhibits let-7 miR processing in liver cells (Heo et al., 2008); in addition to encouraging cell proliferation *in vitro*, Lin28 overexpression is linked to hepatocellular carcinoma (Hill et al., 2009, Guo et al., 2006). The maturity of various antiproliferative miRs has been positively impacted by p53's direct contact with the Drosha maturation complex, It has been shown to communicate with the miR biogenesis apparatus (Suzuki et al., 2009).

8.11 NEUROLOGICAL DISEASE AND TRANSLATIONAL CONTROL

The control of protein synthesis appears to be mainly sensitive to changes in neurological tissue. This might be because of the high metabolic demands placed on central nervous system (CNS) neurons, which makes it difficult for this tissue to tolerate translational flaws that are acceptable in other cell types (Vuppalanchi et al., 2009).

8.12 MUTATIONS IN GLOBAL TRANSLATION FACTORS

Vanishing white matter (VWM) is one of the most well-known neurological illnesses caused by suboptimal translational control (van der Knaap et al., 2006). VWM is a severe hereditary brain disorder that is frequently brought on by trauma or physiological stress and is characterized by a considerable loss of cerebral white matter. Missense mutations in any of the five subunits of eIF2B, the guanosine nucleotide-exchange factor responsible for recharging eIF2, can result in VWM. One of the crucial regulators of translation

initiation is eIF2B (Van Der Knaap et al., 2002). When cells are stressed, phosphorylated eIF2 sequesters eIF2B, which inhibits translation globally. It is interesting to note that symptoms frequently manifest after minor illnesses or head injuries, The subunit mutations appear to be tolerated under normal physiological conditions despite causing deficits in the guanine-nucleotide exchange activities are carried out by eIF2B, and severe neurodegeneration only happens when eIF2 is phosphorylated (Fogli et al., 2004).

The enzymes known as aminoacyl-tRNA synthetases (ARSs) are in charge of attaching amino acids to tRNA molecules, an essential first step in the production of proteins, causing mutations that lead to a number of CNS disorders. Humans contain 36 ARS proteins, and four of these have mutations that have been linked to CNS disorders. The nucleus has the genetic code for every human ARS protein, but only roughly half of them is functional outside of the mitochondria. For instance, two different Charcot–Marie–Tooth diseases are caused by missense alterations in the cytoplasmic ARSs YARS and GARS (Jordanova et al., 2006). Pontocerebellar hypoplasia, lactate accumulation, and leukoencephalopathy with involvement of the brain stem and spinal cord are caused by mutations in the mitochondrial ARSs DARS2 and RARS2 (Edvardson et al., 2007). Neuronal cells may be particularly vulnerable to mitochondrial dysfunction, given their high energy needs (Taylor and Turnbull, 2005). The pathogenesis of Charcot–Marie–Tooth disease may be explained through mutations in YARs and GARs, which reduce translational efficacy in the brain cells cytoplasm. However, several GARS mutations that cause illnessdo not appear to affect the enzyme's ability to acylate amino acids, pointing to potential localization issues and compromised secondary activities as possible causes (Seburn et al., 2006). Neurological problems are also known to result from translation elongation errors. The responsibility of the elongation factor eEF1A is to bring amino-acylated tRNAs to the elongating ribosome. Terminally developed neuronal and muscle cells are the only cells that express eEF1A2 when eEF1A1 is downregulated. The "wasted" phenotype is seen in mice lacking eEF1A2, in which eEF1A expression changes from isoform 1 to isoform 2 in the brain, leading to neurodegeneration (Hafezparast and Fisher, 1998).

8.13 DEFECTS IN NEURON-SPECIFIC TRANSLATION FACTORS

After trisomy 21, the greatest collective reason for mental disability is mental retardation syndrome, which is caused by mental retardation protein

(FMRP) (Jacquemont and al., 2007). It has been proposed that an RNA-binding protein by the name of FMRP participates in the particular translational control of the subset of mRNAs. Theoretically, to do this, mRNA localization is controlled, mainly in neurons which bind with polyribosomes at synapses and influence indigenous translation (Wang et al., 2008). Additionally, it has been demonstrated that FMRP functions as an IRES trans-acting factor, requiring SOD1's effective translation and protecting against oxidative stress. SOD1 has been linked to neurological disorders (Bechara et al., 2009).

8.14 DEFECTS IN IRESS

As was already established, the vast majority of mRNAs require internal ribosome entrance before translation can start, and IRES-mediated translation has been associated to a number of neurological diseases. IRESs are also present in the mRNAs for the proteins tau and amyloid precursor protein (APP), which have been linked to Alzheimer's disease (AD) (Créancier et al., 2000). Connexin 32 translation is impeded by a particular nucleotide alteration in GJB1's 5′ UTR, resulting in the production of CMTX. the myelin protein Connexin 32 is changed, and its translation is also IRES-dependent (Hudder and Werner, 2000).

8.15 MIRS IN NEUROLOGICAL DISEASE

Numerous studies indicate that miRs are crucial for neurogenesis, survival, and synaptic plasticity (Fiore et al., 2009) and that their expression is confined to specific regions of the CNS (Liang et al., 2007). Examples of CNS-specific miRs that mediate the procedures that stop neuronal division include miR-9 and miR-124, which target elements of the chromatin remodeling apparatus (Yoo et al., 2009).

MiRs are currently thought to play specialized functions in human CNS disorders, in line with their activities in neurons. Huntington's disease (HD) has low amounts of miR-9 (Packer et al., 2008). In AD-affected brain tissue, many miRs express themselves abnormally (Sethi and Lukiw, 2009). The -amyloid protein builds up intracellularly in the disease. In cultured cells, MiR-29 targets BACE1, and tissue from AD patients has decreased MIR-29 expression, which correlates negatively with BACE1 expression.

KEYWORDS

- **protein formation**
- **components**
- **role in diseases**
- **inhibitors**
- **MiRs**

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CHAPTER 9

Transposable Elements

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ABSTRACT

Transposable elements (TEs), transposons, and jumping genes are parts of the genome that can move from one location to another. The first TEs discovered in bacteria are insertion sequences or IS elements. These transposons turn out to be the most basic ones. There is roughly 45% of it in the human genome. It inserts itself in a variety of places.

9.1 INTRODUCTION-DISCOVERY

Transposons are genetic components that move. They are non-autonomous DNA segments that can randomly insert into the genome, plasmids, or bacterial chromosomes without relying on the host cell's recombination mechanism. They can integrate at various places in the genome and travel around the genome, or transpose, according to the description of them as introgenic parasites. Transposition is the process of integrating and removing parts that can be transposed. Because of their ability to move around the genome, transposable elements (TEs) are also known as mobile DNAs or TEs. This transposition's capacity to increase their copy quantity throughout the genome gives them a selective role that can turn them into infectious DNAs.

Due to their capacity for replicative transposition via an RNA or DNA intermediary, TEs, particularly in higher eukaryotes, can multiply to occupy

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significant portions of genome sequences. For instance, it is estimated that TE is the source of 25 and 45%, respectively, of the human and rice genome sequences. Due to their functions in genome shape (Lander, 2001; Yu et al., 2002; Pardue et al., 2005), size of genome (Gregory, 2005), genes reconfiguration (Bennetzen, 2005), contributions to host genome (Bennetzen, 2005; Medstrand et al. 2005), and regulator development (Medstrand et al. 2005), TEs are important biological components to be studied. This is in addition to their distinctive mechanisms of replication and sheer abundance.

Many prokaryotes and eukaryotes, as well as other species, have been found to contain transposons. The transposons found in bacteria were the first ones to be examined at the molecular level. In the past 10 years, plant transposons have been discovered and defined. In maize, numerous transposons have been discovered, and they have been categorized into large families. Additional plant types, including snapdragon, petunia, Arabidopsis, as well as others, have also been found to have transposons. Over 50% of the nuclear DNA in many plants with complex genomes is made up of TEs.

9.1.1 DISCOVERY

During research on maize in 1944, Barbara McClintock identified transposable components. The genetic community, however, had a less than enthusiastic response to her discovery. Her 1951 Cold Spring Harbour Symposium lecture was poorly received, if not completely misunderstood (Keller, 1983). With her subsequent works, she had no better success (McClintock, 1950; 1951; 1956). An underappreciated finding was revived once another discovery was discovered, which is not unprecedented in the history of science. In this instance, it was the early 1970s discovery of insertion sequences in microbes by the Szybalski group (Malamy et al., 1972). "Genetic elements were found in higher organisms which appear to be readily transposable from one to another site in the genome," the authors noted in the original research. In maize, McClintock outlined these components, which may be distinguished by their regulating roles. They might resemble the currently being researched IS insertions in some way (Malamy et al., 1972). McClintock's pioneering work was eventually recognized for its significance by the genetics community, which gave her honors, including 14 honorary doctorates and the 1983 Nobel Prize "for her discovery of mobile genetic elements." Although the word had actually been used earlier (Aronson et al., 1960; Ehret et al., 1963), it just so happened that Susumu Ohno promoted the phrase "junk DNA"

simultaneously Szybalski "rediscovered" TEs. Ohno made reference to what are known as "noncoding sequences," as well as more specific, to every segment of DNA that lacks a code for a protein that includes all genetic material derived by transposons. When some researchers began to recognize the evolutionary value of transposable and transposed components in the early 1990s, the negative perception of these elements began to alter (Brosius, 1991; Makalowski et al., 1994). Due to the cycle of fate coming full circle and developments in genomic sciences, the focus of TE research has turned back to the role that mobile elements contributed in the development of the control of genes (Jordan et al., 2003; Thornburg et al., 2006; Feschotte, 2008; Mita and Boeke, 2016; Chuong et al., 2017; Franke et al., 2017; Wang et al., 2017; Venuto and Bourque, 2018).

9.2 CHARACTERISTICS

TEs, or transposons, are sequences of DNA that can move or transpose in a gene. Some fundamental traits of transposable components include the following:

- They are capable of relocating themselves inside a genome. They are able to travel between locations by using the "cut-and-paste" or "copy-and-paste" techniques (McClintock, 1950).
- TEs are frequently numerous in genomes and make up a sizable fraction of the DNA (Lander et al., 2001).
- Two examples of TEs that can be categorized into distinct classes depending on their structure are retrotransposons (Class I) and DNA transposons (Class II) (Finnegan, 1989).
- The lengthy terminal repetitions on the ends of LTR retrotransposons, a particular form of retrotransposon, are important for their transposition and control (Wicker et al., 2007).
- By inducing genetic mutations, promoting genome rearrangements, assisting in the development of different genomes, and affecting genetic expression, transposable components can have a profound effect on the evolution of genes (Kazazian, 2004).
- TEs' activity can be controlled by organisms through mechanisms like DNA methylation, histone changes, and silencing pathways mediated by short RNAs (Slotkin and Martienssen, 2007).
- TEs insert into various genomic regions, resulting in genetic variety and the possibility of adaptation, and this causes genetic heterogeneity among populations (Naito et al., 2018).

- TEs go through the duplication and recombination mechanisms. They lack the location of the replication's origin, though. As a result, they need the DNA or plasmid of the host to replicate.
- Repetitive sequences in organisms' genomes are a result of TEs. Repeated sequences make up 50% of the individual genome.
- Both prokaryotic and eukaryotic species, such as bacteria, fungi, plants, animals, and people, have TEs in their genomes. These elements have a considerable structural and functional importance.
- Transposons and insertion sequences (IS) are two general categories for TEs.
- Bacteria include ISs, which are short lengths of roughly 1000 base pairs.
- The longest sequences, with over a thousand base pairs, are found in transposons.

9.2.1 AUTONOMOUS TRANSPOSABLE ELEMENTS

The proteins required for autonomous TEs to move around the genome can be encoded by them. They possess fully functional transposase genes, which are responsible for producing the transposition-catalyzing enzyme. Examples include DNA transposons in *Drosophila* and maize, such as the Ac/Ds and hAT elements (Lampe and Robertson, 1996).

9.2.2 NON-AUTONOMOUS TRANSPOSABLE ELEMENTS

Since they do not have functional transposase genes, non-autonomous TEs rely on the transposase provided by autonomous elements for mobility. They often have the structural characteristics—such as terminal inverted repeats, or TIRs—but not the coding sequences required for transposition. Examples: Non-autonomous DNA transposons identified in mammals include SINEs and MITEs (Feschotte and Pritham, 2007).

9.2.3 CUT-AND-PASTE TRANSPOSITION MECHANISM

The transposable component is removed from the donor place during cut-and-paste transposition, and it is then inserted into a different chromosomal region. A transposase enzyme that recognizes the ends of TEs and catalyzes excision and reintegration is normally needed. Examples include DNA transposons in bacteria like Tn5 and mammals like the sleeping beauty transposon (Mátés et al., 2009).

9.2.4 COPY-AND-PASTE TRANSPOSITION MECHANISM

In copy-and-paste transposition, the original transposable component is left at the donor place and a fresh duplicate of the element is created and then placed into a different chromosomal region. After reverse transcription of the transposable element RNA into DNA, the cDNA must be integrated into the genome. Examples: Alu elements also long-interspersed nuclear elements (LINEs), two types of retrotransposons found in humans (Kazazian and Moran, 1998).

9.3 TYPES

TEs can be categorized into class I elements (retrotransposons) and class II elements (DNA transposons) depending on how they are transposed.

9.3.1 CLASS-1 (RETROTRANSPOSON)

Class I TEs are replicated twice: first being changed from DNA to RNA and then back to DNA. The copied DNA is subsequently inserted into the genome at a new site. The reverse transcription process is catalyzed by a reverse transcriptase, which is typically encoded by the TE itself. The features of retrotransposons are similar to those of retroviruses, such as the HIV virus.

Typically, retrotransposons are divided into three primary orders:

- Retrovirus-like retrotransposons, which have long-terminal repeats (LTRs) encoding reverse transcriptase, function similarly to retroviruses.
- Long-interspersed nuclear elements (LINEs, LINE-1s, or L1), which encode reverse transcriptase but lack LTRs, are transcribed by RNA polymerase II.
- Reverse transcriptase is absent from short-interspersed nuclear elements (SINEs), which RNA polymerase III transcribes.

TEs can also include retroviruses. For instance, after retroviral RNA is converted into DNA inside the host cell, the newly generated retroviral DNA is incorporated into the genome of the host cell. Proviruses are referred to as these integrated DNAs. A specialized type of eukaryotic retrotransposon called a provirus has the ability to make intermediates of RNA that might exit the host cell and contaminate other cells. Retroviruses and prokaryotic

TEs appear to have no common ancestors, based on their distinct transposition cycles.

9.3.2 TYPE-II (DNA TRANSPOSONS)

Class II TEs use a cut-and-paste transposition method rather than an RNA intermediary. Several transposase enzymes catalyze the transpositions. While some transposases only bind to particular target sequences, others attach to any DNA target site. The transposase, which also generates adhesive ends at the target site, cuts out the DNA transposon and ligates it into the target site. A DNA polymerase fills up the gaps left by sticky ends, and a DNA linker binds the sugar-phosphate backbone. The target site duplicates as a result, and DNA transposons can be identified by their insertion sites by brief direct repetitions, which are necessary for the TE excision by the transposase, followed by inverted repeats, which create an uneven break in the target DNA and then filled by DNA polymerase.

Cut-and-paste TEs may be replicated if TE transposition takes place during the S stage of the cell cycle, while the target site has not yet undergone replication but the donor site has. Such Replications at the target region may lead to gene duplication, which is a vital step in the genomic evolution process (Madigan and Martinko, 2006).

DNA transposons do not always use cut-and-paste transposition. Replicative transposition occurs when a transposon copies itself to a new target site (such as a helitron).

Class II TEs make up just over two percent of the human genome, leaving the majority as Class I (Kazazian and Moran, 1998).

9.4 MECHANISM OF TRANSPOSITION

Depending on their structure and mechanism, transposable components can take numerous different shapes. Based on this theory, there are three transposition mechanisms. Retro-transposition is solely used by eukaryotes; however, the majority of prokaryotes and eukaryotes use either the conservative or replicative transposition methods.

Three Types

- Replicative transposition
- Conservative or non-replicative transposition
- Retro-transposition

9.4.1 REPLICATIVE TRANSPOSITION OR COPY PASTE TRANSPOSITION

Transposons that replicate themselves first and then move one copy to a new location are known as replicative transposons. The TEs are therefore duplicated during replicative transposition, with one copy being placed into the new location and the other staying in the original position. The transposable element itself encodes for the transposase enzyme, catalyzes this transposition. Transposition by copy and paste is another name for it. Prokaryotic creatures are the only ones that have replicative transposition.

For instance, replicative transposons with two steps of transposition are known as non-composite transposons (Tn3). The first step entails the fusion of two plasmids, one of which (donor plasmid) contains Tn3 transposons and another (receiver plasmid), which is catalyzed by transposase enzymes and produces a structure known as cointegrate. One copy of Tn3 is inserted at each place where the two plasmids have joined during cointegration. The tnpR-encoded enzyme resolves the cointegrate into its two component plasmids, each of which has a copy of Tn3, after the second stage of transposition is finished. At the resolution site, this enzyme facilitates a site-specific recombination between the two Tn3 copies.

9.4.2 CONSERVATIVE OR NON-REPLICATIVE: CUT AND PASTE TRANSPOSITION

The "conservative transposons" are those that move within a chromosome by being removed from one place and then put into another. As a result, the transposable element is physically transferred from its original location and pasted onto a different location in a chromosome during conservative transposition. Transposition by cut and paste is another name for it. The transposable element itself carries the encoding for the transposase enzyme, catalyzes certain types of transposition. Prokaryotic and eukaryotic organisms both exhibit conservative transposition.

9.4.3 RETROTRANSPOSONS

Retrotransposons are transposons that have had their RNA reverse transcribed into DNA by the enzyme converse transcriptase and then inserted into a new site on a chromosome. Retro-transposition is the process that causes this

transposition. Retroposons use the reverse transcriptase enzyme of retroviruses to transpose. These retrotransposons include several that are connected to retroviruses. Only eukaryotic organisms include retrotransposons.

9.5 TRANSPOSABLE ELEMENTS IN BACTERIA

Bacteria have been revealed to have genetic instability, which frequently resulted in the finding of TEs. The first molecularly examined transposons were those of bacteria, and they fall into the following categories.

Insertion sequences (IS elements)

Prokaryotic Transposons (Tn): Composite and non-composite transposons

9.5.1 INSERTION SEQUENCES (IS ELEMENT)

Bacterial transposons with insertion sequences, or IS elements, are the most fundamental transposons. They typically consist of short sequences and just one gene that codes for the transposition enzyme. In particular, *E. coli* lac operon variants, IS elements were first identified as spontaneous insertions that silence the gene and prevent transcription and translation. It was determined that the lac operon gene mutation was unstable, and molecular investigation shows that there are additional copies of DNA sequences close to the lac gene. The further DNA sequence is absent during reverse mutation in the altered *E. coli*. A specific form of IS element may appear in many copies on a bacterial chromosome. For instance, the *E. coli* chromosome has 6–10 copies of IS1. Plasmids may also contain IS elements.

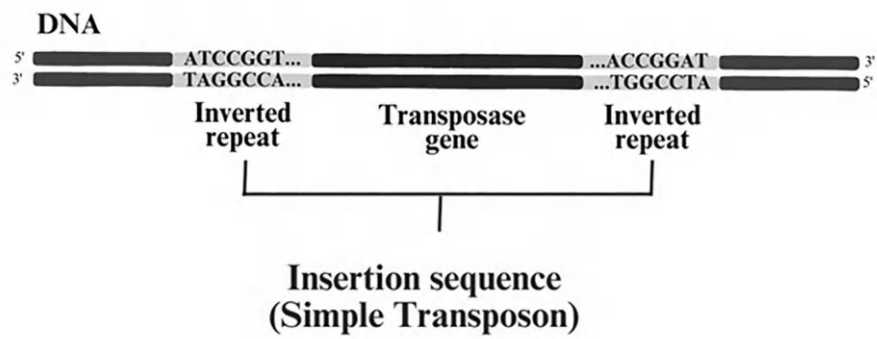


FIGURE 9.1 Simple transposable element.

Source: Modified from Verma and Agarwal, 2004

Only genes (open reading frames) that code for enzymes for regulating transposition are found in IS elements, which are compactly organized and contain roughly 1000 nucleotide pairs. IS1, which has a length of 768 nucleotide pairs, is the smallest IS element. Each type of IS element has a transposon sequence in between the reversed terminal repeats on both ends of the component. The only gene that codes for the transposition of an IS element is a transposon. The majority of IS elements have inverted terminal repeats, which typically have lengths of 9–40 base pairs. Target place duplication is the capability of an IS component to duplicate an inserted sequence at the insertion site.

9.5.1.1 TRANSPOSITION OF INSERTION SEQUENCE IN BACTERIA

One open reading frame (ORF) in the IS element encodes for the transposase enzyme, which catalyzes its own transposition. Similar to a restriction endonuclease, the enzyme transposase binds to the IS element's terminal inverted repeats (IR), which serve as a restriction site. After that the enzyme removes IS components from chromosomes or plasmids. In order to identify the target site for insertion on the same or different chromosomes or plasmids, the excised IS element traverses along the length of the chromosome. Once it has identified the target location, it produces staggered cleavage (which involves cutting the single strand of DNA), creating stickiness, and then it inserts itself. The proofreading process of DNA causes the DNA sequence at the intended insertion site to be duplicated as IS elements are inserted, resulting in one copy of the target DNA being present on one or the other side of the IS element. As a result, IS components aid in target site duplication (TSD).

9.5.1.2 PROKARYOTIC TRANSPOSONS (TN)

Prokaryotic transposons are similar to IS elements, but they are bigger and contain other genes in addition to the transposase gene (primarily antibiotic resistance genes). Transposons have inverted terminal repeats and have a length of several thousand base pairs.

Prokaryotic transposons come in two forms: composite and non-composite transposons.

More advanced than IS elements, composite transposons, and Tn3-like elements contain certain genes that encode products unrelated to the transposition process.

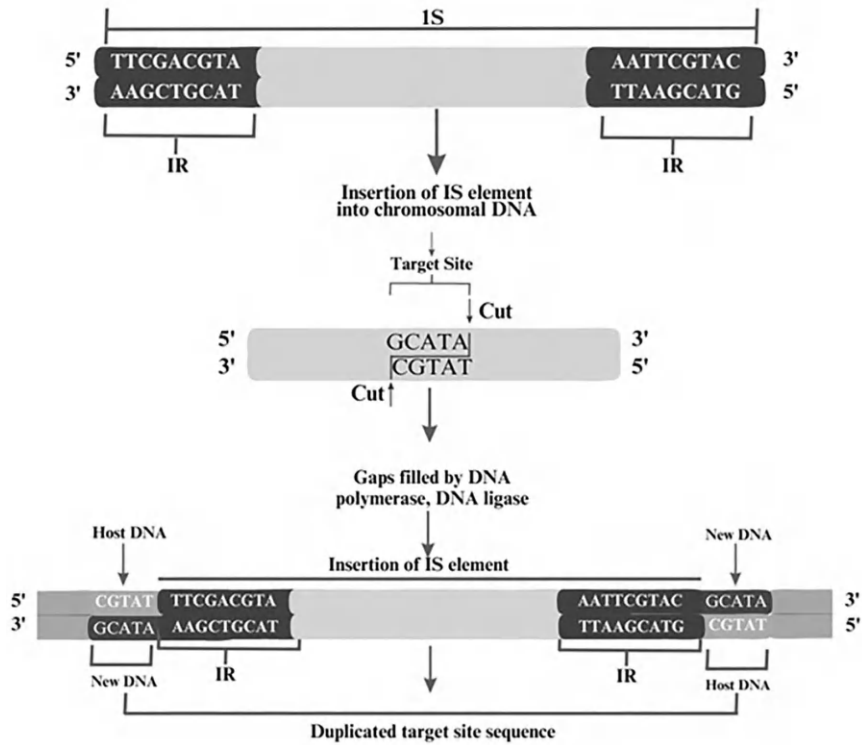


FIGURE 9.2 Transposition of IS element.

Source: Modified from Chawla, 2011.

9.5.2 COMPOSITE TRANSPOSONS

To create a composite transposon, which is represented by the symbol Tn, two homologous IS elements join forces with additional genes (often those encoding drug resistance). As a result, a composite transposon has two "arms" made up of IS elements on either side of its center region, which is where the drug marker(s) are located. The arms could be oriented invertedly or the same way (direct repeat). The composite transposon terminates in the same short inverted repeats because the arms are made up of IS modules, and each module has inverted repeats at their ends.

Following the near insertion of two IS elements, a composite transposon is generated, Thus, the area between the two IS elements can be exchanged when the elements combine. For example, Tn10 is a 9.3 kilobase composite transposon that has 1.4 kilobase TIRs and the transposase and antibiotic resistance genes sandwiched in between.

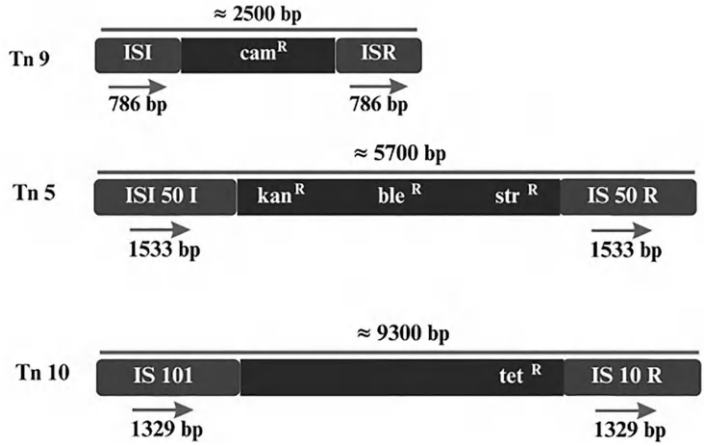


FIGURE 9.3 Composite transposable elements, cam (chloramphenicol), kan (kanamycin), ble (bleomycin), str (streptomycin), tet (tetracycline).

Source: Modified from Chawla, 2011.

9.5.3 NON-COMPOSITE TRANSPOSONS

Non-composite transposons are DNA fragments between TIRs that also include multiple extra genes in addition to the transposase gene. It has simple inverted repeats of 38–40 nucleotide pairs at every termination rather than IS elements at each end like composite transposons do. They are larger than IS elements (about 5000 nucleotide pairs long) and frequently include both transposition-related and auxiliary genes.

For instance, Tn3 is a 5 kb non-composite transposon that carries the genes for the enzymes transposase (tnpA), resolvase (tnpB), and beta-lactamase (bla).

The other two enzymes are crucial for transposition and recombination, whereas the beta-lactamase confers resistance to the antibiotic ampicillin.

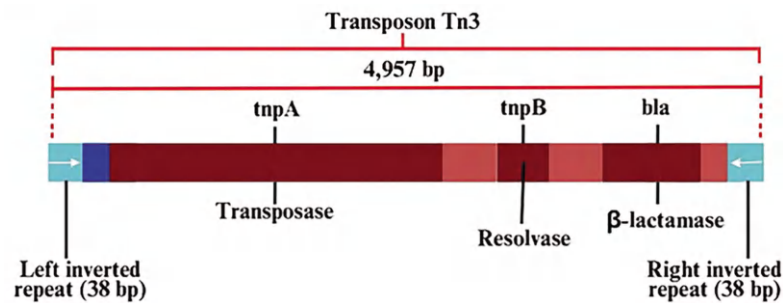


FIGURE 9.4 Non-composite transposable elements.

Source: Modified from Verma and Agarwal, 2004

9.6 TRANSPOSABLE ELEMENTS IN EUKARYOTES

In eukaryotes, transposons come in a variety of sizes, structures, compositions, and behaviors. Basic traits shared by all eukaryotic transposons include target-specific duplication upon insertion and TIRs.

According to structure, included in this are the length and type of the terminal repeats, which can be long or short and either straight (such as CCCAGAC and GGGTCTG) or inverted (such as CCCAGAC and GTCTGGG) repeats. Four different kinds of transposons exist:

I. With long-terminal direct repeats (Copia in *Drosophila*, Ty in yeast, IAP in mice)
II. Long-terminal inverted repeats (FB, TE in *Drosophila*)
III. Short terminal inverted repeats (P and I in *Drosophila*, Ac/Ds in maize, Tam1 in snapdragon), and long-terminal direct repeats (FB, TE in *Drosophila*).
IV. Without terminal repetitions (Alu in mammals), based on the transpositional process.

Based on the transposition technique, this taxonomy classifies TEs into two primary superfamilies.

9.6.1 CLASS 1: RETROTRANSPOSONS

Retrotransposons are TEs that convert an intermediate form of RNA into DNA via reverse transcriptase before autonomously migrating to a new place. Stable mutations are brought about by mobile elements that transfer through RNA intermediates via reverse transcription. They make up the largest mass of TEs in most or all plant species. In maize (SanMiguel and Bennetzen, 1998), retroelements account for nearly 70% of the nuclear DNA, and other plant species with big, complex genomes have an equivalent or higher number of retroelements.

Class I contains three major families of TEs:

I. Retroviruses
II. Long-terminal repeat (LTR) retrotransposons
III. Non-LTR retrotransposons

In plants, only the final two families have been identified.

9.6.1.1 RETROVIRUSES

The single-stranded RNA genomes of retroviruses are replicated by a double-stranded DNA molecule acting as an intermediate. Genomic (duplex DNA) sequences called retroposons/retrotransposons occasionally transpose inside a genome but do not move between cells. The common mechanism for the continuation of retrotransposons and the reproduction of retroviruses is reverse transcription. Retroposons resemble retroviruses in their process of transposition but differ from them in that they do not spread via a separate infectious form.

There are genes for gag, pol, and env in a normal retroviral sequence and are translated into polyproteins that are then split into individual functional proteins. The gag gene produces the proteins that make up the nucleoprotein core of the virion, while the env gene produces the elements of the particle's envelope, resulting in the packing and production of virion. The pol gene produces proteins that are involved in the separation and recombination of nucleic acids. The pol gene encodes reverse transcriptase, which converts the genome into a complementary DNA strand. Additionally, the pol gene has DNA polymerase activity, allowing it to create duplex DNA from a single-stranded reverse transcript of RNA. The enzyme's RNase H activity has the ability to destroy the RNA component of the RNA-DNA hybrid. By switching the template strands, reverse transcriptase copies the 3′ end of the RNA to the 3′ end of DNA and the 5′ end of the RNA to the 5′ end of DNA. The resulting DNA copy is longer than the RNA template. The distinctive LTRs are thus produced. The enzyme integrase inserts linear duplex DNA into the host genome. Additional copies of the RNA sequence are produced by transcription of the integrated DNA from a promoter in the left LTR. Direct target repeats are created by the integration event. Therefore, an inserted provirus has short repeats of the target DNA flanking direct terminal repeats of the LTRs. There are endogenous (inactive) proviruses with these structures in the genomes of mammals and birds.

9.6.1.2 LTR RETROTRANSPOSONS

The retrotransposons family of big retroelements, which includes direct LTRs, is the most prevalent in plants. These range from a few hundred bases to over 10 kb in size, with LTRs that are typically between a few hundred and a few thousand bases long. Due to their early eukaryotic lineage origins

or the possibility of horizontal transmission, LTR retrotransposons are present in all eukaryotes. These have internal domains that express proteins comparable to the group-specific antigen (gag) and the pol polyprotein, and they are surrounded by long-terminal direct repeats, making them resemble integrated copies of retroviruses. The regulatory sequences needed for transcription, the initial phase of transposition, are carried by LTRs. Reverse transcriptase, integrase, and endonuclease domains are conserved in the pol polyprotein (rtint-RNaseH). The Ty 1-copia group and the gipsy group (called after their first members discovered in *Drosophila*) are the two primary subclasses of retrotransposons based on sequence homology and the arrangement of these domains. The location of integrase within the encoded polyprotein varies among them. Although both of these groups are present in plants, the Ty 1-copia group has received more attention. Retrotransposons represent a considerable portion of big plant genomes, according to recent studies. This characteristic led to the identification of the majority of plant retrotransposons as repetitive sequences or insertion elements in cloned DNA. Several Ty 1-copia group components from the plant kingdom have been fully sequenced. These sequences have a very ancient ancestry, as shown by the presence of LTR retrotransposons in algae.

For example, the Ta elements of *Arabidopsis thaliana* have roughly 20 copies per genome, while the Del 1 elements of *Lilium henryi* have about 13,000 copies per genome. LTR retrotransposons are always variable in sequence within particular plant species. The *Vicia faba* species of bean contains one Ty 1-copia subgroup in more than one million copies, while the Bis 1 family represents up 5% of the wheat genome. Retrotransposons are a significant class of TEs in rice, with an estimated 1000 copies total in the genome. About 32 families were recovered, demonstrating the importance of retrotransposons in rice. Although 5 out of 32 families of these retrotransposons were active in tissue culture, they appeared dormant under normal growth circumstances. Grandbastein et al. (1989) reported the isolation of the first transposable element Tnt 1 in higher plants, which appears to be the most fully characterized mobile retrotransposon in tobacco (*Nicotiana tabacum*).

9.6.1.3 TY ELEMENTS IN YEAST

In different strains of yeast (Saccharomyces cerevisiae), a family of scattered repeating DNA sequences known as the Ty elements can be found at various locations. Ty stands for transposon yeast. A Ty transposable element is present in the yeast's haploid genome in roughly 35 copies. These transposons have a

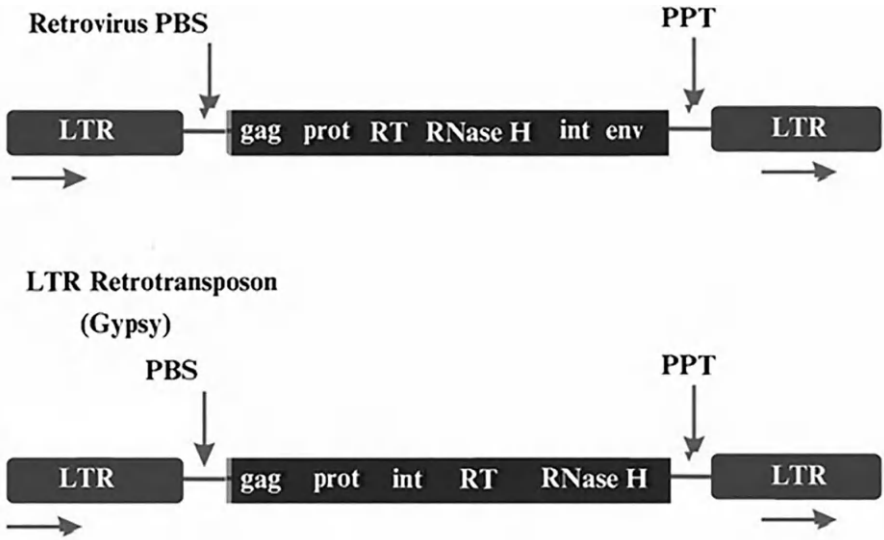


FIGURE 9.5 Class 1 mobile elements, PBS (primary binding site, PPT polyurine tract), sequences required for replication/transposition of LTR elements.

Source: Modified from Chawla, 2011

length of 5900 nucleotide pairs and are enclosed at both ends by a section of 340 base pair-long DNA known as the sequence. Long-terminal repeats, also referred to as LTRs, are sequences that are all oriented in the same way. Ty elements are surrounded by five base pair direct repeats that were produced when the DNA at the Ty insertion site was duplicated. Although the sequence of these TSDs is not known, AT base pairs are frequently found in them. This would suggest that Ty elements prefer to introduce into the gene of AT-rich areas.

The Ty elements' genomic structure mimics that of eukaryotic retroviruses. After entering a cell, these single-stranded RNA viruses create DNA from their RNA. TSD is then produced when the DNA insertion occurs at a certain site in the genome. The three genes *gag*, *pol*, and *env*, which successively encode structural, catalytic, also membrane proteins, are found in the simplest proviruses. Ty elements only have the gag and pol genes of retroviruses as their sole two genes, *TyA* and *TyB*. The result of these two genes can create virus-like particles inside yeast cells, according to biochemical tests. It is unknown, nevertheless, if these particles are indeed contagious. However, it has been demonstrated that Ty elements transpose through an RNA intermediary, which is why Ty elements are referred to be retrotransposons.

9.6.1.4 NON-LTR RETROTRANSPOSONS (RETROPOSONS)

Additionally known as nonviral retrotransposons. Along with other characteristics, this type of transposons can be differentiated by the non-existence of large terminal repeats. An enzyme system that is coded elsewhere will use them as targets for a transposition event. They do not code for transposition-related proteins. Retrotransposons make up a sizable portion of the DNA in genomes, which is moderately repetitive. The number of non-LTR transposons in plants is rapidly expanding, but they have been best studied in mammals and *Drosophila*. Common retrotransposons found in eukaryotes are non-LTR retrotransposons. LTRs are absent from non-LTR retrotransposons' terminal ends. They do, however, have a poly A tail, which is a homogenous sequence of A:T base pairs that results from post-transcriptional alteration. These non-LTR retrotransposons are introduced to the targeted site via an intermediary RNA molecule that is reverse transcriptase, an enzyme that is encoded by the component itself, converts the RNA into double-stranded DNA.

Transposable elements of humans

- long-interspersed nuclear elements (LINEs)
- short-interspersed nuclear elements (SINEs)

9.6.1.5 LINES

The structure of LINEs is similar to an integrated DNA version of an mRNA. A fully functional LINE encodes for the enzyme reverse transcriptase (RT), besides gag proteins, which are important in the intracellular packaging of RNA transcripts. The pol functions have the ability to reverse-transcribe LINE RNA into DNA, and the element also encodes an endonuclease (EN), which is probably involved in integration into the genome. Like all other mobile DNAs, LINE insertions are surrounded by brief direct duplications of the target DNA, which are often produced by a transposase or an integrase.

The LINEs come to an A-rich tract's end. The sequencing of LINEs indicates that the ORF in L1 and RT share similarities. As a result, it is possible that L1 began as a mobile gene that coded for its own transposition. The genomic sequences are inactive because they absence the promoter that was upstream of the initial start point for transcription since LINEs are derived from RNA polymerase II transcripts. Processed pseudogenes are what they are termed because they have the characteristics of the mature transcript.

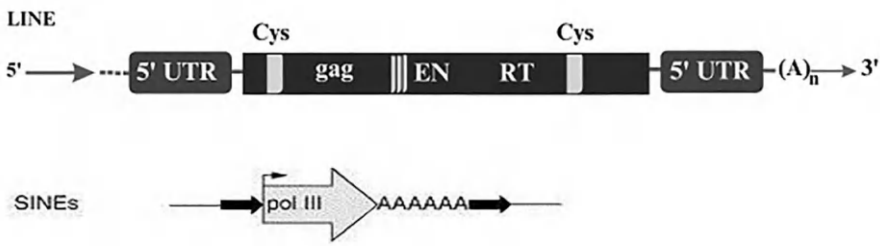


FIGURE 9.6 Structure of plant non-LTR retrotransposons (LINES and SINEs), terminated by a 3 poly(A) stretch. LINEs have two ORFs encoding by a nucleocapsid protein (gag), an endonuclease (EN) and the reverse transcriptase (RT). Cysteine-rich nucleic acid-binding domains (Cys) are represented by colored boxes. The region between the gag and EN reflects the overlapping of ORF1 and ORF2 flanked by untranslated regions (UTR). SINEs are composed of a region with homology to RNA polymerase III promoters.

Source: Modified from Schmidt, 1999

9.6.1.6 SINES

SINEs are rare in the majority of plant genomes but extremely common in animals. SINEs are typically only 100–300 bp in length and seem to be produced from RNA polymerase III products that have undergone reverse transcription. Since they lack recognizable peptides, they must transpose via integrase and trans-acting polymerase activities.

SINEs are often derived from tRNA or snRNA genes that have undergone mutations to develop an integrated structure that can be reverse transcribed. A newly inserted element typically can be translated in any active region of the genome because these RNA polymerase III transcribed genes include a promoter designated within the RNA itself, creating a high potential for amplification. LINEs and SINEs do not have introns since they are made from mature mRNA and typically have an integrated poly(A) tail at their 3′ end. Pseudogenes without introns are thought to be integrated RNA copies.

9.6.2 CLASS 2: DNA TRANSPOSONS

These TEs, such as DNA transpose directly. This group of transposable components is diverse. The Ac-Ds, Spm/dspm (En/I), and Mu maize transposons are the top three members of this class. Ac-Ds elements were the first TEs Barbara McClintock (1951) extensively researched. Initiated in the 1940s, genetic research on maize has revealed modifications to the genome that occur during somatic cell division. Controlling factors, which are recognizable by their mobility from one location to another, are responsible

for these changes. The regulating elements, which McClintock initially identified only through genetic methods, are now recognized as TEs that are identical to those that originate in bacteria. These transposable components, like DNA, undergo direct transposition. This collection of TEs is varied. The three most prevalent members of this class are the Ac-Ds, Spm/dspm (En/I), and Mu maize transposons. The earliest TEs of any species were Ac-Ds elements, which Barbara McClintock (1951) thoroughly investigated. Genetic analysis of maize, which began in the 1940s, has shown changes to the genome that take place during somatic cell division. These changes are caused by controlling variables, which can be identified by their mobility from one place to another. McClintock initially detected the regulatory elements only through genetic approaches; However, it is now known that these are TEs, just as those found in bacteria.

9.7 TRANSPOSABLE ELEMENTS IN MAIZE

9.7.1 AC-DS ELEMENTS

Zea mays shows transposable element system represented by its activator (Ac) and dissociation (Ds) elements. The autonomous *Ac* element is 4565 bp long and encodes a 3.5 kb long main transcript with a lengthy untranslated leader (652 nucleotides) that is rich in GC. Four introns from the main transcript are spliced out to produce a mature mRNA with 3312 nucleotides, which is then translated into a protein with 807 amino acids and a 92 kDa mass. A straight 8 nucleotide pair repeat surrounds the *Ac* element. When the element inserts into a chromosome, a direct repeat is produced. The element itself has more repeat patterns. The most obvious is an 11-bp sequence that is repeated in the opposite direction at each end. It is believed that these TIRs are crucial for transposition. *Ac* may be transposed into an increasing number of transgenic heterologous plant species using the *Ac* product known as transposase. The 200 bp at each end corresponds to the cis determinants for transposition of *Ac*. Mutations in these areas lessen or eliminate the excision of *Ac* elements. DNA-binding tests reveal that the Ac transposase binds to clusters of the hexamer motif AAACGG inside these regions (Kunze and Starlinger, 1989). The 11-bp TIRs, which appear to be recognized by *Ac* transposase, are the other distinct and essential cis determinant for excision. Regarding excision, the irregularity of the two *Ac* ends is significant. *Ac* elements with identical ends exhibit excision defects.

Dissociation (*Ds*), a defective part of the *Ac* family, is a highly diverse group. The 11-bp TIR is present in all *Ds* components. When autonomous

elements are deleted, the transposase that normally works on those sites (termini) is inactivated, resulting in the formation of nonautosomal elements. *Ds* elements are unable to manufacture transposase because of deletions. However, the transposase generated by the *Ac* element is trans-acting and diffusible, which leads to the transposition of *Ds* elements.

Two characteristics of maize have made it easier to track transposition events: (1) controlling elements frequently insert close to genes that have noticeable but nonlethal effects on the phenotype; and (2) maize exhibits clonal development, and as a result, the phenotype can be used to track the timing and occurrence of a transposition event. Variegation is the term used to describe a region of tissue that is produced by the mitotic descendants of a certain cell that remains in the same place but has a different phenotype.

Ac appears to be the model for a family of elements with many members found in plants (Gierl and Saedler 1992).



FIGURE 9.7 Structure of *Ac, Ds* (non-functional transposase) elements in maize.

Source: Modified from Fedoroff, 1989

9.7.2 SPM SYSTEM (SUPPRESSOR MUTATOR SYSTEM)

McClintock also identified the suppressor mutator (Spm) and enhancer (En) families of maize transposons. The autonomous element in this family is known as the Spm, while the non-autonomous elements are known as the dSpm (d stands for deleted or defective, whereas En is autonomous and inhibitor (I) is non-autonomous element). The independent components of Spm and En are nearly identical. At least two functional products are encoded by the 8287-bp long autonomous enhancer (En) or suppressor mutator element (Spm), which was created by alternative splicing from a precursor transcript. There are 13-bp TIRs in En/Spm. These TIRs, which are cis-acting sequences, are essential for excision. They duplicate the nucleotide

pair target site when they insert into a chromosome. Due to the deletion of a portion of their DNA sequence, the dSpm elements are smaller than the Spm elements. It is impossible for the deleted dap elements to promote their own transposition.

The two genes together encode two functional proteins, TNPA (67 Kuda) and TNPD (131 Koda). Although TNPA is around 100 times more prevalent than TNPD, both proteins are necessary for En/Spy transposition. En/Spm also needs the asymmetry at the two ends, just like Ac transposon. An autoregulatory function for TNPA has been hypothesized due to the fact that the innermost TNPA binding motif at the 5′ end matches with the "TATA" box of the single En/Spm promoter (Gierlet al., 1988). The TNPD may interact with 13-bp TIRs and cause endonucleolytic cleavage at the ends of the elements, according to speculation based on comparisons with En/Spm-related elements from other plant organisms. The CACTA family of elements includes En/Spm. These elements share virtually identical 13-bp TIRs and upon insertion, cause a3-bp TSD.

9.7.3 MUTATOR TRANSPOSABLE ELEMENTS

Mutator TEs are among the most prevalent mutagenic transposons due to their extraordinarily high transposition rates and propensity to insert at or near genes (Cresse et al., 1995; Lisch, 2015). The first Robertson's Mutator element (MuDR, "Mutator Don Robertson") was found in maize, where Mutator lines can display mutation frequencies up to 50 times the background rate of spontaneous mutation (Robertson, 1978;Walbot, 1991). 'MULEs' (Mutator-like elements) are the name given to members of the Mutator DNA transposon superfamily (Le et al., 2000). A transposase domain with a catalytic DDE motif and frequently an additional zinc finger DNA-binding motif is enclosed by TIRs at each end, MULEs have a structure that is reminiscent of traditional "cut-and-paste" DNA TEs. MULE TIRs, on the other hand, are often hundreds of base pairs longer than those seen in other DNA TE superfamilies. Some families of MULE (non-TIR MULEs) seem to completely lack TIRs (Feschotte and Pritham, 2007). In MULE elements, TSDs, which are short direct repeats made after transposition and happen immediately outside of the TIRs, are 8–11 bp long (Le et al., 2000), are another feature of MULEs. The acquirement of one or more extra ORFs is what makes MULEs. The first full MULE element found in maize included two ORFs: mudrA, which encodes the transposase, and mudrB, which encodes an unidentified protein that appears to be necessary for integration

into the maize genome (Lisch, 2002; Rudenko and Walbot, 2001). MULEs are most commonly found in plant genomes, particularly those of maize (Le et al., 2000), although they can also be found in a variety of eukaryotic genomes (Lisch, 2015). Numerous non-autonomous Mutator elements, also known as "Pack-MULEs," are found in the genomes of plants and carry pieces of host genes in place of their transposase genes (Jiang et al., 2004).

9.8 TRANSPOSABLE ELEMENTS IN DROSOPHILA

Some mobile elements present in eukaryotes are DNA transposons, and their transpositional mechanism is comparable to that of bacteria. The P elements in *Drosophila*, however, were the first DNA transposons to be molecularly characterized.

9.8.1 P ELEMENT IN DROSOPHILA

The IS element, which includes a single ORF that encodes a single protein (transposase) and briefly inverted terminal repeats at both ends, is comparable to the insertion sequence (P) element found in *Drosophila*. The length of the P elements ranges from 0.5 to 2.9 kb. Margaret Kidwell made the discovery of P elements while researching hybrid dysgenesis.

When laboratory-raised female flies were combined with wild male flies, a process called dysgenesis resulted. The laboratory stocks in these crosses are supposed to have an M cytotype (cell type), whereas the natural stocks are supposed to have a P cytotype. P element is absent in lab flies. Including M (female) P (male) crosses results in F1 progeny that exhibits a variety of unexpected germline characteristics, including infertility and a high rate of mutation, and a high incidence of chromosomal abnormality and dissociation. These dysgenic F1 hybrid offspring are naturally defective. The condition is known as dysgenesis. However, no dysgenesis is shown in the F1 progeny of the reciprocal cross between P (female) and M (male). The simplest explanation for the dysgenesis occurrence is P element transposition. The P element, which silences the transposase genes, is present in all adult P cytotype flies.

When M (female) and P (male) are crossed, the genes are active in the F1 generation, causing dysgenesis. The cross between M cytotype (female flies without P elements) and P cytotype (male flies with P elements) results in gametes with appropriate cytotypes from each parent. Since female gametes

have M cytotype, male gametes supply P elements to the freshly produced zygote, that is in an environment free of silencing. Since the P elements that were obtained from the male genome can now transpose across the zygote genome and inflict a variety of damage when they insert into genes, all F1 children born from such zygotes are dysgenic. On the other hand, no dysgenesis is shown in the reciprocal cross between the P cytotype (female flies, P element) and the M cytotype (male flies, no P element). Because a component is present in the female gamete's cytoplasm, the P element is already silenced in the female gamete even if the female gamete provides the P element to the freshly formed zygote. As a result, dysgenesis is prevented in zygote generated from such a gamete with the P element silenced.

9.8.2 COPIA ELEMENTS

In *Drosophila*, their transposition results in changes that affect eye color. They have direct terminal repeats (DTR) of about 276 bp at either end and are about 5–8 kb in size. There are small inverted repeats (IR) of roughly 17 bp length found within each of these direct repeats. Within the cell genome, there are between 10 and 80 copia elements.



FIGURE 9.8 Copia transposable elements.

Source: Modified from Hassan et al., 2024

9.8.3 FB ELEMENTS

These foldback elements were discovered in the *Drosophila* genome. These have the capacity to fold back to form a stem and loop structure due to their lengthy inverted terminal repeats. Through inducing mutation via insertion or by interfering with the normal gene expression, their transposition leads to an altered expression.

9.8.4 GYPSY ELEMENT

Among *Drosophila* melanogaster's most well-known mobile sequences are the TEs P and gipsy. They are abundant in the repetitive region of the genome

of this species and seem to be permanent members (Bayev et al., 1984; Daniels and Strausbaugh, 1986; Stacey et al., 1986; Daniels et al., 1990; Loreto et al., 1998a). These elements belong to two sizable categories of mobile sequences (Finnegan, 1989): Class I retrotransposons, like the gipsy and 412 transposons, which move Class II DNA transposons like the P and hobo transposons utilizing a transposase and an intermediary RNA molecule and a RT.

Gipsy is a long, 7.3 kb retrotransposon that is extensively distributed in Drosophila and the subgenus Sophophora. It contains 0.5 kb of well-conserved LTRs (Stacey et al., 1986; Loreto et al., 1998b). This widespread distribution is likely due to Gypsy's retroviral properties (Bayev et al., 1984; Terzian et al., 2000; Vázquez-Manrique et al., 2000; Mejlumian et al., 2002; Pélisson et al., 2002; Heredia et al., 2004). Based on the significant pattern similarity across gipsy strains found by Bayev et al. (1984) using Southern blotting, it is conceivable that this element entered the D. melanogaster genome early in this species'' evolutionary history. The same appears to be true for *Drosophila simulans* (Loreto et al., 1998b). The evolution of gipsy element sequences in the genomes of various *Drosophila* populations worldwide is not well understood, nevertheless.

9.8.5 HOBO ELEMENTS

In *Drosophila melanogaster*, the hobo element is a class II active transposable element that can cause gonadal dysgenesis. Some hobo-related sequences (hRSs) are considered to be ancient genomic constituents because they may represent remnants of previous "hobo" invasions. However, several of these hRSs are still active. The expression pattern of hobo and a particular subtype of hRSs, hobo (VAHS), were examined in the current study. It was established that both components express both sense and antisense mRNA transcripts. An expression pattern in whole-mount embryos that resembles several genes that control development was discovered. Here, we propose that hobo sequences contain cis-regulatory regions that are comparable to those in developmental genes. In light of this, hobo mobilization could aid in the creation of novel regulatory networks during genomic evolution

9.9 TRANSPOSABLE ELEMENT OF HUMAN

At least 44% of the repetitive material in the human genome is made up of TEs, which include retrovirus-like elements (8%), retroposons (33%), and various families of elements that transpose by copying and pasting (3%).

Types of human transposons:

i. LINEs
ii. SINEs

9.9 1 LONG-INTERSPERSED NUCLEAR ELEMENTS (LINES)

The main human transposable element, or L1 retroposons, is a member of the LINEs class of long-interspersed nuclear elements. The human genome also contains the L2 and L3 LINEs sequences, although neither of them is capable of transposition. About 6 kb in length, the whole L1 element has two ORFs, an internal promoter that RNA polymerase II can recognize

- A protein that binds to nucleic acids is encoded by ORF1
- while a protein with endonuclease and RT activity is encoded by ORF2.

A full L1 element can be found in 3000–5000 copies of the human genome. An L1 element undergoes transposition in two steps: first, the full L1 element is converted into RNA, and then, second, the RNA is converted back into DNA. The nucleus is where transcription and reverse transcription both happen. But before being reverse transcribed, the L1 RNA travels to the cytoplasm where it is translated into polypeptides (nucleic acid-binding proteins, endonuclease, and RT). When the RNA travels back to the nucleus, the polypeptides are still bound to it. The endonuclease activity of the polypeptide carried by ORF2 is capable of cleaving one part of a DNA duplex at a specific location in a chromosome. RT activity and the polyA tail of the polypeptide combine with the 5' end of the cleaved DNA to create a single DNA strand. The 3' end of the newly synthesized L1 DNA is then used as a primer to create double-stranded L1 DNA. New L1 element is consequently positioned in new location.

9.9.2 SHORT-INTERSPERSED NUCLEAR ELEMENTS (SINES)

SINES, or short-interspersed nuclear elements, are the second-most common class of TEs in the human genome. SINEs have an internal promoter and are less than 400 base pairs long, yet they do not encode proteins. SINEs, like all retroposons, lack terminal repetitions in favor of an A:T base pair sequence at one end. SINEs are transposed by first being reverse transcribed from DNA into RNA and then back into DNA. But there is not an ORF for

any enzymes in SINEs. RT and other enzymes therefore rely on LINEs-type elements. As a result, in order to replicate and insert themselves into the genome, SINEs depend on LINEs. The human genome contains members of the Alu, MIR, and Ther2/MIR3 families of SINEs. However, only the Alu components are actually active during transposition.

9.10 EFFECT OF TRANSPOSONS

DNA sequences that can migrate or "transpose" within a genome are referred to as transposons, jumping genes, or TEs. Transposons can have a variety of impacts when they travel and insert themselves into different regions of the genome, some of which include:

Transposons can modify the DNA sequence in a population, resulting in genetic diversity. A transposon can alter a gene's normal function or trigger the synthesis of several protein isoforms when it inserts itself into a gene or its regulatory regions. Individual phenotypic variations may come from this.

By inserting themselves into the coding or noncoding sections of genes, transposons can result in mutations. A transposon can alter the reading frame or introduce premature stop codons if it inserts itself into a coding sequence, which results in non-functional proteins. Transposon insertions can have an impact on how genes are regulated in noncoding areas.

Chromosome rearrangements, such inversions, deletions, and duplications can be facilitated by transposons. A transposon may transport nearby genomic sequences when it moves from one place to another, changing the chromosomal organization. Gene expression and genomic structure may be significantly impacted by these rearrangements. The evolution of genomes has benefited greatly from the presence of transposons. They may add additional genetic material to a genome, which could result in the emergence of new genes or gene families. Transposons can also play a role in genomic reorganizations, the development of novel regulatory components, and the evolution of gene expression patterns. Some transposons are linked to illness. As an illustration, some transposons, like retrotransposons can lead to genetic problems by interfering with genes or encouraging genomic instability. They have been linked to diseases like muscular dystrophy, cancer, and hemophilia. It is important to note that while transposons can have these effects, they are not always harmful or detrimental. In fact, transposons have been an important source of genetic innovation throughout evolution and have contributed to the diversification of genomes.

9.11 USES OF TRANSPOSONS

Transposons can alter the structural and functional properties of the genome by shifting where they are located within it. TEs can change the genome through insertion, deletion, etc. It has a beneficial impact on evolution since it significantly affects how the genetic makeup of organisms changes. They can be used in gene cloning as cloning vectors as well. P elements, for instance, are often utilized as transgenic delivery vehicles in *Drosophila*. While mapping the genomes, transposons may potentially be used as genetic markers. To find and isolate a specific gene, transposon-mediated gene tagging is used.

Vectors for genetic exchange between different organisms:-

Additionally, many transposons' drug resistance genes are helpful in the creation of plasmids as cloning tools. Due to insertional inactivation, the use of transposons increases the rate of mutation.

9.12 CONCLUSION

In conclusion, transposons are intriguing elements within the genome that have significant implications for genetic variation, gene regulation, genome rearrangement, evolutionary innovation, and genetic engineering. They contribute to the diversity and evolution of species by introducing mutations, altering gene expression, and facilitating the generation of new genes and functional elements. Transposons can be harnessed as valuable tools in genetic engineering and biotechnology, but their activity must be carefully regulated to prevent detrimental effects on genome stability and proper gene function. The study of transposons continues to provide insights into the complexity and dynamics of genomes and their role in shaping the characteristics and adaptations of living organisms.

All organisms have transposons in their genomes, which can make up a significant portion of the total DNA sequence. They are a significant contributor to genomic rearrangements and mutations. The majority of macromolecular rearrangement is caused by TEs' capacity to insert and produce deletions and inversions. They create mutations that are employed to produce fruits like grapes, maize, and other berries that are varied colors. They are therefore employed in genetic research.

KEYWORDS

- **transposable element**
- **jumping genes**
- **genomics**
- **insertion sequences**
- **human genome**

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CHAPTER 10

Chromosomal Aberrations

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ABSTRACT

The study of chromosomes and chromosomal aberrations is known as Cytogenetics (Gersen, 2013). These aberrations in chromosomes can be both structural and numerical. Human beings have 23 pairs of chromosomes, out of which 22 pairs of chromosomes are homologous autosomes. The remaining set is of sex chromosomes, which are homozygous X in females and heterozygous X and Y in males (Milani and Tadi, 2022). The anomalies that arise in the number of chromosomes are called numerical abnormalities and they are more common than the structural abnormalities, which as the name suggests occur due to changes in chromosomal structure (McFeely,1993) According to the World Health Organization, 240,000 newborns die every year within a month due to congenital disorders. Additionally, congenital disorders contribute to an additional 170,000 child deaths occurring between the ages of one month and five years. Numeric and structural chromosomal abnormalities are present in roughly 0.6% of newborns (Shaffer and Lupski, 2000). These abnormalities often lead to physical abnormalities, birth defects, and developmental impairments. These observable traits result from an unequal distribution of one or more genes that are sensitive to changes in their quantity on a specific chromosome or segment of a chromosome. Such gene imbalances can have severe consequences, being responsible for 25%

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of all cases of miscarriage and stillbirth, as well as 50–60% of miscarriages occurring during the first trimester (Hassold et al., 1980).

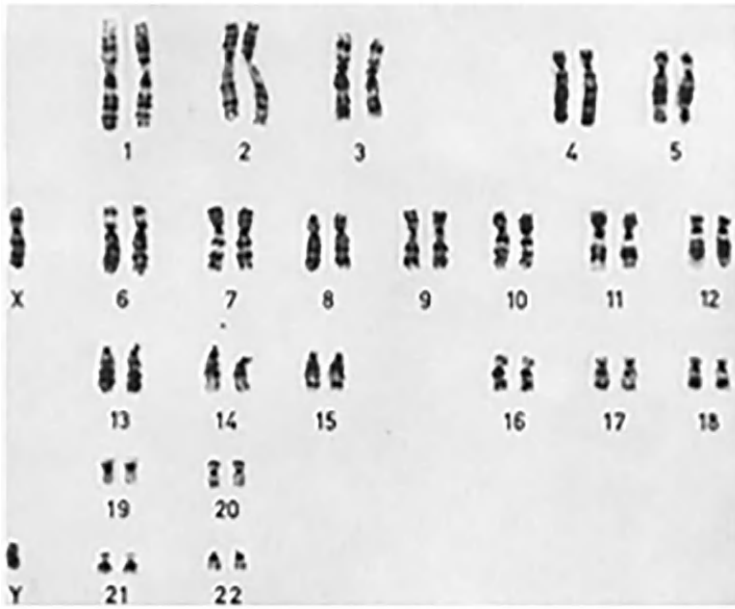


FIGURE 10.1 The human karyotype- all the chromosomes arranged on the basis of their size.
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10.1 NUMERICAL ABERRATIONS OF CHROMOSOMES

For a given species, the number of chromosomes tends to remain the same. When numerical abnormalities occur, the number of chromosomes deviates from the normal one. This may result in the presence of an extra chromosome (supernumerary), or a missing chromosome. In some cases, the complete haploid set of chromosomes multiplies. The structure of the chromosomes during numerical aberrations however does not change.

A Polish–German Botanist named Eduard Strasburger first coined the terms "haploid" and 'diploid' which refer to the single 'n' as in gametes and double '2n' as in chromosome set of somatic cells, respectively. The term "ploidy" refers to the total chromosomal content present in the cell. The term euploidy (*eu = true; ploid = unit*) is used to describe the chromosome complement that is an exact multiple of the haploid 'n' number. The euploid variation that involves more than two sets of the chromosomes is called polyploidy, as in triploid (3n), tetraploid (4n), and so on. A chromosome

set that comprises one or more chromosomes added or deleted results in a condition called aneuploidy. Euploidy exists in natural form as haploid in fungi, diploid in mammals, and polyploids in case of plants.

10.2 MONOPLOIDY

Monoploidy is a genetic condition where organisms possess a single set of chromosomes in their genome. In most diploid organisms, the monoploid number (denoted as x) is equivalent to the haploid number. For example, in barley, x = 7, and in corn, x = 10. It is noteworthy that monoploidy is rare in animals, with male bees being an exception as they develop from unfertilized eggs.

The monoploid number corresponds to the haploid number in diploid organisms. All monoploids can be considered haploid, not all haploids are necessarily monoploids. Another perspective on this concept is that 'haploidy' represents the chromosome number found in the gametes of any species, whereas 'monoploidy' characterizes the chromosome number in diploid species.

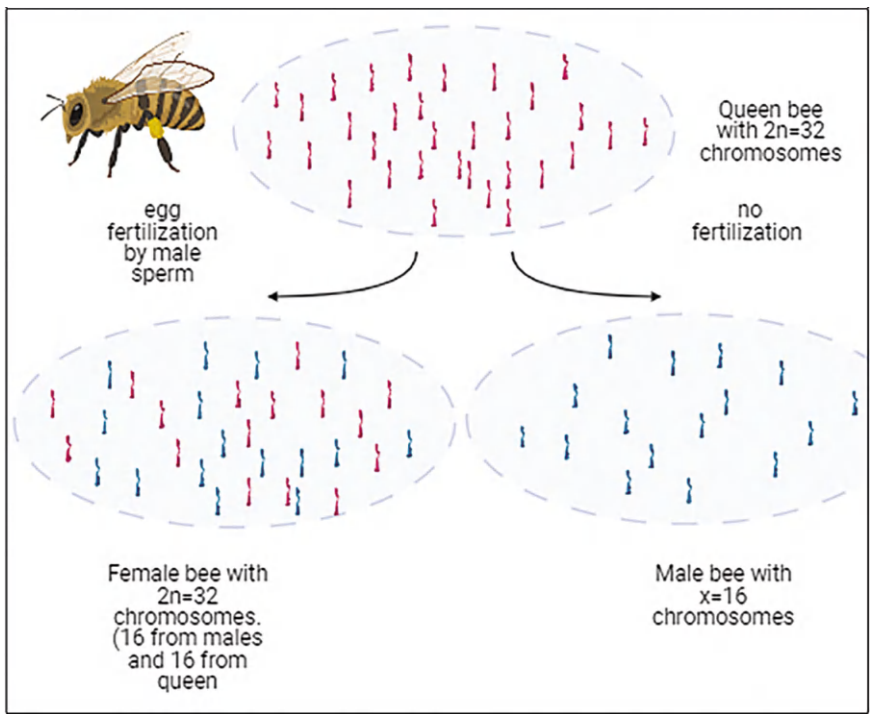


FIGURE 10.2 Fertilization in honey bee. Females are diploid while drones are monoploid

In nature, monoploidy can result from parthenogenesis, a natural form of asexual reproduction that does not require fertilization by sperm, as seen in male bees (drones). Female bees are diploid. Occasionally, monoploidy can spontaneously arise in flowering plants (angiosperms), leading to parthenogenetic egg development. A comparison of monoploid maize with diploid one showed that monoploid plants are not only smaller in size but also had fewer components (Chase, 1964).

However, it is important to note that monoploidy is often lethal in animals due to the potential expression of lethal alleles. Monoploids lack a complete set of chromosomes, preventing normal meiosis and the production of viable gametes, rendering them sterile.

Monoploidy can also be induced. Some artificial ways to do so are use of anti-mitotic agents like oryzalin, colchicine, and trifluralin (Sarachek, 1983). These agents can be applied in a control laboratory environment to induce monoploidy. Some other means to achieve these goals are: delayed pollination, X-ray treatments, temperature shock, and distant hybridization.

Monoploides have multiple uses especially in the field of plan biotechnology. It provides ease in performing genetic experiments. As monoploids only comprise a single set of chromosomes, each gene has only one allele to express. Weather dominant or recessive, all the genes are expressed. Monoploides can aid in exploring the arrangement, chemical makeup, and function of various chromosome segments, manipulating which can result in enhanced productivity (Shakoori, 2017).

10.3 POLYPLOIDY

Polyploidy refers to the condition where an organism possesses more than the typical two sets of genomic chromosomes (>2n). This often results in larger cells and, consequently, higher yields and increased plant size. However, it is important to note that for each genus, there exists an optimal number of polyploid individuals. Polyploids can take various forms, including triploids (3n), such as in certain seedless watermelon varieties; tetraploids (4n), as observed in salmonidae fish; pentaploids (5n), exemplified by the kenai birch; hexaploids (6n), seen in crops like wheat and kiwi, and so on. Beyond a certain level of polyploidy, the additional chromosomes can lead to reduced production and growth.

There can be various factors behind polyploidy including spontaneous duplication of somatic cells that results in the presence of multiple sets of

chromosmes in an organism (Dar and Rehman, 2017). Another cause can be total non-disjunction of homologous meiotic chromosomes (Moghe and Shiu, 2014). Polyploidy can also be induced using drugs that obstacle cell division (Sun et al., 2018). Polyploidization is a phenomenon that occurs in both plants and animals as part of their developmental processes. If it occurs within specific tissues of an organism, it is referred to as somatic polyploidy. This is distinct from germline polyploidy, which occurs in plants and in some amphibians and fish.

Polyploidy plays a significant role in plant speciation and can be categorized into three types:

1. Autopolyploidy
2. Autoallopolyploidy
3. Allopolyploidy

For example, consider a plant named 'Flora' with a diploid chromosome number of 2n = 8. If a mutation spontaneously results in an individual with four sets of chromosomes (4n = 16), it remains the same 'Flora' species but has undergone autopolyploidy. Autopolyploidy occurs when an individual within a species has multiple sets of chromosomes from its own species.

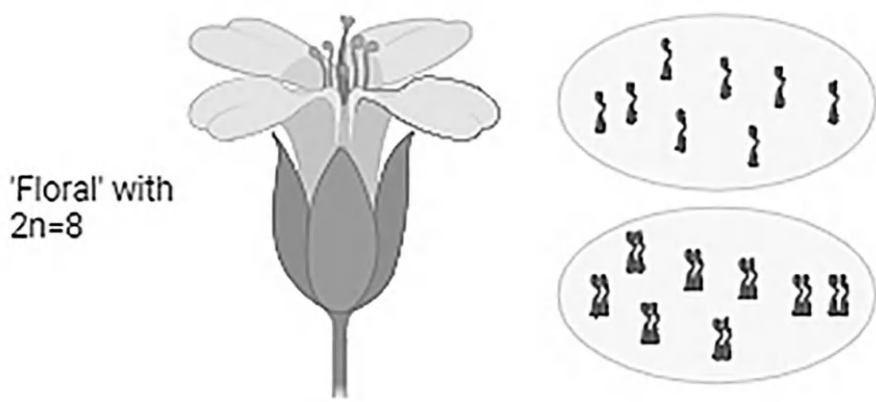


FIGURE 10.3 Autoploidy.

Now, if 'Flora' acquires an additional set of chromosomes from a closely related species like 'Flora 2' (with a genome of 2n = 6), this condition would be termed autoallopolyploidy. The resulting individual would possess both genome sets, resulting in 2n = 14.

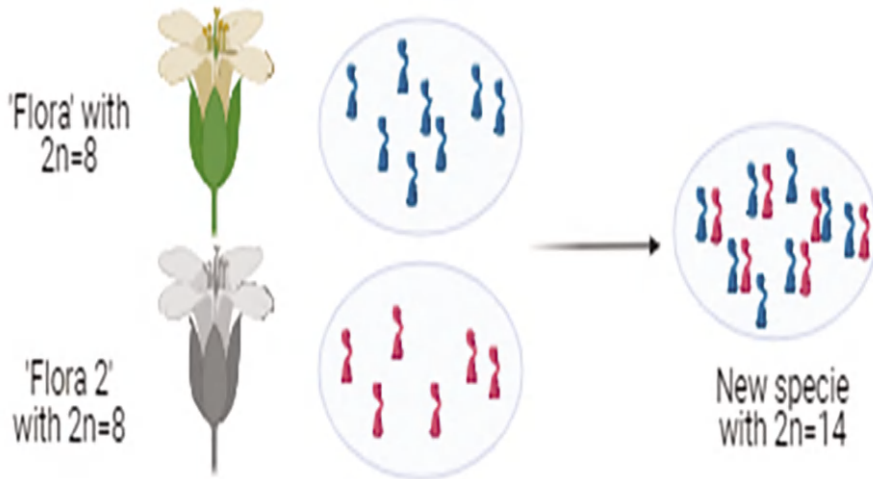


FIGURE 10.4 Autoallopolyploidy.

Allopolyploidy, on the other hand, occurs when two distinct species undergo hybridization to produce an individual with double the typical chromosome number. For instance, if 'Flora' crosses with a different species individual, let us say 'Floris' with a chromosome number of (2n = 10), the new individual will have a chromosome set of 2n = 18, and this new individual is considered a different species.

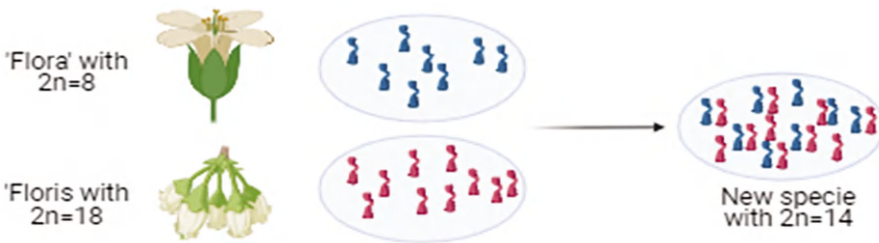


FIGURE 10.5 Allopolyploidy.

It is worth noting that polyploidy is relatively rare in the animal kingdom, and the exact reasons for this rarity remain uncertain. It may be related to the delicate balance of chromosomes involved in sex determination or the infrequency of self-fertilization in animals. In the case of humans, polyploid zygotes typically do not survive, accounting for approximately 20% of spontaneous abortions.

10.4 POLYPLOIDY EFFECT ON PHENOTYPES

Polyploidy has a range of phenotypic effects, some of which include delaying the expression of mutations and increasing the frequency of recessive mutations in the standing genetic variation (Sun et al., 2018) This alteration of plant genomes and phenotypes extends to traits associated with root and nodule development in legumes (Forrester and Ashman, 2018). However, it is important to note that polyploidy can have both positive and negative consequences. On the positive side, it may lead to increased enzymatic activity and gene expression (Larkin et al., 2016). Nevertheless, it can also entail potential drawbacks related to organismal ecology and the genomic mutational load (Larkin et al., 2016).

Specific examples of polyploidy effects can be observed in plants like *Dendrobium nobile*, where it results in taller and wider flowers, but with a reduced number of flowers per pseudobulb and delayed flowering (Vichiato et al., 2014). Additionally, polyploidy can induce changes in cell size, genetic and phenotypic traits, as well as alterations in metabolism and gene transcription (Mayfield-Jones et al., 2013). Notably, it can even influence early centromere association during anther development, potentially shortening meiotic prophase and impacting gene expression (Martinez-Perez et al., 2000). These diverse effects underscore the complexity of polyploidy's role in shaping the biology and characteristics of organisms.

10.5 ANEUPLOIDY

Aneupolidy (aneu = uneven; ploid = unit) is marked by the possession of irregular chromosome number. Aneuploidy can originate through various mechanisms. If we talk about meiosis, premature seggregation of sister chromatids or chromosome non-disjunction can cause errors in oogenesis (Stohl and Platt, 2018). These errors are prone to produce aneuploid oocytes which are significant cause of aneuploidies arising in embryos and fetuses (Fragouli et al., 2013). There are other factors that contribute to the occurrence of aneuploidies in oocytes including fluctuation in hormonal levels, advanced maternal age, and exposure to certain environment agents (Delhanty, 2010). Aneuploidy is not confined to meiosis, it can also result due to faulty mitosis, particularly during initial phases of cell division that follow fertilization (Stuppia, 2013). Aneuploidy can result in congenital disorders, diminished reproduction, etc (Compton, 2011).

If the cause of aneuploidy is loss of one or more chromosomes, it is known as hypoploidy; however, if it is due to addition of one or more chromosomes,

it is called hyperploidy. Examples of hypolploidy are *(i)* monosomy, (Taketa et al.) nullisomy. Similiarly, hyperploidy includes *(i)* trisomy, (Taketa et al.) tetrasomy.

10.5.1 MONOSOMY

Loss of single chromosome is called monosomy. It can be shown as (2n-1). A human being with monosome will have 45 chromosomes. Autosomal monosome is not tolerated in human beings. X-monosomy is observed in case of Turner's syndrome which was first investigated in 1959 by C.E. Ford who found only one X-chromosome in a 14-year-old girl. Turner's syndrome or monosomy X is a neurodegenrative disorder marked by complete or partial absence of an X-chromosome. Specific physical and medical characteristics associated with this disorder are reduced height, lack of estrogen, cardiac problems, etc. as for cognitive and behavioral characteristics, individuals show weak verbal skills, and difficulties are visuospatial perception and emotional processing. Individuals with Turner's syndrome are females who do not undergo puberty, and hence the secondary sexual charateristics do not develop properly. Pubic hair growth is scarce, with little breast development, and apsence of menstruation cycle. These women have usually short stature, low hairline, skin folding on neck, and broad chest. Monosomy X can be shown as XO. About one-half of females with Turner syndrome have a monosomy (45,X) with only one X chromosome. The rest of the cases are due to 45,X/46,XX mosaicism indicating that some cells have one chromosome while the others have normal 2.

10.5.2 NULLISOMY

In nullisomy, a whole set of chrmosomes is lost. It is represented as (2n-2). Polyploid plants may survive it. This condition is lethal in human beings. Sperm nullisomy results in male infertility. Failure of chromosomes to segregate properly during meiosis may lead to nullisomy (Begemann et al., 2022). Nullismy in plants lead to sterility, loss of sustainable seed and pollen, and reduced vigor (Taketa et al., 2005).

10.5.3 TRISOMY

Trisomy occures when an individual has one extra chromosome (2n+1). Trisomy can occur in both autosomes and sex chromosomes. The most

common trisomies in human beings is of chromosome 13, 18, and 21, with 21 being most prevelant (Down's syndrome). A trisomic individual will have three homologous copies of a chromosome. For plant, each chromosome has a distint effect on the phenotype of plants. This allows for identification of trisomic individuals.

There can be multiple reasons of how a trisomic individual may originate. The fundamental principle however is an extra chromosome present in a gamete (n+1) getting fertilized with a normal gamete (n).

There are various types of trisomies.

i. Primary trisomy refers to the presence of the extra chromosome that is normal and completely homologous to a pair of homologues within the chromosome set. For instance, *Datura stramonium* shows trisomy (2n + 1 = 25), and the type of chromosome was identified on the basis of variations in shape and size of capsule, size of plant, flower shape, leaf size, length and size of spines, and form of stigma (Blakeslee, 1921). Primary trisomy can take place when autotriploid is crossed with a diploid. As in case of cabbage, where primary monosomics were selected from the progeniy of triploid–diploid crosses, the individuals obtained had pimary monosomy and unique charateristics (Zhang et al., 2006). Primary trisomics in soybean can also form due to desynaptic and asynaptic mutants (Palmer, 1976). Primary trisomy has also been produced using ionic radiation (Simeone et al., 1985).

10.6 PRIMARY TRISOMICS AS GENETIC MARKERS IN PLANTS

Primary triaomics have been recognized as genetic markers in a variety of plant specie including cucumber (Diao et al., 2009), cabbage (Zhang et al., 2006), and grass pea (Talukdar and Biswas, 2007). In case of diploid plants, these trisomic chromosomes can be visually recognized. The individuals with trisomic chromosome show unique phenotypic traits including seed coat color, floral structure, pollen sterility, and leaflet structure that can be categorized as genetic markers (Cregan et al., 2001). In case of soy bean, a complete primary triomic set has been established that fascilitates the correlation of linkage groups with specific chromosomes. These primary trisomics have aided in pointing the Mendelian genes on a particular chromosome and formation of genetic map of soybean (Xu et al., 2000).

ii. Secondary trisomy refers to the condition where additional chromosome is an isochromosome. An isochromosome has two identical arms and typically involves one of the two arms of a normal chromosome. This isochrome can be result of centromere misdivision. Secondary trisomics can be formed in a diploid plant, due to the presence of chromosomes that do not pair during meiosis also known as univalents. The plants that carry more than one univalent chrmosome, usually produce progeny that has a significant number of trisomes. Secondary trisomes can also be present in the progeny of primary trisomics. Misdivision of centromere in a univalent chromosome can form an isochromosome. This misdivison can result in the formation of two isochromosomes, one for the each arm. So for a primary trisome that exists, it can result in two types of secondary trisomies.

iii. The third type is telocentric trisomy. In such trisomy, the individual has a normal set of chromosomes along with an extra telocentric chromosome. Therefore, the fragment of telocentric chromosome corresponds to a single arm of a chromosome pair within the typical chromosome complement. In nature trlocentric trisomics have been found to exist in plants and non-human specie like chimpenzee (Hiltgen et al., 1996; Dhar et al., 1990). Telocentric trisomics also originate due to centromere division-gone-wrong. These are prevelant in the plants with the univalent chromosome. The phenotypic effect of telocentric trisomies is less obvious as compared to primary and secondary trisomics. During the division, the fragment of the telocentric chromosome can pair up with the normal chromosme (homologous arm) to form a trivalent. Their effect is less deleterious than entire chromosome or iso-chrmosome. These trivalents can be rod-shaped, Y-shaped, or zigzag. They are typically identified via Karyotyping by Giemsa-C or Giemsa-N banding pattern.

iv. Tertiary trisomics mean that the extra chromosome has been present as a result of trans-location. In such conditions, the chromosome end is identical to non-homologous chromosomes. If it is reciprocal translocation, in which two chromosomes have exchanged segments, two potential varieties of tertiary trisomies exist. The extean chromosme has the centromere of one chromosome and a

translocated segment from the other chromosome. In some conditions, it may carry the centromere of the other non-homologous chromosome and traslocated portion from the first chromosome. In maize, it occures due to the reciprocal translocation between the normal chromosome and supernumerary B chromosome (Auger and Birchler, 2002). Another reason can be malsaggregation of a reciprocal translocation, that leads to partial trisomies with two distict chromosomes (Braddock et al., 2000). The rate of transmission of this extra chromosome in the tertiary trisomic varies and is effected by genetic factors and inbreeding (Janse and Sybenga, 1992). The uneven chromosome distribution and aberrant meiosis can lead to change in physical properties and reduction in fertility (Lakshmi and Nalini, 1989).

v. Sometimes when the diploid organism is devoid of a single chromosome, this deficiency is compensated by the presence of two chromosomes that together provide the equivalent function of the missing chromosome. This new chromosome can be telocentric or result of tranlocation. These studies have been done on rye and buckwheat. In case of rye, the meiotic behavior of the compensating trisomics has been closely correlated with length of the involved chromosome segments (de Vries and Sybenga, 1989). Their frequency can be determined by noting how frequently the segments of translocation chromosomes associated at first meiotic metaphase are exchanged (De Vries, 1985).

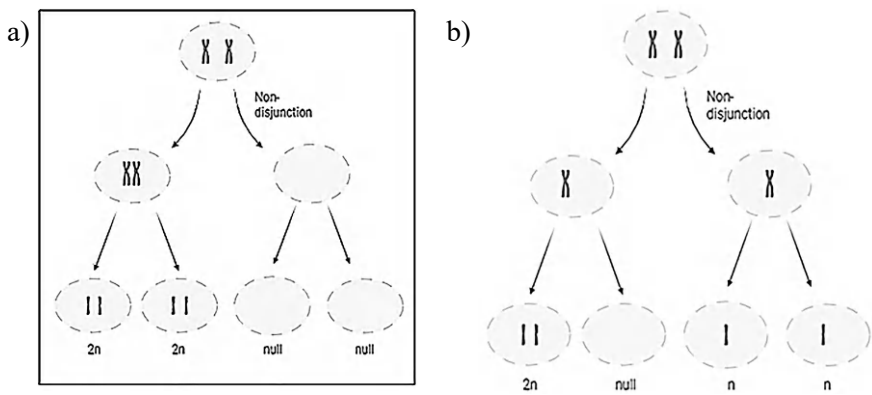


FIGURE 10.6 (a) Non-disjunction at Meiosis I. (b) Non-disjunction at Meiosis II.

10.7 TRISOMY IN HUMANS

10.7.1 TRISOMY 13

Trisomy 13 is also known as the Patau's syndrom and was first recognized in 1960 (Cammarata-Scalisi et al., 2019). It is a severe condition with a high mortality rate in newborns. It covers a vast horizon of abnormalities including facial anomalies, brain retardation and malformation, high prevelence of cardiovascular disorders, and genital abnormalities (Kara et al., 2013). This aneuploidy is relatively rare with the chance of 1 in 10,000 to 20,000 live birth (Satgé et al., 2017). Trisomy 13 can occur as mosaicism, partial, and complete expression (Cammarata-Scalisi et al., 2019). The most prevelant type is the complete trisomy in which three copies of chromosome 13 are present and constitute about three-fourths of the patients (Miryounesi et al., 2016). In the partial expression, Robertsonian translocation might be present, i.e., t(13;14). During mosaicisim, a certain proportion of cells retain trisomy, while the other cells show normal euploidy (Cammarata-Scalisi et al., 2019).

Nondisjunction of germ cells during meiosis I and II of either parent gives rise to trisomy 13 (Hall et al., 2007). Nondisjunction during meiosis I in the maternal chromosomes accounts for 91% of Patau's syndrome cases. This nondisjunction is due to aberrant chromosomal recombination which has a high chance of occurrence in the women of age above 35



FIGURE 10.7 Newborn with Patau's syndrome.

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(Hall et al., 2007). Trisomy occurs due to nondisjunction of homologous chromosomes during gamete formation. In this situation three copies of autosome 13 are present in the germ line and somatic cells (Macias and Riley, 2016).

Sondgrass in 1966 introduced histological anatomy of trisomy13. The two categories introduced were based upon the basis of absence and presence of holoprosencephaly. Another common feature associated with trisomy 13 is postaxial hexadactyly (Moerman et al., 1988). Trisomy 13 often results in internal organs midline malformation, cardiac defects in septa region, and uterus didelphys. When aborted trisomic 13 fetuses were examined, metanephric differentiation of embryonic structures was found consistent. The normal development of craniofacial and forebrain structure takes place during the third week of embryogenesis. So many of the malformations in the embryo can be traced back to the migration of mesoderm cells and presence of olfactory aplasia (Moerman et al., 1988).

10.7.2 TRISOMY 18

Autsome 18 trisomy is called Edward's syndrome and it was first recognized in 1960. It is the second most frequent autosomal disorder to occur with the occurrence of 1 in 3600 to 10,000 individuals (Crider et al., 2008). Edward syndrom can also be of three types: complete, partial, and mosaic expression. In the complete trisomy 18, all the cells contain an extra copy of chromosome 18q arm. The presence of extra chromosome is due to chromosomal nondisjunction in meiosis II. It is responsible for about 94% and usually maternal originated. As the age of mother increases, so do the chances of nondisjunction. During the partial trisomy that is reason for only 2% cases, only a portion of 18q is present in triplicate which may result from inversion or balanced translocation in either of the parents. The phenotype of this trisomy is variable depending upon the extent of the segment and location. Mosaic expression accounts for less than 5% of total cases of Edward's syndrome. During mosaicisim some cells have complete trisomy 18, while the other cells have normal chromosome number (Baty et al., 1994). The diagnosis of Edward's syndrome cases can be done prenatally through antenatal screening, ultrasounds, and maternal serum marker (Kroes et al., 2014). Various abnormal features have been associated with Edward's syndrome including nerological disorder (seizure, apnea, neonatal hypotonia, mental retardation), craniofacial abnormalities (microcephaly, microphthalmia, iris coloboma, short palpebral fissure, distinct nasal

bridge, choanal atresia, cleft lip and palate, microtia, microstomia) (Rosa et al., 2013), skeletal anoomalies (short neck and sternum, growth retardation, scoliosis, dislocated hip, pectus excavatum, arthrogryposis) (Rosa et al., 2012), cardiac and pulmonary defects, gastrointestinal problems (omphalocele, ileal atresia, diastasis recti, malrotation), and gastrourinary (micropenis, horshoe kidney, hydronephrosis, clitoral hypertrophy) (Balasundaram and Avulakunta, 2023).

10.7.3 TRISOMY 21

Trisomy of autosome 21 is called Down's syndrome. It was described in 1866 by John Langdon Down and then associated with chromosome 21 after a century by Dr. Jerome Lejeune (Holmes, 2014). It is the most common aneuploidy marked by the presence of an extra complete or just a part of chromosome 21 (Gardiner et al., 2010). This can be due to unability of chromosome 21 to detatch during gamete formation some other reasons can be formation of ring chromosome or Robertsonian translocation (Asim et al., 2015). Different phenotypes are associated with Down's syndrome. There are various hypotheses to relate these physical features to the respective genotype. For example, gene doasage imbalance results in enhanced expression of Hsa21 due to an extra copy of chromosome 21 (Antonarakis et al., 2004). Another hypothesis suggests that trisomic genes create genetic imbalance and increase the expression and regualtion of a number of genes (amplified development instability hypothesis) (Antonarakis et al., 2004; Pritchard and Kola, 1999). There are specific areas of chromosome 21 that are linked to the partial trisomy of Has21 known as Down Syndrome Specific Chromosomal Areas. One of the positions specified is 21q21.22. But this cannot be contributed to a single point. Rather multiple criticle gene regions collaborately put up this phenomenon (Holland et al., 2000). The frequency of Down's syndrome varies in different populations. But its occurrence increases with the advancement in maternal age. The incidence of other chromosomal trisomies is higher as compared to chromosome 21. The reason we do not know about them is that they do not survive pregnancy (Roper and Reeves, 2006). The gene density on chromosome 21 is relatively low. Pateints with Down's syndrome have a large variety of symptoms associated with them including cardiac defects which are also a major cause of morality within the first two years of birth (Benhaourech et al., 2016). Commonly observed anomalies are congenital heart defects (atrioventricular septal defect in 40% cases, ventricular septal defect in 32% cases, secundum atrial defect in 10%

cases, Fallot tetralogy in 6%, and PDA in 4% of the cases) (Asim et al., 2015; Wiseman et al., 2009), GI track anomalies (Hirschsprung disease, intestinal obstruction, duodenal atresia gastroesophageal reflux, intermittent diarrhea, chronic constipation) (Amiel et al., 2008; Wallace, 2007; Asim et al., 2015; Holmes, 2014), hematologic diseases (neutrophilia, thrombocytopenia, polycythemia, myeloproliferative disorder, high risk of leukemia) (Akhtar and Bokhari, 2018; Hasle et al., 2000; Henry et al., 2007; Hord et al., 1995), neurological disorders (hypotonia-hallmark of Down's syndrome, joint laxity, Lennox-Gestaut syndrome, myoclonic seizures, early onset of Alzheimer's, dementia in patients older than 45) (Ferlazzo et al., 2009; Hunter et al., 2004; Lott, 2012; Menéndez, 2005; Pueschel et al., 1991), endocrinological disorders (hypothyroidism, crytochidism) (Haddow et al., 1999; Hawli et al., 2009), disorders of skeletal muscles, visual abnormaalities (Akhtar and Bokhari, 2018), and otorhinolaryngological disorders (Shott, 2006). Early prognosis can lead to better health care and longer lifespan of children with Down's syndrome.

10.8 STRUCTURAL ABNORMALITIES OF CHROMOSOMES

Structural abnormalities of chroosome basically refer to the changes in structure and gene rearrahngements in the chromosome. These can be categorized into four categories: (i) Deletion, (ii) Duplication, (iii) Inversion, (iv) Translocation. There can be various reasons as to how these structural aberrations take place in chromosomes. They ususally occur when a part of the chromosome breaks away and then rejoins incorrectly. During meiotic prophase I of the cell division, the regions of chromosomes that are homologous to each other demonstrate a high affinity to pair and usually go through different morphological contortions in order to do so. It results in a variety of perplexing structures especially in the cells that contain both a normal chromosome pair and an aberrant chromosome. Another main reason is exposure to chemical or physical agents that lead to chemical lesions. These structural abnormalities are responsible for a vast array of diseases in human beings.

The karyotype is considered normal if the total genetic content of the chromosome remain the same and some parts of the chromatin material have just been traversed. In case of balanced rearrangemnt of chromosome, the person stays perfectly healthy. They usually have an adverse effect in case the breakage point of chromosome lies amidst a gene. This can hinder the gene function. Such people have difficulty in concieving and even if they are able to reproduce, the child can have a genetic disorder. But this is highly

dependent upon the part of chromosome involved and the genes residing on those parts. However, if some information goes missing or gets duplicated, the karyotype becomes unbalanced. The lost information is called deletion and the duplicated is called insertion. The effect inflicted by unbalanced chromosomal aberration is also hinged upon the chromosomal portion involved. This can happen in the offsprings of the parents who have either normal chromosomes or balanced rearrangements.

10.8.1 DELETION (PART OF CHROMOSOME MISSING)

Deletion or defficiency of chromosomal content simply takes place due to breaks in chromosome. These breaks can cause the loss of chromosome from the terminal region (terminal deletion) or middle region (intercalary region) of the chromosome. Terminal deletion requires one break, while the intercalary deletion requires two breaks. Deletion is an irreversible process (a gene lost is lost forever). These deletions are heritable from one generation to the next one and can vary diversely in size. Small deletions are usually less fatal than the large deletions. The large deletions are easy to recognize due to noticable deduction in chromosome size. If this deletion is hetrozygous, in order for the normal chromosome to pair with it during synapsis step of prophase I, it has to loop out in order to align with the chomosome that has undergone deletion. Heterzygous deletions can have

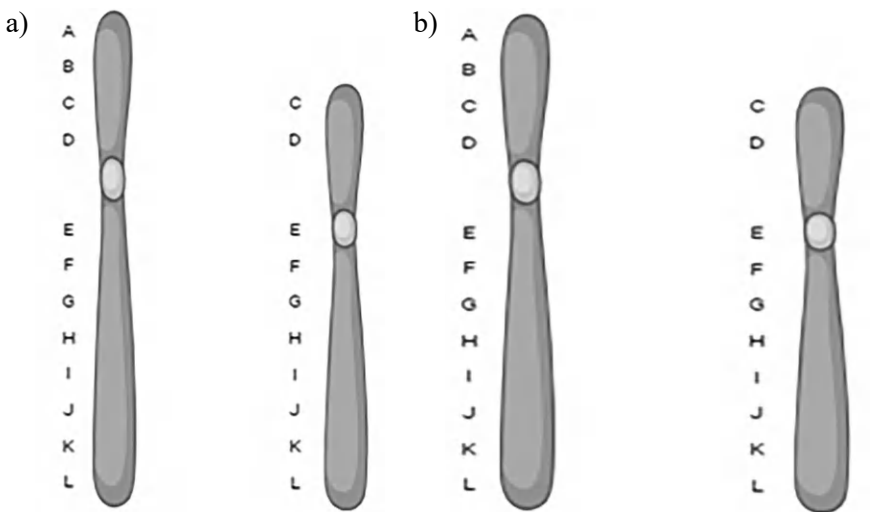


FIGURE 10.8 (a) Terminal deletion, (b) Intercalary deletion.

various effects. Due to the presence of a single copy of certain genes, the gene imbalance can result. If the deleted portion comprised of the dominanat wild-type allele, it can result in the expression of the recessive mutation allele (pseudodominance). In case of haploinsufficiency where genes need to exist in two copies for normal functioning, the loss of function mutaions is dominant.

When the centromeric region is also involved in the deleted portion, the chromsome will be eventually lost, as it would not be able to seggregate during meiosis and mitosis. In case of homozygous deletion, all the essential genes get deleted (Pierce, 2012). If the number of base pairs deleted is nondivisible by three, the result can be frameshift mutation, and translation of such genes produces faulty product protein.

The chromosomal region 7q11.23 is associated with Willian-Beuren syndrome (WBS) critical region or WBSCR. A microdeletion of 1500–1800kb within this region results in William-Beuren syndrome, which is an autosomal-dominant disorder (Pober, 2010). WBS is a rare multifunctional disorder with complete pentrence and high occurrence of 1 in 7500 to 10,000 live births (Strømme et al., 2002). This disorder is characterized by the presence of peculiar congenital anomalies, with recognizable craniofacial features, and distinct behavioral and cognitive traits along with other neurological and cardiovascular diseases (Beuren et al., 1962; Osborne and Mervis, 2021). WBSCR has 25 to 27 genes and their individual contribution to the certain phenotype of WBS is not yet fully comprehended (Osborne and Mervis, 2021). There is elastin gene (*ELN*), within WBSCR that is responsible for phenotype related to cardiac condition (Johnson et al., 1976). During WBS, *ELN* is lost which results in narrowing of the ascending aorta (Osborne and Mervis, 2021).

Another reported deletion is in chromosome 21q which is very rare (1 in 1,000,000). The phenotypic variation in this deletion is dependent upon the position and size of the deletion. Three breakage points have been proposed for this region. Point 1 ranges from centromere to upto 21q11.2 - 21q22.11 which harbors abot 50 genes, point 2 from 21q22.11-21q22.12 with 80 genes, and point 3 from 21q22.12-21q22.3 with more than 130 genes (Lyle et al., 2009). Typical clinical menifestation of deletion of 21q is mental retardation, delay in growth and development, microcephaly, cerebral atrophy, clinodactyly, scoliosis, epicanthal folds, and cardiovascular defects (Lyle et al., 2009; Roberson et al., 2011; Su et al., 2018).

Interstatial deletion in long arm of chromosome 14 is a rare condition that result in a number of congenital abnormalities like heart defects, congenital

brain retardation, and dysmorphic phenotype (Alina et al., 2016). Deletion in short arm of the autosome 18 results in developmental delay, short heights, and disorders related to movement like dystonia (Kumar et al., 2017).

10.8.2 DUPLICATION

In this type of aberration, the chromosoal content gets duplicated at specific points. If this duplicated region borders the original portion, it is known as tendem duplication. The duplicated region if it displaces and lies somewhere else on the same or different chromosomes is known as displaced duplication. Furthermore, this duplication can be inserted in the same orientation as the original one, or it can get inverted, in which case it is called reverse duplication. The homologous rearrangements are present on homologous chromosomes but in the case of heterologous rearrangements, where mutation is present only on one chromosome, the problems arise at meiotic prophase I as the chromosomes that are to align do not have same length. So for the snapsis, chromosome loops out. No genetic information goes missing. The only difference is the copy number variation of the gene which results in imbalanced gene dosage. Sometimes gene expression is dependent upon the gene copy number. Developmental processes often require different protein interactions. Protein concentration variations can vary the normal cell function (Pierce, 2012).

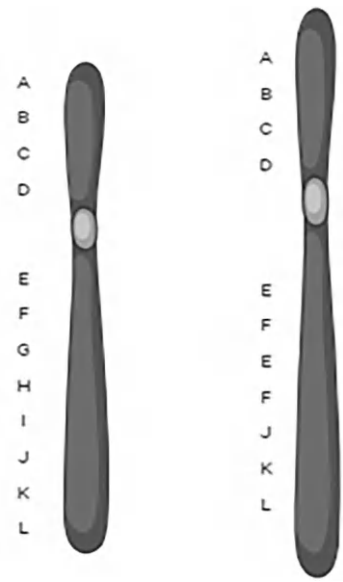


FIGURE 10.9 Chromosomal duplication.

Gene duplication can play a significant role in evolution. The duplicated gene provides evolutionary material for the novel gene function as it has higher and less-regulated possibilities of natural selection to introduce a new gene function (Long, Betrán, Thornton, and Wang, 2003).

There are different mechanisms through which gene duplication can arise. Most commonly occuring are unequal crossing over, polyploidization, duplicated DNA transposition, and retrotranspositon.

Unequal crossing over can result in the formation of the tandem repeats. The duplicated region might comprise of a single or multiple genes. The consequences of crossing over with duplications can vary if the duplication is present only on one chromosome (Zhang, 2003).

- If the duplicated gene pairs with its homologue on the other chromosome without the regard of other homologous segments
- If the segment had undergone reverse duplication, the pairing and crossing over forms a dicentric chromosome along with an acentric fragment.
- If the segment has undergone dispersed duplication, crossing over with this duplicated region results in the production of two interchange chromosomes (Gupta, 2007).

Polyploidization is another mechanism of gene duplication. The proof for this in vertebrates was provided by Ohno who pointed out the two rounds of duplication that had taken place during the course of evolution (Ohno, 2013). There is a strong indication that compact genome in *S. cervisiae* was once a tetraploid. Duplication of the whole genome followed by a large gene loss is considered an impactful adaptive development (Kellis et al., 2004).

Transposition of duplicated DNA can follow two pathways. i) Non-Allelic Homologous Recombination (NAHR) or ii) Non-homologous End Joining (NHEJ). This difference relies on whether the homologous region is utilized as a template during the repair of the double-strand break. This method can also be used to deduce the mechanism through which separate genes undergo duplication. These nonoallelic homologues when recombined can form duplicated intervening regions which can result in further duplications. As for the NHEJ, as observed in many cases in human beings, long homologue sequences and repetitive DNA have not been found at the breakpoints where duplication has occurred. Tandem duplication is relatively lower in human beings, transposition of duplicated DNA is considered a major source for duplication.

10.9 RETROTRANSPOSITION

Reverse transcription of the mRNA to construct the complementary DNA and its insertion in the genome is known as retrotransposition which can take place through various mechanisms. The potential reasons include missing gene introns and regulatory sequences, sequence with multiple adenines, and flanking short repeats. It is distinct from the unequal crossover. In retrotransposition, if the introns are present in the original gene, the duplicated gene will retain the duplications but it would be absent in the retrogenes (Brosius, 1991). The cDNA resulting from the retrotransposition undergoes insertion at random places as the duplicated genes have no link to the original genes (Long et al., 2003). The retrogenes that get integrated in close viscinity of the coding genes are potent for expression (Vinckenbosch et al., 2006).

Reciprocal duplication in the chromosome 16p13.11 can result in various anomalies including autism, seizures, mental retardation, various congenital disabilities, and abnormal physical features (Ramalingam et al., 2011). Dup15q that takes place in chromosome 15q11.2-q13.1 if contains even one extra copy of meternal derived Prader-Willi/Angelman critical region results in autism spectrum disorder, hypotonia, motor function delays, and attention deficit/hyperactivity disorder (Devinsky et al., 1993).

10.9.1 INVERSION

Chromosomal inversion takes place as a rresult of breakage of a chromosomal segment and then its reinsertion at the same location but in the reverse position (Morin et al., 2017). Inversions can be of the two types depending upon the region of the chromosomes involved. i) Pericentric inversions and ii) peracentric inversions. Pericentric inversions invlove both arms of the chromosomes along with centromeric region that result in the formation of the dicentric chromosomes (chromosomed with two centromeric regions). Paracentric chromosomal inversions take place in the single arm and do not involve the centromere (Nussbaum et al., 2015). The crossing over in such conditions results in duplications, deletionsa, and formation of acrocentri chromosomes.

Inversions are balanced anomalies as no genetic information is lost or gained. However, the order of genes is changed. The inversion can result in breakdown of two genes, consequently effecting the gene product. The genes that are regulated in a position-dependent manner, even if intergenic break does not occur but break between gene occurs, can also affect the overall expression. If inversion changes the gene position, they may get expressed at random time and occasions also known as **position effect**.

The homozygously inverted chromosome can undergo meiosis in a normal way—normal pairing and separation. The herteozygously inverted chromosome during meiosis has to form an inversion loop (indication of inversion) in order to pair and align. Genes allocated in the inverted region undergo less recombinations. These portions are capable of recombination, but the resulting gametes are not feasible (Pierce, 2012). Chromosomal inversions can play a significant role in speciation and adaptation and can be retained as polymorphed sequences over a vast period of time (Nosil et al., 2023).

10.9.2 TRANSLOCATION

Translocation in the chromosome results in the structural varients of the genome that can take place when two breaks coexist on the different chromosomes and end up in the joining of the incorrect ends—in other words two chromosomes undergo breaks in the same space and time. The broken pieces of the chromosome join in a *trans* manner rather than *cis* manner. Translocations are mostly of two types: i) nonreciprocal translocation, ii) reciprocal translocation, and iii) Robertsonian translocation. In nonreciprocal translocation, the part of a chromosome breaks away and gets attached to one of the two ends of a non-homologous chromosome.

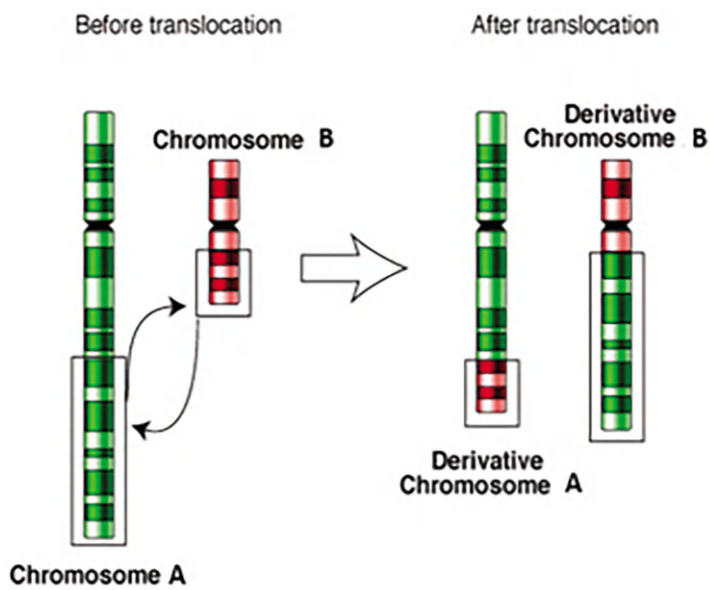


FIGURE 10.10 Reciprocal translocation.

In reciprocal translocation the exchange of chromosomal segments takes place between the non-homologouse chromosomes. No genetic material is lost so these translocations are rendered harmless. However, individuals who carry these translocations may form a gamete with unbalanced translocations during meiotic saggregation which can result in miscarriages. They are also prone to being infertile and have recurrent pregnancy lost or RPL. Men who have reciprocal translocation on X chromosome are more suscptible to spertmatogenic arrest as the X-chromosome does not undergo complete inactivation (Braekeleer and Dao, 1990; De Braekeleer and Dao, 1991). Prenatal diagnosis for reciprocal translocations is possible (Milunsky and Milunsky, 2015). In the general population there is high occurrence of particular rearrangements between the regions that are closely similar to eachother. Examples of some major hotspots are at 11q23, 17q11, and 22q11 that results in the translocation as t(11;22) and t(17;22) (Kurahashi et al., 2010). This site of chromosome 22 is also assocated with velocardiofacial syndrome.

Robertosonian translocation is the centromeric fusion of the acrocentric chromosomes that take place in chromosome 13, 14, 15, 21, and 22 (Gardner et al., 2012; Nussbaum et al., 2015). This type of translocation occurs 0.1% in the typical population, 1.1% in the people who show recurrent pregenany losses, and 3% of all the cases of infertile men (Fryns and Van Buggenhout, 1998; Therman et al., 1989). Robertsonian translocations are more observable in non-homologous chromosomes. The shorter arms of the chromosome (p-arm) of these chromosomes fuse together, but they are eventualy lost. The gene density on the p arm is usually low, so there is no major effect. Meiosis takes place in the long arms of the chromosome and results in the formation of trivalents (Scriven et al., 2001). These trivalents are a potential source of nullisomic and disomic gametes that in case of fertilization produce zygotes with trisomy and monosomy. Robertsonian translocations have mostly been reported for chromosomes 13 and 14 (der(13;14)(q10;q10). Almost 75% cases account for such mutations (Therman et al., 1989). Feasible gestation can be produced between individuals with Ptau's syndrome and this translocation, but the conceptions can result in early pregnancy loss. However, the chance of viable gestation is significantly higher for chromocsome 21 robertsonian translocation. But this risk is again higher if the origin of this translocation is maternal (Boue and Gallano, 1984).

10.9.3 RING CHROMOSOMES

In eukaryotic genome, rarely there is a presence of round DNA molecules known as ring chromosomes. They are typically formed by breaking of the

linear chromosomes, and then fusion of the broken ends. This can take place naturally in a sponatenous manner and can also be induced via radiations. The presence of ring chromosome can result in the presence of a number of symptoms and usually depend upon the type of chromosome involved. Common menifestation is in the form of mental retardation and physical disability. It has incidence of occurrence in 1/50,000 newborn individuals. These ring chromosomes tend to be unstable during meitic phases and often lead to secondary chromosomal abnormalities including aneuploidy (Nikitina et al., 2021; Pristyazhnyuk and Menzorov, 2018).

10.10 THERAPIES FOR CHROMOSOMES

One in every hundred and a fifty newborns is a victim of chromosomal aberraations (Hsu, 1998). Currently, the treatment exists only for the symptoms of the syndrome and no therapies exist for eradicating the root cause of the syndrome. With the passage of time progress has been seen in the treatment for the disorders that involve single genes including genome editing and gene therapies. The latest focus of gene therapy is on the insertion of new gene and not so much on the correction of the abnormal one. There are certain ways to make directed small changes to the genome of human beings that might include ninc-finger nucleases (ZFNs) (Gaj et al., 2013; Palpant and Dudzinski, 2013), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) (Gaj et al., 2013; Zetsche et al., 2015), or Transcription Activator-Like Effector Nucleases (TALENs) (Gaj et al., 2013; Joung and Sander, 2013). These therapies can target monogenic syndromes. These advancements are commendable. However, we still lack in devising the technique that can target a chromosomal aberration that involves multigenic origin. A number of *in vitro* techniques have been formed that can silence, lose, or replace the abnormal chromosome (Amano et al., 2015; Bershteyn et al., 2014; Kouprina et al., 2014; Li et al., 2012). These techniques are called 'chromosomal therapies' and can be a potential way to rectify chromosomal aberrations.

As mentioned above, the goal of this therapy is to pacify the consequences of chromosomal aberrations. Now trisomy, or the presence of a large duplicated region, usually results in the higher expression of the certain product that leads to gene overdose. And the genes on the chromosomes inside a nucleus are present in a well-arranged order. So in oder for a cell to fuction properly, there come forth two requirements: correct amount of gene product

and correcct gene position. The presence of extra genetic material can be the leading cause of alteration of structural organization of chromosome and ultimately the normal genetic functon (Cremer et al., 2006; Hancock, 2014). So, the therapy required for chromosomal aberrations must be able to remove or silence the unrequired chromosomal material to eliminate its counterproductive impact. For the large deletions, the chromosomal material has to be replaced. Gene replacement can be done through gene therapy (Naldini, 2015) and human artificial chromosomes (HACs) can be a potential means to introduce the gene that got lost during interstitial deletion.

In 2013, a study was conducted that utilized genome-editing techniques to integrate an inducible form of XIST gene in the single copy of autosome 21 using zinc-finger nucleases into the induced pluripotent stem cells from the patient of Down's syndrome (Jiang et al., 2013). XIST gene plays a role in gene silencing of X-chromosome in mammalian females (Brown et al., 1991). The insertion of XIST gene in the autosome 21 using doxycyclin can silence most of the genes. The result was correction of cell phenotype *in vitro* including increase in proliferation rate and neural differentiation. The limitations included continuous treatment of doxycyclin to keep the chromosome in the silenced state. Also if the genes escape the XIST-mediated silencing, there can be a potential of occurrence of undeside impacts (Jiang et al., 2013).

Another technique applied is the use of insertion of TKNEO transgene into the extra copy of the chromosome 21 instead of silencing the chromosome. It was then proceeded through rounds of antibiotic selectivity. The gene *Neo* provides resistance against a drug that is a toxix analogous for the mammalian cells—G418 (positive control). Another gene *HSV-TK* gene induces sensitivity to gancyclovir and is used as negative control—cells selected if they lose this gene. Once the TKNEO transgene was introduced, the cells were selected that contained it. The cell population was propagated in such a way that it underwent spontaneous nondisjunction leading to the loss of chromosome 21. Gancyclovir then selected against the cells that had transgene with the extra chromosome. In this way eupolid cells were isolated (Letourneau et al., 2014; Olmos-Serrano et al., 2016).

Alternative therapeutic stratagies for trisomies like Down's and Edward's include treating fibroblast cells of the patients with the ZSCAN4—a mammalian gene that maintains the telomere and stability of genome. This treatment enahanced a number of cells containing normal chromosome number by 24%. This ZSCAN4 can be induced either via nontransmissible Sendai viral vector or synthetic mRNA. The euploidy is promoted without

any unnecessary complications including gene disruption or off-target effects (Amano et al., 2015).

Chromosomal therapies can expand the horizon of abnormalities that can be cured. With more experimentation and high-put technology, this might just become possible one day. However, there are a whole lot of ethical issues contained as it does not allow any alteration of the germline cells.

KEYWORDS

- **aberrations**
- **trisomy**
- **polyploidy**
- **Down'syndrome**
- **chromosome**
- **crossing over**
- **retroposition**

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Index

A

Abiotic stress, 184–186
Achiasmy, 53–54
Agricultural science, 175
  challenges, 196
    biodiversity, 196–197
    crops as food, 197–198
    farmers, 198–199
  CRISPR
    maize, 191
    nitrogen fixation, 191–192
    rice, 190–191
    soybean, 187–190
  demands, 177
    abiotic stress, 184–186
    biofortification, 177–178
    biotic stress management, 178–184
  gene editing
    camelina, 193
    citrus, 194–195
    cotton, 194
    potato, 193–194
    tomato, 195–196
    wheat, 193
  methodologies, 186–187
  in plants, 128
  successes
    CRISPR, 187
    gene editing, 192
    TALEN, 192
Agriculture
  crops, 92–93
  livestock species, 93
Allosomal linkage, 30
Aneuploidy, 283–284
  monosomy, 284
  nullisomy, 284
  trisomy, 284–285
Aquaculture species
  bio-informatics, 87–88
Artificial selection, 105–106, 144–145
Autosomal linkage, 29

B

Bacteria, 254
  composite, 256–257
  insertion sequences (is element), 254–255
  non-composite, 257
Bacteria artificial chromosome (BAC), 85
Breeding
  genetic variability, 165
  impact, 168
  isolations, 166–168

C

Chromosomal aberrations, 277
  aneuploidy, 283–284
    monosomy, 284
    nullisomy, 284
    trisomy, 284–285
  effect, 283
  monoploidy, 279–280
  numerical, 278–279
  polyploidy, 280–282
  primary, 285–287
  retrotransposition, 296
    inversion, 296–297
    ring chromosomes, 298–299
    translocation, 297–298
  structural, 291–292
    deletion, 292–294
    duplication, 294–295
  therapies, 299–301
  trisomy in humans
    trisomy 13, 288–289
    trisomy 18, 289–290
    trisomy 21, 290–291
Chromosome-based linkage
  allosomal linkage, 30
  autosomal linkage, 29
Coupling phase (CIS phase), 30
CRISPR
  maize, 191
  nitrogen fixation, 191–192

rice, 190–191
soybean, 187–190
Crossing over, 32
centromere effect, 42
complex crossing over, 34–35
crossover assurance, 43–44
crossover interference, 42–43
distribution, 41–42, 44–45
E0 tetrads, 44
mixed crossing over, 34
paracentric synapsis, 35
preterminal synapsis, 35
random synapsis, 35–36
simple crossing over, 34
theories of, 33–34
Crossover-dependent linkage
complete linkage, 26
incomplete linkage, 29–30
Thomas Hunt Morgan, 27–28

D

DNA recombination
evolutionary explanation, 55
meiotic recombination, 52–53, 129
neutral hypothesis, 55
sex dimorphism in
achiasmy, 53–54
heterochiasmy, 54–55
sexual selection, 55
Drosophila, 267
copia elements, 268
(P) element, 267–268
foldback elements (FB), 268
gypsy element, 268–269
hobo elements, 269

E

Eukaryotes
genetic code, 219–220
initiation, 218
polymerases, 217–218
termination, 218–219
Eukaryotic gene
exons, 77–78
introns, 78
Evolution, 134
classical antiquity, 135
Darwinian revolution, 136–137
factors affecting
artificial selection, 144–145
genetic drift, 148–149
migration, 145–146
mutation, 147–148
natural selection, 143–144
middle ages, 135–136
modern synthesis, 137
pangenesis and heredity, 137
pre-Darwinian, 136

F

Foldback elements (FB), 268

G

Gene
biochemical standpoint
multiple alleles, 68
eukaryotic gene
exons, 77–78
introns, 78
genetic perspective, 67
jumping genes, 73
LAC operon
regulation of, 76–77
Mendel's laws, 66
nucleotides
classification of gene, 71–72
eukaryotic gene, fine structure, 70
regulation of
inducer, 76
LAC operon, 74
operator region, 75
promoter gene, 75
regulator gene (1), 75–76
repressor, 76
structural genes, 74–75
section
cistron, 70
muton, 69–70
recon, 69
split genes, 72–73
splitable, 68
Gene configuration-based linkage
coupling phase (CIS phase), 30
repulsion phase, 30

Gene conversion, 50, 127
genetic engineering in plant, 51
safety considerations, 51–52
Gene editing
camelina, 193
citrus, 194–195
cotton, 194
potato, 193–194
tomato, 195–196
wheat, 193
Genetic diversity, 88
adaptability, 91
in agriculture
crops, 92–93
livestock species, 93
crop improvement
plant breeders, 91–92
evolution, 93
classical antiquity, 94
Darwinian revolution, 95
middle ages, 94
modern synthesis, 96
pangenesis and inheritance, 95–96
pre-Darwinian, 94–95
genomic variability, 90
importance of, 89–90
mutations, 90–91
neutral and modification, 89
plant genetic materials (PGRs), 92
Genetic engineering, 175
challenges, 196
biodiversity, 196–197
crops as food, 197–198
farmers, 198–199
CRISPR
maize, 191
nitrogen fixation, 191–192
rice, 190–191
soybean, 187–190
demands, 177
abiotic stress, 184–186
biofortification, 177–178
biotic stress management, 178–184
gene editing
camelina, 193
citrus, 194–195
cotton, 194
potato, 193–194
tomato, 195–196
wheat, 193
methodologies, 186–187
in plants, 128
successes
CRISPR, 187
gene editing, 192
TALEN, 192
Genetic engineering in plants, 128
Genome sequencing
bacteria artificial chromosome (BAC), 85
diversity of, 86
ferns, 86
land plants genome structure, 85
maize, 87
next-generation sequencing (NGS), 85
Genomic diversity
adaptation, 106
aquaculture species
bio-informatics, 87–88
chloroplast and evolution, 84
chromosome
defined, 82
factors affecting, 104
gene flow, 109
gene pool, 109–110
genetic drift, 110–111
genetics, 82
genome sequencing
bacteria artificial chromosome (BAC), 85
diversity of, 86
ferns, 86
land plants genome structure, 85
maize, 87
next-generation sequencing (NGS), 85
green plants, 83–84
leguminosae, 88
mammals
coalescent and combined phyla, 83
migration, 108–109
mutations, 107–108
selection
artificial selection, 105–106
natural selection, 105
tobacco and alcohol, 88
Genomic recombination
in eukaryotes, 125

interchromosomal recombination, 125
intrachromosomal recombination, 125

I

Independent assortment *vs* linkage
allele pairs, 14
complete linkage, 13
physical basis for, 14–15
Interchromosomal recombination, 125
Intrachromosomal recombination, 125

L

Land plants genome structure, 85
Law of dominance
cross-fertilization, 7
dominant trait, 6
recessive trait, 6
Law of independent assortment, 11
dihybrid cross, 12
Punnett square, 12
Law of segregation
monohybrid cross, 9
Punnett square, 10
Linkage
analysis, 30
limitations, 32
parametric linkage analysis, 31–32
chromatid segments, 36
chromosome-based linkage
allosomal linkage, 30
autosomal linkage, 29
crossing over, 32
centromere effect, 42
complex crossing over, 34–35
crossover assurance, 43–44
crossover interference, 42–43
distribution, 41–42, 44–45
E0 tetrads, 44
mixed crossing over, 34
paracentric synapsis, 35
preterminal synapsis, 35
random synapsis, 35–36
simple crossing over, 34
theories of, 33–34
crossover-dependent linkage
complete linkage, 26
incomplete linkage, 29–30
Thomas Hunt Morgan, 27–28
defined, 20
gene configuration-based linkage
coupling phase (CIS phase), 30
repulsion phase, 30
genetic recombination
eukaryotes, 48–49
history of, 21
linkage map, 25–26
linkage value, 25
linked genes, 21
X-linked gene, 22
Y-linked gene, 22
meiotic crossing, 37–38
crossover and non-crossover routes, 38–40
D-loop resolution, 40–41
recombination, 45
homologous recombination, 46
illegitimate or non-homologous recombination, 46
replication recombination, 47
site-specific recombination, 46–47
Reginald Crundall Punnett theory, 21
RNA recombination
type I (homologous) recombination, 47
type II (aberrant homologous) recombination, 47–48
type III (non-homologous or illegitimate) recombination, 48
terminalization, 36–37
tetrad formation, 36
theories of crossing over, 33
chiasma type theory, 34
classical theory, 34
unlinked genes, 23
Linked genes, 21
X-linked gene, 22
Y-linked gene, 22
Long-interspersed nuclear elements (LINES), 270

M

Maize
AC-DS elements, 264–265
mutator transposable elements, 266–267
suppressor mutator system (SPM), 265–266
Meiotic crossing, 37–38

crossover and non-crossover routes, 38–40
D-loop resolution, 40–41
Mendel's experiments
blending inheritance, 3
dominant trait , 2
factors
alleles, 6
genotype, 6
heterozygous organisms, 6
homozygous, 6
phenotype, 6
independent assortment *vs* linkage
allele pairs, 14
complete linkage, 13
physical basis for, 14–15
law of dominance
cross-fertilization, 7
dominant trait, 6
recessive trait, 6
law of independent assortment, 11
dihybrid cross, 12
Punnett square, 12
law of segregation
monohybrid cross, 9
Punnett square, 10
pea *Pisum sativum*
characteristics, 5
true-breeding, 4
phenotypes and genotypes, 7
comparison, 8
plant hybridization, 3
recessive trait, 2
test cross
half heterozygotes and half recessive, 10
Mutations and mutagens, 157
breeding
genetic variability, 165
impact, 168
isolations, 166–168
induced
chromosome, 162
gene, 162–163
genome, 161–162
induction, 163
mutagens, 163–164
plant materials, 163
recompences, 164–165
types
induced, 160–161
spontaneous, 160

N

Neutral hypothesis, 131–132
Next-generation sequencing (NGS), 85

P

Pea *Pisum sativum*
characteristics, 5
true-breeding, 4
Plant genetic engineering, 128
Plant genetic materials (PGRs), 92
Prokaryotes
elongation, 216–217
initiation, 215–216
polymerase, 215
termination, 217
Prokaryotic transposons (TN), 255–256
Protein synthesis, 209
components, 210
DNA, 210–211
ribosomes, 212–213
three types, 211
defects, 239–240
eukaryotes
genetic code, 219–220
initiation, 218
polymerases, 217–218
termination, 218–219
fundamental, 230
create, 231–232
inhibitors, 229–230
IRESs, 240
micro-RNAS in cancer, 236–238
MIRS, 240
mutations, 238–239
neurological disease, 238
prokaryotes
elongation, 216–217
initiation, 215–216
polymerase, 215
termination, 217
regulation, 235–236
role, 232–233
dysregulated, 233–234

IRES-mediated, 234–235
sickle cell disease, 233
steps involved, 213
chain termination, 228
chemical groups, 224–225
cleavage, 224
complex molecules, 225–226
covalent bonds, 226
eukaryotes, 217
folding, 226–227
polypeptide chain elongation, 227
posttranslational modifications, 223–224
prokaryotes, 215
transcription, 213–214
translation, 220–221
translocation, 227–228
three types
mRNA, 211
rRNA, 212
transfer RNA (tRNA), 211–212
trafficking, 229
transcription
posttranscriptional modifications, 214–215
translation
activation, 221
elongation, 222
initiation, 221–222
termination, 222–223
Punnett square, 12

R

Recombination
evolutionary explanation, 132
factors affecting
artificial selection, 144–145
genetic drift, 148–149
migration, 145–146
mutation, 147–148
natural selection, 143–144
homologous recombination, 122
importance of
bacterial recombination, 50, 126–127
chromosomal crossover, 50
chromosome crossover, 127
mechanism, 49, 126
non-homologous recombination, 123
replication recombination, 123
RNA recombination
Type I (homologous) recombination, 124
Type II (aberrant homologous) recombination, 124
Type III (non-homologous or illegitimate) recombination, 124–125
sex dimorphism
achiasmy, 130
heterochiasmy, 130–131
sexual selection, 132
site-specific recombination, 123
in viruses
DNA and RNA viruses, 56–58, 133–134
mechanism of, 56, 132–133
Reginald Crundall Punnett theory, 21
Retrotransposition, 296
inversion, 296–297
ring chromosomes, 298–299
translocation, 297–298
RNA recombination
type I (homologous) recombination, 47
type II (aberrant homologous) recombination, 47–48
type III (non-homologous or illegitimate) recombination, 48

S

Sex dimorphism
achiasmy, 53–54, 130
heterochiasmy, 54–55, 130–131
Short-interspersed nuclear elements (SINES), 270–271
Speciation
ecological divergence in, 142
ecological speciation, 101, 141–142
plant speciation, 101–102, 142
plants
ecological divergence in, 102
types
allopatric speciation, 103, 142–143
sympatric speciation, 102–103, 142–143
Species, 137
evidence
analogous features, 139
anatomy and embryology, 138–139
biogeography, 140–141
fossil record, 141
homologous genes, 140
molecular biology, 140

evolution
analogous features, 98–99
anatomy and embryology, 97–98
biogeography, 100
fossil record, 100–101
homologous features, 98
homologous genes, 99–100
molecular biology, 99
origin, 96–97, 138
Suppressor mutator system (SPM), 265–266

T

Thomas Hunt Morgan, 27–28
Transposable elements, 247
bacteria, 254
composite, 256–257
insertion sequences (is element), 254–255
non-composite, 257
characteristics, 249–250
autonomous, 250
copy-and-paste, 251
cut-and-paste, 250
non-autonomous, 250
class 1retrotransposons
LINEs, 262–263
LTR retrotransposons, 259–260
non-LTR retrotransposons (retroposons), 262
retroviruses, 259
SINEs, 263
Ty elements in yeast, 260–261
discovery, 248–249
Drosophila, 267
copia elements, 268
(P) element, 267–268
foldback elements (FB), 268
gypsy element, 268–269
hobo elements, 269
effect of transposons, 271
eukaryotes, 258
class 2: DNA transposons, 263–264
class 1: retrotransposons, 258
human, 269–270
long-interspersed nuclear elements (LINES), 270
short-interspersed nuclear elements (SINES), 270–271
insertion sequences (is element)
prokaryotic transposons (TN), 255–256
transposition, 255
maize
AC-DS elements, 264–265
mutator transposable elements, 266–267
suppressor mutator system (SPM), 265–266
mechanism, 252
conservative, 253
replicative, 253
retrotransposons, 253–254
types, 251
class-1 (retrotransposon), 251–252
type-II (DNA transposons), 252
uses, 272
Trisomy in humans
trisomy 13, 288–289
trisomy 18, 289–290
trisomy 21, 290–291