CYTOLOGY AND GENETICS

V R DNYANSAGAR

CYTOLOGY AND GENETICS

V R DNYANSAGAR
Department of Botany
Nagpur University

Tata McGraw-Hill Publishing Company Limited
NEW DELHI

This book has been subsidised by the Government of India, through the National Book Trust, India, for the benefit of students

NBT Code : 45-113/1982

Rs 39.00

Publishing Manager: Rajiv Beri
Editorial Assistant: Deepa George
Production Supervisor: B L Dogra

Published by Tata McGraw-Hill Publishing Company Limited
12/4 Asaf Ali Road, New Delhi 110 002, and printed at
Rajkamal Electric Press, 4163 Arya Pura, Delhi 110 007

Preface

While teaching cytology and genetics in B Sc classes, I felt the need for a suitable textbook which would include both these branches of biology. I had prepared detailed notes on these subjects, which proved very useful to my students. So, when I was assigned the task of writing a book on cytology and genetics in Marathi for B Sc students, these notes became the basis of my work. At this time, I was also thinking of writing a similar book in English. It was only when Tata McGraw-Hill Publishing Co. Ltd, New Delhi, showed keen interest in publishing this book that I earnestly started on the work of writing it.

Both cytology and genetics have made tremendous progress in recent years. Some discoveries in the field of genetics have been so startling that even laymen have evinced keen interest in them. It is, therefore, obvious that B Sc students pursuing the study of cytology and genetics are expected to possess information in some detail. A special chapter on genetic engineering has been included in this book for them. It is often not possible for the majority of students to purchase books dealing with cytological techniques. It was therefore thought that including a chapter on cytological techniques would be useful. The rest of the chapters deal with the topics prescribed in the syllabi of most Indian Universities.

I have made every effort to include the latest relevant work, on the basis of available literature, so as to make this book as up to date as possible. In addition to the comprehensive bibliography given at the end, selected references (selective reading) are given at the end of each chapter. Teachers should encourage students to refer to such literature. A summary is given at the end of each chapter. It is hoped that this will prove useful to students while revising each topic.

I take this opportunity to express my grateful thanks to the National Book Trust, New Delhi, for subsidising the publication.

I am grateful to Prof. G B Deodikar, Retd Director, Maharashtra Association for Cultivation of Science (MACS), Pune; Prof. S S Bir, Head of the Botany Department, Punjabi University, Patiala; and Dr R V Pandit, Veterinary College, Nagpur, for valuable suggestions.

I am obliged to Prof. G B Deodikar and Dr V P Patil of MACS, Pune; Dr Joginder Singh, Indian Agricultural Research Institute (IARI), New Delhi; Prof. R P Roy, Patna University, Patna; Dr S H Tulpule (Botany Department) and Dr A K Indurkar (Zoology Department), Poona University, Pune; Dr A B Sapre, Marathwada University, Aurangabad; Dr Kakde, Associate Dean and Prof. S B Lal, Punjabrao Agriculture University (College of Agriculture, Nagpur) and my old students, viz. Dr A Dhanaraj, Dr C D Chowta, Dr A R Pingle and Dr R J Thengane (Poona University) for photomicrographs and photographs. I am also thankful to Shri Vilas Andhare, Director, Maharashtra Universities Book Production Board, Nagpur; Dr K M Mann, Editor, Scope Publications (Upjohn Co., Kalamazoo, Michigan, USA); Prof. R A Popham, Business Manager, *American Journal of Botany* and McGraw-Hill Book Co., for permission to reproduce certain figures.

Dr S H Tulpule, Dr V L Bakale (Institute of Science, Nagpur), Dr R J Thengane, Dr V S Kothekar (Marathwada University), Dr A D Choudhary, (Nagpur University), Shri Naveen Chandra and Shri T Srinivasu (Institute of Science, Nagpur) helped me much in the preparation of the bibliography, and Dr Choudhary in going through the typescript. I extend my grateful thanks to them.

My wife Kusum and my daughter Rucha helped me in various ways during the preparation of the book. I express my sincere thanks to them.

V R Dnyansagar

Contents

Introduction

Genetics

Genetics is defined as the science of heredity. It considers the transmission of characters from generation to generation. It was in 1906 that Bateson proposed the term 'genetics' for that branch of biology which deals with "the elucidation of the phenomena of heredity and variation." Although the history of genetics can be traced from the ancient period, its background was laid only in the seventeenth century, and modern genetics was born when Mendel's paper embodying the results of his hybridization experiments was published in 1866. Mendel is regarded as the Father of Modern Genetics as his paper laid the scientific foundation of genetics. However, his work remained unknown to the scientific world for 34 years. In 1900, his paper was rediscovered independently by De Vries, Correns and Tschermak. Genetics is, therefore, one of the younger branches of the biological sciences. However, its progress has been astonishingly rapid, especially after the Second World War.

Cytology

Cytology is the science of cells. Since the study of cell requires a microscope, the history of cytology began only after the invention of the microscope. It had its beginning in 1665, when Robert Hooke first observed cork cells under a microscope made by him. After this a series of studies followed leading to the formation of the cell theory by Schleiden in 1838. According to this theory, all organisms are made of cells. It was made applicable to animals by Schwann in 1839. The cell theory was regarded as one of the great generalisations of experimental biology. In 1858, Virchow established conclusively that a cell never arises *de novo*, but that it arises from another cell (the theory of cell lineage). In the beginning of the twentieth century, the research stream took a turn when a close link between embryology and genetics was discovered. In 1902, Sutton, who was the first to propound the chromosome theory, pointed out the parallelism of behaviour between chromosomes in meiosis and the Mendelian factors in heredity. Thus cytology was linked to genetics and the branch of cytogenetics was born.

History of Cytology

The period of the nineteenth century is considered as the classical period of cytology as it was during this period that its foundations were being laid. The modern period begins with the rediscovery of Mendel's work on genetics in 1900. Cytology therefore had a beginning with a very long descriptive period passing into the modern experimental period.

However, one must not forget that the cytologist is primarily a morphologist. As such, descriptive cytology continues to be an important part of cytological research. O. Hertwig and R. Hertwig's studies on fertilisation in 1887 marked the beginning of experimental cytology. When Wilson's *The Cell in Heredity and Development* was published in 1925, the relationship between cytology and genetics had become closer. The development of the chromosome theory by the *Drosophila* workers (1910-20) of the Morgan school, the discovery of giant chromosomes, the use of heteroploidy and polyploidy as cytogenetic tools, and the advances made in the cytogenetics of maize contributed to the creation of more avenues of approach towards a better understanding of the role of chromosomes in cell division and heredity.

The present phase of experimental cytology is actually an extension of the earlier work. During and after the Second World War, cytological researches have been chiefly on the chemical side and a branch called cytochemistry or biochemical cytology has developed. With the isolation of DNA (the hereditary substance) by Avery. Macleod and McCarty in 1944 and the model of its structure proposed by Watson and Crick in 1953, the researches have been mostly on the (i) nature of gene, (ii) its mode of replication, and (iii) its manner of action and the determination of characters.

Our knowledge of intercellular activities has now advanced in a spectacular manner. After the formulation of the concept of catalytic activity by Ostwald and the discovery of cell enzymes, physics and chemistry have been increasingly employed for researches on cell physiology, and cytology has now come to be known as *cell biology*.

As regards energy input, plants and animals resemble each other closely apart from the direct source of energy upon which they have to depend. The former are autotrophs and the latter heterotrophs. However, it should be noted that the living matter is capable of presenting its highly improbable-appearing individuality at the expense of large amounts of free energy obtained from the environment. It has been shown that the laws of thermodynamics can be applied to the living matter, meaning thereby that it is an integral part of the physical world. Studies are also concentrated on the mechanism by which enzymes and other proteins are manufactured in a living system and how information for their synthesis is carried from one generation to the other.

With finer cytological techniques, single cells as well as their parts can now be isolated. With the invention of the electron microscope, the study of cells in minute details started and it has led to tremendous progress in cell biology. It has now been possible to resolve cell structure at the molecular level, and a separate branch called *molecular biology* has come into existence. Although it may appear that the development of separate branches such as cytochemistry, cytogenetics, or molecular biology may result in narrow specialisations, they are actually tending to approach unity, since the cell is the common denominator or basic module of the living system.

Information on the nature and manner of transmission of genetic specificity (chromosomal heredity) prior to 1940 was based on the series of developments of the theory of the gene. Müller started studies on mutation in 1920, and in 1927 he discovered the mutagenic effects of radiation in *Drosophila*, and stadler in maize. This discovery was a significant step in the progress of genetics. Just prior to the Second World War, ideas from physics were introduced by workers like Frank-Kammenetski, Friedrich-Freska, Jorden and Zimmer-Delbruck in the sphere of genetics, especially in connection with the problem of size, mutability and self-replication of genes. Müller and Timofeef-Ressovsky acted as biological interpreters. Morgan put forth an ingenious idea of crossing over and suggested that the genes are situated in the chromosome in a linear fashion. This formed the basis of the classical concept of the gene according to which the gene is the ultimate unit of heredity, mutation and recombination. A gradual change has taken place in the outlook on genetics, especially the concept of gene, since 1940. Though the classical theory of gene is convenient to use in everyday genetics, it is no longer tenable as the works of Benzer, Bonner, Pontecarvo have shown that the gene is further divisible. Benzer has suggested the terms *muton, recon* and *cistron* with reference to mutation, recombination and function, respectively.

The present astonishing progress in genetics has been owing to the development of refined techniques to study microorganisms which have been found to be very useful tools in the screening of mutagenic activity of various agents and the studies aimed at quantitative aspects of such activity.

The works of Beadle and Tatum indicated that genes act through enzymes in the development of characters. For this, biochemical methods were used. Since then, a closer relationship between biochemistry and genetics has developed. It has enabled the workers to crack the genetic code. The operon concept propounded by Jacob and Monad in 1965 has helped a great deal in our understanding of gene action. The latest landmark in the progress of genetics is the development of a branch called *genetic engineering* which has fascinated even the layman.

In the following sections, a brief history of cytology and genetics is first traced after which the various topics included under these branches of science are dealt with.

1. History of Cytology and Genetics

Every branch of science has a history. When we study a certain branch, it is first necessary to have an idea about its history. It enables us to know how it developed step by step. This knowledge helps us to gain an insight into the fundamentals of the branch. If we look at the history of cytology and genetics, it is seen that these were established recently as compared to the other main branches of science. Modern genetics was born in the beginning of the twentieth century.

Since cytology and genetics are intimately linked, their history will be dealt with together in this chapter.

Invention of Microscope and Beginning of Cytology

In order to study a cell, it is necessary to have a *microscope*. The history of cytology thus began with the invention of the microscope by Robert Hooke (England). When he cut a piece of cork into fine sections and examined one such section under his microscope, he found that it had a compartmentalised structure like that of a honeycomb. Hence, he termed the compartments observed in the cork section as cells. While describing a cell, he stated that it was bounded by a wall and contained a natural fluid. He presented his observations in the form of a paper to the Royal Society of London in 1665. This paper laid the foundation of *cytology* or the science of cell. After this Grew started the study of plants with the aid of microscope. Malpighi (Italy), who studied animal cells laid the foundation of descriptive embryology (1650-70). Both these scientists observed that the structure of plants was made up of utricles or vesicles which possessed a wall of their own. Free cells were first discovered by Leeuwenhoek (1677), a Dutch scientist. In 1677, his disciple John Ham was the first to observe spermatozoa of mammals. After this, for about a century or so, there were no noteworthy additions to the knowledge of cytology.

Background of Genetics

The beginning of the seventeenth century saw a new spirit of scientific scepticism. This was followed by actual experiments to verify statements. In 1676, Grew wrote about the nature of ovules and pollen grains. Camararius worked on sex in plants and published a 50-page letter which convincingly proved that plants are sexual organisms. A short time before 1717, Fairchild (England) produced the first artificial plant hybrid. These discoveries of sexual organs in plants led to the establishment, mainly through the efforts of Vilmorin, of the famous French company Vilmorin-Andrieux et Cie in 1727 for seed breeding. In the following years, there was a lot of interest in plant breeding. During 1761-66, Kolreuter (Germany) published his extensive work on plant hybridization. It included the results of 136 experiments in artificial hybridization.

In England, Bakewell began experiments in 1760 on domestic animals with a view to improve their characters. After 35 years of experimentation, he concluded that inbreeding was not always harmful and was the quickest way to fix types. His experiments formed the basis of the development of many of the modern breeds of livestock. In 1773, Sprengel observed that cross-pollination occurred in plants through the agency of insects. By this period, knowledge was gained to some extent in the field of human genetics, although man is least amenable for experimentation. A British divine, Michel Lort, studied the peculiar inheritance of colour blindness and reported his findings to the Royal Society in 1779.

In 1809, Lamarck attempted to formulate a comprehensive theory of evolution. He made a very important statement that if organised parts of a thing were not made up of cell tissues, they would not have life. A more or less similar idea was expressed by Mirbel in 1802, Mayen in 1830 and Von Mohl of Germany in 1835. Darwin and Buffon, both contemporaries of Lamarck, studied in this period the various problems connected with the development of individuals, race and species.

In 1820 the German scientist Nasse became interested in haemophilia, a disease prevalent in the royal families of Europe. He studied its inheritance and propounded the law of sex inheritance. In England, Goss (1822) was studying how characters were transferred from generation to generation. He reported dominance, recessiveness and segregation in pea hybrids but was unable to interpret them. In 1823, Knight, a plant breeder, also observed dominance, recessiveness and segregation in pea. He was the propounder of the Knight-Darwin law of cross-breeding. With the help of this law, the value of crossing to produce better plants could be determined. The term 'dominant' was first used by Sageret of France in 1826. He classified contrasting characters in the parents of a cross in pairs and cited unit characters in the human eye colour. During 1840-50, Vilmorin made many genealogical studies while carrying out breeding work in wheat, oat and sugarbeet, and developed the progeny test. In 1843, Couteur (Island of Jersey) published a summary of his work on wheat breeding. According to De Vries, this formed the basis and origin of variety testing. Methods similar to those of Couteur were also independently developed a little earlier by Sheriff (Scotland), who is credited with the production of many outstanding varieties.

The year 1859 is very important in the history of biology. It was in this year that Darwin's *Origin of species* was published which revolutionised the outlook of contemporary biologists. It was the turning point in biological thought, and an era of evolutionary and experimental approach began. Darwin established that plant and animal species are evolved from pre-existing ones gradually and natural selection is an important factor in the process of evolution. His theory of evolution was instrumental in disposing of the prevailing doctrine of fixity of species.

In 1863, Godron and Naudin, both from France, independently published their results on plant hybridization. In his paper, Naudin confirmed the findings of Sageret. He also discussed the work of earlier hybridizers. He reported dominance and segregation in *Datura* hybrids, although he did not work with a single character at a time. He also did not make statistical analysis of his data on the second generation. In spite of these drawbacks in his work, it may be said that he was a forerunner of Mendel.

Progress of Cytology in the Nineteenth Century

In 1833, Brown used the term 'nucleus' to define the centrally-located, darkly-stained body in the cell. The year 1835 was an important year from the point of view of progress in cytology. It was in this year that Von Mohl described the division of cells. Another important step in the progress in cytology was the pronouncement of the cell theory by Schleiden in 1838. According to this theory, all organisms are made up of cells. This theory is regarded as one of the greatest generalisations of experimental biology. Schwann (Germany), however, made it applicable to only animals. Certain assumptions made in this theory are not, however, correct. Both Schleiden and Schwann gave more importance to the cell wall than the inner fluid. They thought that new cells were formed by the method of free-cell formation. Their idea was that there was a structureless and continuous material called cytoblastome from which cells were formed by a process similar to crystallisation.

In 1840, Purkinje (Bohemia) coined the term 'protoplasm' for the cell fluid enclosed within the cell membrane, and which has been in use since then. Before him, it was Dujardon who first gave any importance to the inner contents of the cell. He called it 'sarcode'. He tried to study its chemical properties and solubility. He stated that sarcode was a completely homogeneous matter which was elastic, contractile, transparent, and gelatinous in nature. It was insoluble in water and did not show any signs of organisation. In 1840, Payen (France) and Cohn (Germany) emphasised the essential similarity between protoplasm of plant and animal cells, and regarded it as the physical basis of life. In 1841, Von Kollikar (Switzerland) proved that spermatozoa arise from the parent body and are not parasites, as was formerly believed.

It is interesting to note that in 1848 Hofmeister (Germany) had figured chromosomes as unstained bodies but he did not realise their importance and ignored them.

In 1849, Owen (England) enunciated the principle of continuity of germ plasm. This principle was further developed by Virchow, Weismann and others. Ultimately, it took shape in the form of the modern gene theory. In 1858, Virchow established conclusively a very important principle that a cell never develops *de novo*, i.e. a cell always develops from another cell and does not arise anew. This was indeed the basic biological principle that contributed to the completion of the cell theory. It established the theory of continuity of all life from a remote beginning, and thus dealt a final blow to the theory of spontaneous generation.

In 1861-62, Schultz and De Bary (Germany) proved that there is essential unity in all living cells. In 1865, Schweigger-Seidel and Valette, both of Germany, independently proved the unicellular nature of a spermatozoon and showed that it contains cytoplasm and a nucleus.

Birth of Modern Genetics

The above was the background when Mendel read his paper in 1865 in the meeting of the Brünn Natural History Society. The paper was published in the proceedings of the society in the following year. It is difficult to state how much knowledge of the work done in cytology and genetics was known to Mendel, but the fact that he was in constant touch with Nageli, the renowned biologist of his time, and was also using the library containing the works of Darwin and biological literature including the work of plant hybridization, indicates that he must have benefited by them.

The year 1866 is regarded as the year of the birth of modern genetics as it was in this year that Mendel's paper embodying the results of his hybridization experiments was published in the *Proceedings of the Brünn Natural History Society* (Verhandlungen der Natur Forschenden Verein in Brünn). This paper laid the scientific foundation of genetics. However, for 34 years this work remained unknown to the scientific world. It was only in 1900 that it was rediscovered simultaneously by De Vries (Holland), Correns (Germany) and Tschermak (Austria).

Work in Post-Mendelian Period

Chromosomes were first described in 1875 by Strasburger (Germany). In the same year, Hertwig (Germany) experimentally proved that fertilisation is brought about by the union of the spermatozoon nucleus and egg nucleus, and in this sexual process the male and female gametes have an equal share. His work indicated clearly that genetics is essentially a problem of cell physiology.

In 1882, Flemming (Germany) stated that during cell division, chromosomes split longitudinally. In 1883, Van Benedin (Belgium) discovered that when gametes are formed, the number of chromosomes is reduced to half to that present in the body cells. At about 1884-85, independent researches of Hertwig, Strasburger, Kollikar, Weismann and Flemming contributed considerably to the knowledge of cell division. In 1885, Rabl (Austria) proved the individuality of chromosomes. In the same year, Weismann published an important theory which explained the behaviour of chromosomes during meiosis and fertilisation as well as the earlier observations of Van Benedin, Strasburger, Flemming, etc. The theory predicted two kinds of cell division, mitosis and reduction, in which the chromosome number would be reduced to half by a separation of paternal and maternal chromosomes. Boveri (Germany) (1887-88) verified this theory in respect of reduction division and proved its correctness by his work on *Ascaris*.

In 1889, Galton (England) proposed the law of ancestral inheritance. It indicates how characters in offspring are determined and the relative influence of parents, grandparents, etc., in their inheritance. Weismann published his germ plasm theory in 1892. It helped to dispose of the prevailing theory of inheritance of acquired characters formulated by Lamarck.

In 1894, Bateson (England) laid emphasis on the study of discontinuous variations in solving the problem of heredity. This was an approach to the idea of Mendelian units of heredity. At this time, Spillman (USA) who was working on wheat had also reached almost the same conclusions as Mendel did.

In 1899, Cuenot (France) who was working on animals and Strasburger on plants put forth a theory that the sex is not affected by environment but is controlled within the reproductive cells.

In 1902, Montgomery (USA) showed that during meiosis, the homologous paternal and maternal chromosomes pair before actual reduction in the number of chromosomes and formation of gametes has taken place. Sutton (USA) was the first to propose the chromosome theory and in 1902 he showed parallelism of behaviour between chromosomes in meiosis and Mendelian factors an heredity. In this manner he linked cytology to genetics, and the branch of cytogenetics thus arose.

In 1904, Davenport (USA) proved that the inheritance of polydactyly was as per Mendelian laws. In the same year, Morgan began working as a professor of zoology at the Columbia University. In collaboration with his students, he started important researches in the field of genetics. This group later on became famous as the Columbia group. Shull and East (USA) started work independently on inbreeding in maize. Their experiments helped to prepare the theoretical as well as experimental background of genetics. In 1906, Bateson coined the term 'genetics' for the science of heredity. In the same year, the American geneticists, Woodworth and Castle found that the fruitfly, *Drosophila* was the best experimental material for genetic research. The Morgan group used *Drosophila* extensively and contributed significantly to the progress of genetics. In 1906, Lock (England) suggested the possibility of a relation between chromosomes and linkage. In 1907, Correns put forward the theory that there are two types of male gametes, the male determiner and the female determiner. In the same year, Strasburger used the term 'haploid' for defining the reduced number of chromosomes and the term 'diploid' for the double number of chromosomes. It was proved by Lutz (USA) that in the gigas mutation of *Oenothera lamarckjana*, the chromosome number is double the number in *O. lamarckiana*, i.e. it is tetraploid. This work gave impetus to the study of polyploids. The Swedish geneticist Ehle worked on the inheritance of colour of wheat grains and laid the foundation of research in quantitative inheritance.

In 1909, Janssens (Belgium) put forward the theory of crossing over. In 1915, Morgan and his students, Sturtevant, Bridges and Müller published *The Mechanism of Mendelian Heredity* based on their researches of the genetics of *Drosophila*. This book is regarded as a very important step in the progress of genetics. In 1916, Shull observed hybrid vigour in F_1 hybrid of maize. He used the term *heterosis* for this vigour. This discovery stimulated rapid progress in plant hybridization.

Between 1911 and 1922, several scientists, for instance, Pearl, Collins, Kempton, Goldschmidt, Cuenot, Gates, Jennings, etc., tackled multifold problems in genetics and contributed a great deal to its progress. In 1923, Wright (USA) published a book on the application of coefficient of inbreeding to the irregular system of inbreeding. From then on several attempts were made at using Mendelian inheritance in reproduction of organisms. The works of Wright, Fisher, Haldane and others are significant contributions in this direction.

In 1927, Müller, Stadler and Goodspeed independently observed that the rate of mutation was increased by x-rays and thus it was possible to induce mutations artificially. This was a very important discovery in the field of genetics and is regarded as a very significant step in its progress. In the same year, Hanson (USA) also realised that it was possible to induce mutation in genes by using radium. In 1934, Painter (USA) found giant chromosomes in the salivary glands of *Drosophila* and realised their genetic value in making a detailed study of the chromosome structure.