# GENETICS
# Fundamentals and Applications

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# Preface

Genetics is an integrated domain of any advanced field of biology in 21st century. However, successful development of future applications will depend on the basic domain knowledge. **Genetics: Fundamentals and Applications** covers maximum aspects of genetics and it is aimed at readers from multidisciplinary fields of biology.

It is an effort to use simple language and examples throughout the book for better understanding of principle of genetics and its application in modern era. Few specific chapters have been incorporated to emphasize the application aspects of genetics in disease pathology and therapeutic areas.

Basic principles of genetics (first 19 chapters) have been described in a cognized way with simplest example sets for beginners at **under graduate** and **post graduate** level. In contrary, Application part (last 5 chapters) especially chapters covering cancer genetics, apoptosis and stem cell are written elaborately for **post graduate** students and **researchers** in biomedical sciences.

**Topics and Features**

- Consists total 25 chapters covering maximum aspects in genetics.
- Simple understanding of genetic principles in plants and animals.
- Simple example for complex genetic problems.
- Includes a chapter for different terminologies used in genetics.
- Includes a chapter from agricultural point of view.
- Includes a chapter for cancer biology, genetics, diagnosis and treatments.
- Explains different process and gene families in cancer.
- Highlights the challenges in conventional cancer chemotherapy.
- Includes a chapter for various aspects of apoptotic including pathways and gene families in model organisms.
- Highlights cancer chemoprevention through apoptosis by dietary phytochemicals.
- Includes techniques in detection of apoptosis.
- Describes in death biology, markers, plasticity, trans-differentiation and therapeutic applications of different stem cells.
- 64 illustrations for easily understanding of the subject matter.

**Dr. H C Srivastava**
**Dr. Debmalya Barh**

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Dr. Srivastava dedicates this book to his Late Grand Father & Grand Mother Mr. & Mrs. Matabadal Lal Srivastava; Late Father & Mother Mr. Daya Shankar Lal Srivastava, Mrs. Indrakali Devi Srivastava; Late Uncle Mr. Badri Prasad Srivastava and Late Younger Brother Dr. Sushil Kumar Srivastava.

Dr. Debmalya dedicates the work to his loving parents Mr. Purnendu and Mrs. Mamata.

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*Part I*

# Fundamental of Genetics

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# Chapter 1

# Introduction

Genetics is an important branch of biology. It means study of heredity and variation. Heredity relates to that science by which living beings resembles to their relatives and ancestors. Variation means the science by which living being differs from their relatives and ancestors. Thus genetics is the science to study how various characters are transferred from parents and develop into offsprings. Now-a-days the sphere of genetics is extremely wide. It studies how genes are developed, what is their chemical structure and how these effect the development and behaviour of living beings. Genetics has been divided into six branches: 1. Cytogenetics, 2. Physiological genetics, 3 Biochemical genetics, 4. Population genetics, 5. Eugenics and 6. Applied genetics including cancer genetics, apoptosis & stem cell.

**Importance of genetics:**

Genetics is comparatively a recent science. Its development has happened at fast speed. Genetics has been widely used to develop improved types of cereals, vegetables, fruits, ornamental plants, medicinal & aromatic plants, spices etc. and various animals. In addition to this, genetics is closely associated with other sciences such as morphology, taxonomy, embryology, cytology, biochemistry, ecology, evolution, bacteriology, statistics etc., as briefed below:-

1. **Genetics and Morphology:** Morphologies studies various morphological features of living beings. Growth and development of tissues are effected by mutations - a branch of genetics
2. **Genetics and Taxonomy:** Classification of various living beings, their species, genus, family and order depends on their genetics. Development of a living being is governed by its genetical constitution.
3. **Genetics and Embryology:** Theoretical and practical embryology is governed by its genetics. Genetical constitution of a living being conditions its development.

4. **Genetics and Cytology**: Cytology of a living being is concerned with its genetics. Studies on Cytology and genetics are collectively grouped as cytogenetics. Dunn (1960) has rightly mentioned that cytology is the pillar of genetics.
5. **Genetics and Biochemistry**: To understand the nature and function of genetic material a geneticist has to take help of biochemistry. Now a days genetics helps in biochemical research. For example due to reduction of an amino acid tyrosine by an enzyme, homogenestic acid is formed in human body which causes a disease Alkatopnuria in which urin on keeping in air turns black. This disease is controlled by a simple genetic factor. Another example of controlling enzyme by genetic factor has been found in the fungus Neurospora in which formation of spores gets stopped.
6. **Genetics and Ecology**: Ecology means study of living being in natural environment. Now a day's significant progress has been made in ecology by the help of genetics. Acclamatization of various polyploids in different environment reveals close relationship between ecology and genetics. This fact reveals that ecology is dependent on heredity of the organism.
7. **Genetics and Psychology:** In modern days genetics is used to solve psychological problems. Studies of inheritance and DNA help to solve several psychological problems.
8. **Genetics and Statistics**: Biometry helps significantly in genetical research. Dunn (1960) has rightly mentioned that statistics has greatly helped in development of genetics.
9. **Genetics and Bacteriology:** Several scientists have done remarkable progress on physiology, pathology, biochemistry etc., of bacteria. But complete information on their life cycle could not be obtained. It was afterwards completed by geneticist, the invisible cytological facts were proved by practical experiments. For example sex linked inheritance is expressed in presence of sex chromosomes, though cytologically it may or may not be visible. Similarly, genetics has been helpful in studies of virus.
10. **Genetics and Botany & Zoology:** Botany and Zoology are different sciences for studies of plants and animals. However, genetics correlates both these sciences. Both express similarly in inheritance.

**Application of Genetics:**

Genetics has been used for varietal improvement of various plants & breed amelioration of various animals, for treatment of diseases, and for betterment of human race. Brief descriptions of various applications are mentioned below:

**1. Improvement of agricultural plants:**

*a.* ***Improvement in disease resistance:*** One of the main agricultural problems to the whole world is occurrence of diseases which cause economic loss to crops. The best solution to this problem is development of disease resistant varieties using the principles of genetics.

*b.* ***Amelioration of insect resistance:*** Crops also suffers serious economic loss due to various types of insects such as aphids, sucking insects, mites, nematodes etc. This problem can also be solved by breeding for resistance following the principles of genetics.

*c.* ***Synthesizing of plants with multiple qualities:*** With the help of genetics now a days plant breeder are synthesizing plants with multiple qualities such as high yield, superior quality, resistance to diseases & insects and more adoptability to the existing agro-climate. Breeders do it by multiple crossing.

Details are discussed in the chapter on plant breeding. **Plant breeding** is a subject which is related to genetics, cytology and cytogenetics and has its own special features.

**2. Use of genetics for improvement in animals:**

*a.* ***Improvement for disease resistance:*** Due to occurrence of some diseases in animals the farmer suffers economic loss. Several medicines are invented to control these diseases. A better method of control is to develop disease resistance breeds. For example, typhoid resistant breeds of poultry.

*b.* ***Improvement in utility of domesticated animals:*** Such improvement in animals can be possible by research on quantitative inheritance & by help of proper hybridization and selection.

**3. Genetics for treatment of diseases:**

**Treatment of cancer** is done by **gene therapy**, surgery, radiation therapy, chemotherapy, hormone therapy, immunotherapy /

biological therapy, adjuvant therapy - combining two or more treatments, prophylactic or preventive treatment. Diseases and maladies treated using adult stem cells are brain cancer, ovarian cancer, skin cancer, testicular cancer, tumors abdominal organs, chronic myelogenous leukemia, acute lymphoblastic leukemia, breast cancer, cancer of the lymph nodes, soft tissue sarcoma, various solid tumors etc.

**4. Genetics for betterment of human race:**

This subject was named as **Eugenics** by Galton in 1904. In this science geneticist investigate those factors which effect hereditary characters in human and which can make such characters more useful to mankind. Genetics can help to predict the future life of an infant. To improve human being two methods may be used: first method is euthenics. It means by improving the environment and surroundings. The second method is eugenics. Under it improvement in hereditary constitution are studied. This method is of two types- positive eugenics and negative eugenics ie. to get rid of undesirable germplasms Details are given in chapter on eugenics.

Hence it is apparent that progress of genetics has been associated with progress of other sciences. Beyond academic importance, genetics has tremendous applications for human welfare.

# Chapter 2

# Brief History

Centuries before Christian era, in Egypt and Mesopotamia few people had some idea of genetics because they attempted cross breeding in animals and plants. Example of artificial pollination in date palm was available in history of that time. But more development in this science started from beginning of twentieth century.

In eighteenth and nineteenth century geneticist were trying to understand that how characters are transferred from one generation to another. But actual knowledge of heredity did not come till details of reproductive process were not clear. In 1760 Kolreuter a botanist in Germany, on hybridization of one type of tobacco with other type of tobacco (by transferring of pollen from one type to pistil of other type) has observed that in the hybrid the characters came from both the parents. From this observation it was clear that characters are transferred from gametes of the parents. Therefore, gametes had been said as a unit of heredity.

Based on these facts the Austrian botanist John Gregor Mendel has experimented with sweet pea in his garden and found unprecedented success. Earlier workers in this experiment failed. The reason for his success was that at one time he selected one contrasting character. He counted the offsprings of various types and kept the account properly. First he experimented with plants having green & yellow seeds and wrinkled & smooth seed coat. From these experiments he concluded that inheritance is based on certain principles. He has expressed that if pedigree of two parents is correctly known then it will be possible to forecast the type and number of progenies. In the end he concluded that inheritance is controlled by units or factors present in the cell. He has also expressed that in somatic cells of plants and animals there are two units or factors present but in their sex cells i.e., pollen and ovule or sperm and egg cells only one unit or factor is present. Mendel was unable to find out that how two units or factors in somatic cells become only one unit in their sex cells. This mystery was solved by the experiments of Fleming (1882). He has told that it happens by the cell division mitosis and meiosis. Oscar

Hertwig (1885) has told that in combining of sex cells it is not that two cells unite but the nucleus of male sex cell unites with the nucleus of female sex cell. Therefore, he concluded that the unit of inheritance is nucleus. Immediately after knowledge of meiosis and fertilization Devries from Holland, Correns from Germany and Von Tschermark from Austria experimented separately on the laws of inheritance proposed by John Gregor Mendel. These scientists have designated the laws as Mendel's laws of inheritance.

Despite these information it could not be explained that how so many characters get transferred from one generation to another. To solve this mystery efforts were made by the famous scientist Charls Darwin in 1895. Later on he proposed the theory of Pangenesis. It suggested that every cell of the body produces its agents known as Gemmules. These gemmules come in the blood stream. Then they reach in the sex cells (ovaries and sperms). These sex cells unite and ultimately develop a living being. Each gemmule helps in expression of different character. But this theory was proved to be wrong by Galton (1822-1911) and other scientists.

At almost same time the famous scientist Lamark (1844-1929) did research on same problem of heredity. He experienced that a living being or any of its organ gets changed due to use or no use or environmental effect. For example if any organ is used constantly it becomes strong, but if not used it becomes weak. Lamark thus proposed a "theory of acquired characters". He told that acquired characters of parents reach to their offsprings. This theory became famous as 'Lamarkism".

Weismann (1835-1914) could not accept Lamarkism. After experimentation he concluded that reproductive cells are different from other cells. So reproductive cells can be called as 'germplasm'. He told that germplasm can give rise to somatoplasm. No changes in the somatoplasm can go in germplasm. Therefore, acquired characters cannot be inherited. Weismann has also proved it wrong by simple experiments. He has cut tail of rats for several generations. But still the new generation have normal tail. In muslims skin over the tip of penis is removed from every child but still the newly born muslim children have skin over tip of their penis. Weismann (1835-1914) has proposed Germplasm Theory. It has significantly helped in development of genetics.

Significant progress of genetics has started in 1875 when Strasburger has invented that at cell division nucleus is changed to several

longitudinal structures to which he has given the name chromosome. The chromosomes are understood as the base of heredity. In 1902 Sutton has discovered that each chromosome is made up of globular structures which are known as genes. Genes are arranged longitudinally and control all the characters or traits. Afterwards, especially after 1920 genetics progressed at tremendous speed.

Cancer is multistage carcinogenesis processes during which normal dividing cells accumulate multiple somatic mutations i.e. point mutations and mutations due to chromosomal damage, and then become cancer cells by several pathway events. Most human tumors are spontaneous but some are age and sex related, and a few are inherited or familial. There are many etiologic factors such as genetic history, diet, lifestyle, and environment may contribute to the development of these tumors. In human latency period ranges from a few years to 30 years or more.

Apoptosis is the term coined by Andrew Wyllie in 1972 to describe morphological description of a dying cell that contrast with necrosis. Programmed cell death (PCD) is a term used to describe cells that die at predictable time and place during development. Programmed cell death is a gene directed cellular suicide mechanism that eliminates unwanted, superfluous cells during development as well as tissue homeostasis, control proliferation and differentiation and defends against viral infection. All Programmed cell deaths are apoptotic and so these terms are used interchangeably. Programmed cell death is associated with ALPS- atoimmunity, myoicordial infraction, stroke, diabetes, neurodegenerative diseases, alzheimer's disease, infertility, sepsis, viral infection etc.

Characters of apoptosis are cytoplasm shrinks with out membrane rupture, blabbing of plasma membrane and nuclear membrane chromatin condenses and migrates to nuclear membrane, inter-chromosomal cleavage in nucleosomal size by $Mg^{+2}$ dependent endonuclease leads to laddering of DNA at nucleosomal repeat length of 200bp, fragmentation of cell with retention of cell membrane, flipping up phosphatidal serine from outer plasma membrane, cell contents are packed in membrane bounded bodies (apoptotic bodies), internal organneles still remain functional, mitocondria swells and engulfed by neighbours, epitopes appear on plasma membrane, making cell as phagocytotic target and no spillage and inflammation.

There are 3 different mechanisms of apoptosis: 1-death signals generated within the cell. 2-activation of death activators by extrinsic death signals. 3-by reactive oxygen species and DNA damage.

Cancer is a multistage carcinogenic process where there is a net accumulation of atypical cells arising from excess proliferation, an insufficient apoptosis or a combination of the two. There are also various side effects of chemotherapy. Thus from therapeutic point of view, the best strategy is " induced apoptosis in the neoplastic cell line without affecting the normal cells of the body. Several diatary phytochemicals are now a days used for chemoprevention.

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have three general properties: 1-they are capable of dividing and renewing themselves for long periods; 2-they are unspecialized; 3- regulated by intrinsic signals and the external microenvironment and can give rise to specialized cell types.

Ability of stem cells is self-regeneration, to divide and produce more stem cells. During early development, the cell division is symmetrical i.e. each cell divides to gives rise to daughter cells each with the same potential. Later in development, the cell divides asymmetrically with one of the daughter cells produced also a stem cell and the other a more differentiated cell.

Stem cells are involved in three processes - development, repair of adult tissue and cancer. An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Some scientists now use the term somatic stem cell instead of adult stem cell. Unlike embryonic stem cells cells, which are defined by their origin, the origin of adult stem cells in mature tissues is unknown.

Bone marrow (BM) contains hematopoietic stem cells, which differentiate into every type of mature blood cell; endothelial cell progenitors; and marrow stromal cells, also called mesenchymal stem cells, which can differentiate into mature cells of multiple mesenchymal tissues including fat, bone, and cartilage. Recent findings indicate that adult BM also contains cells that can differentiate into additional mature, nonhematopoietic cellsof multiple tissues including epithelial cells of the liver, kidney, lung, skin, gastrointestinal (GI) tract, and myocytes of heart and skeletal muscle. Experimental results obtained

in vitro and in vivo are the subject of this review. The emphasis is on how these experiments were performed and under what conditions differentiation from bone marrow to epithelial and neural cells occurs. Questions arise regarding whether tissue injury is necessary for this differentiation and the mechanisms by which it occurs. We also consider which bone marrow subpopulations are capable of this differentiation. Only after we have a better understanding of the mechanisms involved and of the cells required for this differentiation will we be able to fully harness adult stem cell plasticity for clinical purposes

Eugenics word was evolved by Sir Francis Galton in the year 1883. He defined it as - eugenics is the study of those factors by which development of mental and physical characters takes place in human beings. In other words we can say that the branch of genetics in which the laws of inheritance are used to improve human beings is called eugenics. Eugenics is a Greek word meaning well born. Aims and ideals of eugenics are improvement for: 1- mental and physical healths, 2-intelligence, 3-moral character and 4- to attain specialization in knowledge is very useful to the person and society. Generally the capacity for specialization is hereditary.

Plant breeding is based on principles of genetics and studies in details the technologies of crop improvement. A good plant breeder has good understanding of genetics and cytogenetics. In addition to these he/ she should know agronomy, horticulture, plant pathology, plant physiology, statistics, agricultural chemistry and entomology. Plant breeding has been defined by G.M. Poehlman and D.N. Borthakur (1959) as a science to improve heredity of plants. When by hybridization between close relatives offspring are produced, it is known as inbreeding. But if the parents are least related or distantly related then the breeding is known as out breeding.

Under plant breeding stigma of other plant is artificially pollinated and offspring are produced for various types of selections. Therefore plant breeder has to know merits and demerits of the plant and requirement of the country.

Objective of plant breeding is to combine various useful characters into one variety of plant. The useful characters which a plant breeder must understand are:high yield, early maturity, high quality. It should have sufficient nutrient, attractive colour, should be tasty and easily digestible, non shedding of grain, resistance to drought and frost, disease and insect resistance, more medicinal property. In case of

breeding of medicinal & aromatic crops the objective is to increase yield & medicinal properties, more yield of superior quality essential oil and resistance to biotic & abiotic stress. In addition to above mentioned objectives a plant breeder makes efforts to develop varieties which by application of more fertilizers and manure can produce more yield, the fruits should have uniformly good size and colour. The crop should have resistance to lodging and flood.

Main methods of plant breeding are plant introduction, selection, hybridization, mutation and polyploidy. Hybridization in self pollinated crops are pedigree selection method, bulk method, back cross method, and multiple cross method. Hybridization procedures in cross pollinated crops are 1-single cross method 2- double cross method. 3-triple cross method. Depending on parental relationships hybridization can be of 3 types: intervarietal cross, interspecefic cross and intergeneric cross

Chapter 3

# Important Definitions of Genetics

In foregoing pages of this book several technical words have been used. It is very important todescribe those technical words Otherwise it will not be possible to understand the subject. In addition to it these definitions will help the students to answer several questions asked in their practical exams.

In every cell chromosomes are present in pairs. Therefore two genes of each character are present on these chromosomes. These genes are expressed as capital letter (dominant gene) and small letter (recessive gene). For instance tallness is expressed as TT and dwarfness as t t.

At the time of reproductions pollen or semen and eggcell or ovum are formed. In these only one gene is found which is written by only one letter. For example tallness is expressed as T and dwarfness is expressed as t only, in such reproductive cells.

Important technical words of genetics are explained & defined below.

**Acquired Characters:** Modification of character due to environment or artificial means in life of an organism is called aquired character.

**Allele or allelomorph:** Each gene responsible for contrasting character in organism is called allel or allelomorph. For example in heterozygous plants for height, responsible gene is L and for dwarfness the responsible gene is l. Both these genes are called alled or allelomorph.

**Ameioses:** It is a special type of meiosis in which number of chromosomes is not reduced to half as happens in normal meiosis. In ameiosis the chromosome number remains 2 n.

**Amitosis:** It is special type of mitosis in which without differentiation of chromosomes the nucleus is divided.

**Autosome:** Except sex chromosomes other chromosomes are called autosomes.

**Allosome:** Sex chromosomes can be also called as allosome.

**Acclimatization:** Ability of organism to adjusts with changed environment is called acclimatization.

**Autopolyploid:** It is that polyploid in which all chromosomes are similar in sets.

**Allopolyploid:** It is that polyploid in which chromosomes are different in sets.

**Amphidiploid:** It is a polyploid produced from that diploid which has been development by cross of two dissimilar diploids.

**Amphimixis:** It is the mixing of male and female gametes during fertilization.

**Apomixis:** Development of organism from female gamete without fertilization with male gamete is called apomixis.

**Aneuploid:** Improper multiplication of chromosomes number is called aneuploid . It is always minus or plus of a few chromosomes.

**Asexual Reproduction:** It is a process of reproduction not requiring any gamete .

**Asynopsis:** During meiosis absence of pairing between homologues chromosome is called asynopsis.

**Autogamy:** Self fertilization is also known as autogamy.

**Back cross:** Crossing of hybrid with any parent is called back cross.

**Backcross Breeding:** It is a procedure of breeding. The hybrid is continuously (recurrent) crossed with a parent followed by selection of the desirable character.

**Balance:** It is a situation when genetic factors exist in such a proportion so that development of the organism is normal.

**Basic number:** It is the chromosome number of a very old member of an organism. Basic number is expressed as x.

**Biometry:** Use of statistical procedures in biology is called biometry.

**Biotype:** Organisms of uniform genetical constitutions are called biotype. It may by homozygous or heterozygous.

**Bivalent:** In first meiotic cell division, pairing of chromosomes is called bivalent.

**Breeder's seed:** Seeds produced by variety developing organisation or individual is called breeders seed. From this seed foundation seed is produced.

**Breeding:** It is the science to change the genetic structure of plant or animal.

**Bulk Breeding:** It is a procedure of breeding. Self pollinated plants of different genotypes are grown in a bulk plot and then single plants selection is done.

**Centromere:** It is a point on chromosomes on which spindle fiber is attached. During cell division movement of chromosomes is governed by centromere.

**Character:** Trait developed by gene is called character.

**Chiasma:** While pairing of chromosome attachment with in two chormatid is called chiasma.

**Chromatid:** In beginning of meiosis each chromosomes is divided into two threads like structures. These threads are called chromatid.

**Chromomere:** Chromomere are points arranged linearly in chromosomes. Inside chromomeres gene is located.

**Chromosome:** Thread like structures present in nucleus are called chromosomes. At various stages of meiosis shape of chromosomes changes. For each kind of organism number chromosomes is same.

**Clone:** Organism developed by mitotic cell division or asexual technique is called clone.

**Combining ability:** It is of two types- 1.general combining ability. 2. Specific combining ability.

**General combining ability:** In cross fertilization capacity of a parent to combine is called general combining ability.

**Specific combining ability:** Fertilization capacity of a parent is called specific combining ability.

**Covariance:** Covariance is statistical unit to measure variability in a population.

**Crossing over:** It is the exchange of segment between chromatids of a chromosome. It happens because of break and join in chromatids during meiosis.

**Cytoplasm inheritance:** Inheritance of characters from one generation to other through cytoplasm is called cytoplasm inheritance. Another name is molecular system of heredity.

**Chimera:** Plant part or plant made of two or more genetically different tissues is known as chimera. Reasons may be mutation, irregular mitosis or plastid segregation.

**Deficiency:** Removal or absence of segments or gene from chromosome is called deficiency.

**Detassel:** Removal of male part from maize crop is called detassel.

**Diallel cross:** Crossing of several genotypes in all combination is called diallel cross.

**Dihybrid :** Hybridization between male and female having two pair of contrasting characters is called dihybrid.

**Diocious:** If male and female are present on different plants then it is diocious.

**Diploid:** It is that organism which has two sets of chromosomes.

**Diplotene:** In meiosis after pachytene the stage of diplotene comes. In diplotene each bivalent form 4 chromatids. These four chromatids appear in pairs, but are attached at chiasma.

**Disjunction:** Separation of chromosomes at anaphase.

**Dominance:** Out of two contrasting characters, the character expressed in F1 generation is known as dominant the phenomenon is known as dominance.

**Donor Parent:** In backcross breeding, the parent which gives gene to the recurrent parent is called donor parent.

**Double cross:** It is the cross between two F1 hybrids.

**DNA:** Its full form is deoxy ribo nucleic acid. It is found mainly in nucleus . It has adenine, guanine, cytosine and thymine.

**Duplication:** Double occurrence of a segment in a chromosome, is called duplication.

**Duplex:** See the multiplex

**Emasculation:** Removal of another before dehiscence or opening of flowers is called emasculation.

**Epistasis:** Dominance of gene over expression of non allelic gene is called epsitasis. That gene whose effect is masked is called hypostatic.

**Error Variance:** Error caused by unknown or uncontrolled gene is called error variance.

**Expressivity:** Capacity to express genetic character is called expressivity.

**Eugenics:** Study of genetic for human improvement is known as eugenics .

**F1:** Its full name is first filial generation. It is the first generation obtains by hybridization.

**F2:** It is the second generation obtained on self fertilization of F1.

**F3:** It is the third generation obtained on selffing of F2 hybrids.

**Family:** It is the group of organisms produced by one parent.

**Fertilization:** Union of nucleus of male and female gametes is called fertilization.

**Foundation seed:** Seeds produced from breeder seed is foundation seed. After growing it the certified seed is obtained.

**Gamete:** Cells formed after meiosis are called gametes.

**Gene:** Genes are located in chromosomes. It is made of D.N.A. and expresses & inherits characters.

**Gene Frequency:** It is the proportion of expression of alternative allele of an organism.

**Gene Interaction:** Gene interaction is the change in expression of a gene by non-allelic gene.

**Genetics:** Study of inheritance of organism is called genetics.

**Genome:** Haploid set of chromosome is called genome.

**Haploid:** Gametic chromosome number or n chromosome number is haploid.

**Heritability:** The quantum of variability which is due to heredity is called heritability.

**Heterosis:** If character of F1 is superior to parents then it is known as heterosis.

**Heterozygous:** Presence of unlike allels on corresponding loci of homologous chromosome is known as heterozygous.

**Homozygous:** Presence of like allels on corresponding loci of homologous chromosomes is called homozygous.

**Hybrid:** Hybrid is the organism borned by crossing parents of different genotype.

**Inbreeding:** Fertilization between members of close relatives.

**Inbred line:** It is the near homozygous line obtained by repeated inbreeding and selection.

**Inbred-variety cross:** It is the cross between inbred line and a variety.

**Intersex:** Intersex is the organism showing features of male and female.

**Inversion:** Rearrangement in segments of chromosome resulting alteration in sequence of genes.

**Irradiation:** Exposing radiations like x-rays, gamma rays etc. to part of organism of full organism are called irradiation. Irradiation may cause mutation.

**Isogenic lines:** Isogenic lines are those lines which are uniform on all loci.

**Isoallele:** Allels in which difference is not clear is known as isoallel.

**Kinetochore:** It is that place on chromosome where spindle fibers are attached.

**Linkage:** Certain genes located very close on chromosome are passed on together in next generation. This phenomenon is called linkage. Such genes are known as liked genes.

**Linkage value:** It is the recombination fraction which indicates the ratio of occurrence of parental type and cross over type in the progeny.

**Locus:** Locus is the position of genes on chromosome.

**M1, M2, M3:** It indicate the number of generation after treatment with mutagen.

**Male sterility:** Absence or inactivation of anther (male organ) in organism.

**Mass pedigree method:** It is method of breeding where the population collectively raised for some generations. Afterwards pedigree selection is done.

**Mass selection:** It is method of breeding where desirable plants are selected; seeds from these are collected and grown for selection.

**Mating system:** It is the system for sexual reproduction. It of several types such as random i.e. any parent do mating with the other, genetic assortative mating i.e. mating between parents of similar genetic makeup, genetic disassortative mating i.e. mating between parents of dissimilar genetic makeup, phenotypic assortative mating i.e. mating between phenotypically similar parents, phenotypic disassortative mating i.e. mating between phenotypically dissimilar parents.

**Mean:** Average of several observations is known as mean.

**Meiosis:** It is that type of cell division where number of chromosomes

is reduced to half .By meiosis cell division gametes are formed.

**Metaphase:** It is that stage of mitosis or meiosis where chromosomes arrive in centre of spindle.

**Metaxenia:** Effect of pollen on maternal tissue is called metaxenia.

**Median:** It is that point in observation on its both sides lower or higher figures of observations exist.

**Mitosis:** It is that type of cell division which do not change number or characters of chromosomes.

**Mode:** It is the value of observation in frequency distribution.

**Modifying genes:** These are those genes which changes the expression of non allelic genes.

**Monohybrid:** It is the hybrid produced by hybridization between parents having one contrasting character.

**Monoecious:** Occurrence of male and female flowers separately on one plant.

**Monoploid:** It is that organism which has the basic chromosome number.

**Monosome:** Deficiency of one chromosomes in diploid chromosome compliment such as 2n-1.

**Multiple Allele:** More than two alternative forms of a gene is called multiple allele.

**Multivalent:** Holding of more than two homologous chromosomes by chiasmata is called multivalent.

**Mutation:** Sudden and heritable change in structure of gene or chromosome is mutation.

**Nulliplex:** Nulliplex means that a gene is recessive on all chromosomes. If except one or other chromosomes there is recessive gene then it is called simplex, if recessives are on two chromosomes then it is duplex, if recessives are on three chromosomes then it is triplex and if recessives are on four chromosomes then it is quadriplex.

**Nullisomy:** Absence of a pair of chromosome in a cell is called nullisomy.

**Pachytene:** It is the double thread stage of chromosome in meiosis.

**Parthenogenesis:** Development of organism without fertilization of

sex cells is parthenogenesis.

**Pedigree:** Record of past generations of an organism is called pedigree.

**Polysomic:** If in a diploid organism there is more than two homologous chromosomes then it is called polysomic.

**Polyembryony:** Occurrence of more than one embryo in seed is called polyembryony.

**Position Effect:** Neighbouring gene effects the expression of the gene. Therefore if position of a gene is changed there is a change in the expression of the gene. It is called position effect.

**Pedigree Breeding:** It is a method of breeding when from a cross between suitable parents, in the produced segregants selection is made based on individual behaviour and pedigree record.

**Phenotype:** Visible form of an organism is called phenotype.

**Physiological race:** Physiological race is such pathogen of a species which are similar in appearance but are different in pathogenecity.

**Polycross:** Independent mating in group of selected parent's generation is polycross.

**Ploygenes:** These are such genes that have least effect independently but when come together then causes variation.

**Polyploidy:** An organism is a polyploid which has more than two basic set of chromosomes.

**Progeny test:** Study of progeny to test the genotype of the organism is called progeny test.

**Protandry:** Maturity of anthers before stigma is called protandry.

**Protogyny:** Maturity of stigma before anther is protogyny.

**Pureline:** Homozygosity at all loci in chromosomes is pureline.

**Qualitative Character:** Qualitative character is that character which has discontinuous variation.

**Reciprocal cross:** Reciprocal hybridization between male and female parents.

**Recombination:** New combination of genes due to hybridization of parents caused by crossing over and segregation is known as recombination.

**Recurrent parent:** In backcross breeding the parent which is used for repeated crossing is called recurrent parent.

**Recurrent selection:** It is a method of plant breeding. Selection is done from segregants of desirable parents. Selected lines are further inter crossed. Again selection of desirable ones is made. This cycle is repeated.

**Registered seed:** From foundation seed the registered seed is developed. Registered seed is further used to develop certified seed.

**Recessive:** From contrasting characters, the character which is unable to express in offspring is called recessive character.

**Segregation:** In meiosis maternal or paternal separate out resulting in separation of genes. It is called segregation

**Selfing:** Selfing is the fertilization of male and female gametes of same plant.

**Self fertility:** Capacity to produce seeds on self fertilization:

**Self incompatibility:** On self fertilization inability to produce seed due to some genetical reason is called self incompatibility.

**Sib:** Offspring of single parent is called sib. If one parent only is common then the offsprings are known as half sibs.

**Single cross:** It is a cross between two genotypes.

**Sport:** Occurrence of mutation in vegetative bud is known as sport.

**Somatoplastic sterility:** Death of zygote due to disturbance in relationship between embryo and endosperm is called somatoplastic sterility.

**Species:** It is unit of taxonomy. Species is a division of genus.

**Synapsis:** Synapsis is conjugation of homologous chromosomes at pachytene and zygotene stages of meiosis.

**Telophase:** Telophase is the last stage of mitosis and meiosis. Afterwards resting stage comes.

**Test Cross:** It is the hybridization of $F_1$ with recessive parent.

**Tetraploid:** A cell or organism with four sets of basic chromosomes is called tetraploid.

**Top Cross:** Top cross is crossing of a selection or clone with inbred line/variety/single cross. It is also known as inbred variety cross.

**Transgressive segregation:** Segregation of such offsprings which are better or inferior than their perents is transgressive segregation.

**Translocation:** Transfer of segment of a chromosome on the same

chromosome or other chromosome is known as translocation. If it happens mutually on two chromosomes then it is known as reciprocal translocation.

**Triploid:** A cell or organism with 3 sets of basic chromosomes.

**Trisomic:** A cell or organism with triplication of one chromosome is known as trisomic.

**Univalent:** During meiosis non pairing chromosomes are called univalent.

**Variety:** It is sub division of species. A variety is different morphologicaly or physiologicaly from other varieties of a species.

**X:** It means the basic chromosome number.

**Xenia:** It is the effect of pollen on embryo or endosperm.

**Zygote:** It is a cell formed by fertilization of maleand female gametes.

**Zygotene:** Zygotene is the stage of meiosis where thread like chromosomes do pairing.

Chapter 4

# Chromosome and its Structure

Body of living organism is made of cells. Nucleus is the most important part of cell. During cell division, nucleus under goes division. At that time the covering nuclear reticulam breaks off and in the nuclear sap thread like structures appears. Hofmeisfer (1848) Working on Tradescantia for the first time observed this structure in cells. Later on Waldeyer (1888) named these as chromosome. Investigations on chromosomes got intensified with the progress ir science of genetics. Number of chromosome is fixed for every type of organism. During cell division in the nuclear sap several thread like structures can be seen. These are called chromonemata (Fig-1) (Chromonema-singular). Every chromonemata is double.

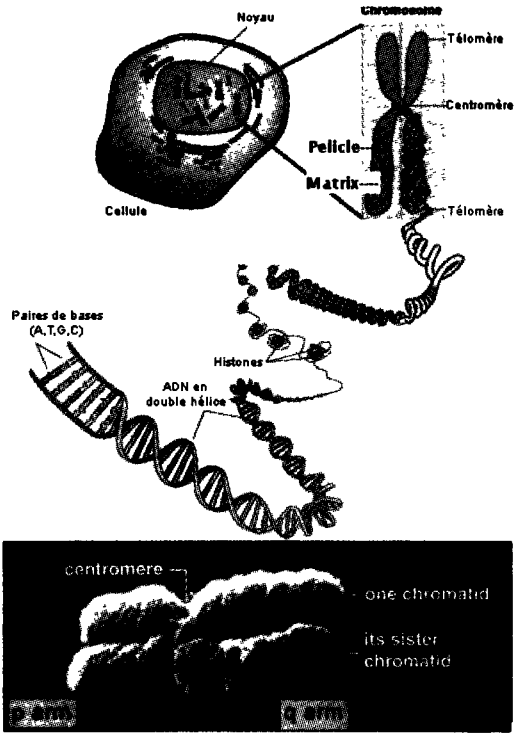



**Fig.1: Structure of a typical chromosome.**

Every chromosome has a kinetochore or centromere which is slightly constricted. Both sides of kinetochore are called arms. At one end of chromosome there is another constriction- known as secondary constriction. Upper portion of nucleolar organizer is called satellite or nucleolar organizer. Some cytologists have expressed that : chromonemata is enclosed in achromatic matrix and covered by a membrane -pellicle. Chromosomes have a large number of chromomeres which have genes. Now we will discuss various components of chromosomes.

1. **Chromonemata:** Number of chromonemata in a chromosome may be two or more. In some cases the number of chromonemata goes up to 32.. Reason for increased number is the division of chromonemata. One important thing is that whatever is the number of chromonemata but they behave as two chronemata only. During cell division each chromosome produces only two chromatids.
2. **Matrix:** Chromonemata remain in matrix which is covered by a covering pellicle. Both of these materials serve as protection to chromonemata.
3. **Centromere:** It is one very important part of chromosome which leads to primary constriction.

In absence of centromere, during cell division the chromosome is unable to reach at metaphase. During anaphase normal chromosomes move towards poles. But centromere less chromosomes lag behind. Ultimately such chromosomes get lost resulting reduction in number of chromosome.

Depending on the position of centromere shape of chromosome is determined (Fig-2). If the centromere is located in centre then shape of the chromosome is V at anaphase. If the centromere is located at the end then shape of chromosome is I during anaphase. If the location is sub median i.e. between end and middle then the chromosome take J shape at anaphase. If the centromere is located at the end then shape of the chromosome is I during anaphase. Detail study of centromere under electron microscope was done by Depharia in 1956.

4. **Secondary constriction:** It is located in one or both arms of chromosomes. Secondary constriction and satelite are related to nucleus. Heitz (1931) reported that nucleolus is related to secondary constriction. In polygonatum change in shape of chromosome and little breakage resulted due to secondary constriction.

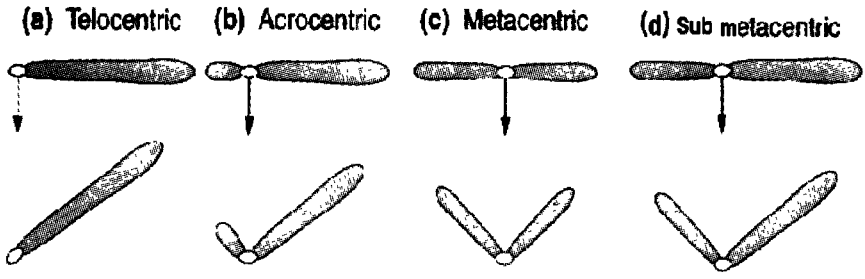



**Fig.2: Shape of chromosome depending on centromeric position.**

5. **Telomere or satellite:** It is the small portion above secondary constriction. It is also called nucleolar organizer. Some cytologists have expressed that in formation of nucleoli at telophase stage some material is used from chromosomes. Somehow this work is done by nucleolar organizer. During metaphase nucleolus disappears. Perhaps its material gets deposited on chromosome or gets transferred to cytoplasm. Nucleolus contains dioxide ribonucleic acid (DNA) and ribonuclic acid (RNA).
6. **Chromomere:** Chromomere appears like beads in chromonema. Number, shape and location of these beads are constant in various organisms. Balbiani (1876) and Pfitzner (1881) named these beads as chromomere. These chromomeres have genes which are essential unit of heredity and variation.

**Special types of chromosomes:**

In nature following special types of chromosomes are found.

**1. Compound chromosomes:**

Compound chromosomes are found in *Ascaris megalocephalia* . In this organism during meiosis only one pair of chromosome is visible. But during mitiosis the two chromosomes gets divided into numerous very small segments. As written earlier only one pair of chromosome is visible in meiosis. Therefore it is called compound or aggregate chromosome.

**2. Salivery gland chromosome:**

Balbiani (1881) reported giant chromosomes in salivary gland cells of Midga larva. Afterwards Kostoff (1930), Heitz & Bauer (1933), Painter (1933), Mechelke (1953) and Breaur & Pavan (1958) have observed similar giant chromosomes in salivary gland cells of *Drosophila* ( Fig 3

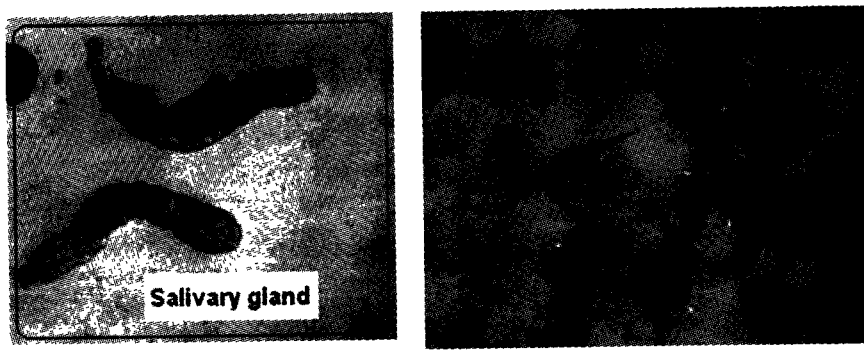



**Fig. 3 a. Fruit fly Slivery gland and salivary gland chromosome under microscope**

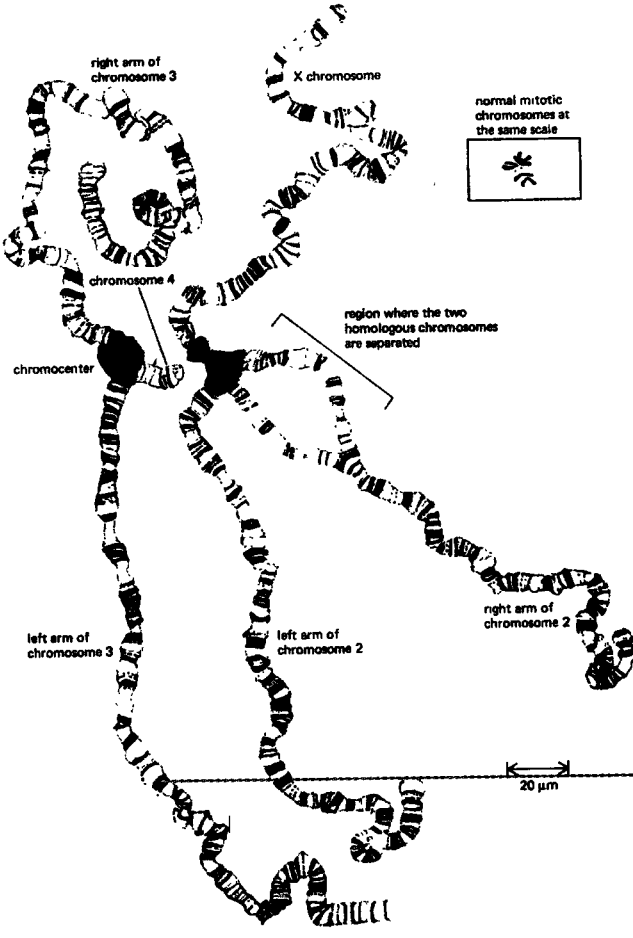



**Fig. 3 b: Salivary gland chromosomes of fruit fly (*Drosophila melanogaster*)**

a & b) and other insects. These were 100 to 200 times larger than ordinary chromosomes. In volume these are 1000 to 2000 times more than ordinary chromosomes. Therefore the name giant chromosome has been given

One more speciality of this chromosome is that there are numerous dark staining and non staining bonds & cross bonds whose sequence is predetermined. Darkstaining bands have DNA containing nucleoprotein but light staining on non staining bond have nucleoprotein free from DNA.

**3. Lampbrush chromosomes:**

Lampbrush chromosomes are elastic in nature because by micro needle it can be expanded several times without any breakage. On leaving, it assumes original length. Lampbrush chromosomes were studied by Duryee (1941 & 1950). Call (1952, 1954 & 1956) and Alfert (1954) studied such chromosome in some fishes, birds and amphibian. Ris (1954) has observed this chromosome in spermatocyte of some invertebrates.

**4. Accessory chromosome:**

In certain organism besides fixed number of chromosomes there are are one or more accessory or supernumerary chromosome. These chromosomes segregate at random. Sometimes these express non disjunction. Occasionally accessory chromosome gets broken into fragments. In 1905 Wilson reported accessory chromosome in cells of Metapodius. Darlington (1935), Ostergren (1947) and Melander (1950) have observed accessory chromosomes in several plants.

Chapter 5

# Mitosis and Meiosis Important Phenomenon of Genetics

## Mitosis

Growth is any living organism is because of increase in number of cells without change in its characters. Such growth starts from zygote. Nucleus and cytoplasm of the zygote divide and forms two cells. In the same way each cell again divides-so the two cells forms four cells. This process goes on till the growth of the organism is not complete. This type of cell division is called mitosis. Due to mitosis in all the cells number and characters of chromosomes are identical to the mother cell. In mitosis cell division nucleus undergoes several changes. Scientists have divided the changes in to four stages such as prophase, metaphase, anaphase and telophase. Between mitotic cell division there is resting stage or also known as interphase (Fig-4). All the stages are described below:

1. **Interphase or Resting stage:** Chromosomes appear as interwoven thin threads. Nucleolus and chromosomes are almost dark stained
2. **Prophase:** At prophase nucleus enlarges, chromosomes appear clearer. Chromosome becomes shorter and thicker. From beginning of prophase all chromosomes split into two. Each part is called chromatid. The chromatids are interwoven in full length. This interwovenship is known as relational coiling. Chromosomes are seen scattered in the nucleus. Nuclear membrane gradually starts disappearance.
3. **Metaphase:** Complete disappearance of nuclear membrane is the beginning of metaphase. Formation of spindle takes place. Cytologists differ on origin and number of spindle. The spindles arrange all chromosomes in equator.

Chromatids of each chromosome are together and through the centromere is attached to the spindle. The Centromere remains undivided.

If somehow it gets split even then this acts as unit only. In mitosis cell division nucleus undergoes several changes. Scientists have divided the change into four stages such as prophase, metaphase, anaphase and telophase. Between mitotic cell division there is resting stage or also known as interphase **(Fig.4).** All the stages are described below:

Chromatids of each chromosome are together and the centromeres are attached to the spindle. The centromere remains undivided, if somehow it gets split, even then this acts as one unit only.

**4. Anaphase:** At this stage centromere divide into two centromeres, these have repulsive action. Due to this repulsion both the chromatids move towards poles. In absence of centromere the chromatid is unable to move and remain at the equator.

**5. Telophase:** At this stage all the chromatids reach to respective poles. New nucleus are formed at both the poles, in nucleus (plural nuclei) nucleolus is developed. Spindle fibres disappear. Chromosomes loose the capacity to get stained.

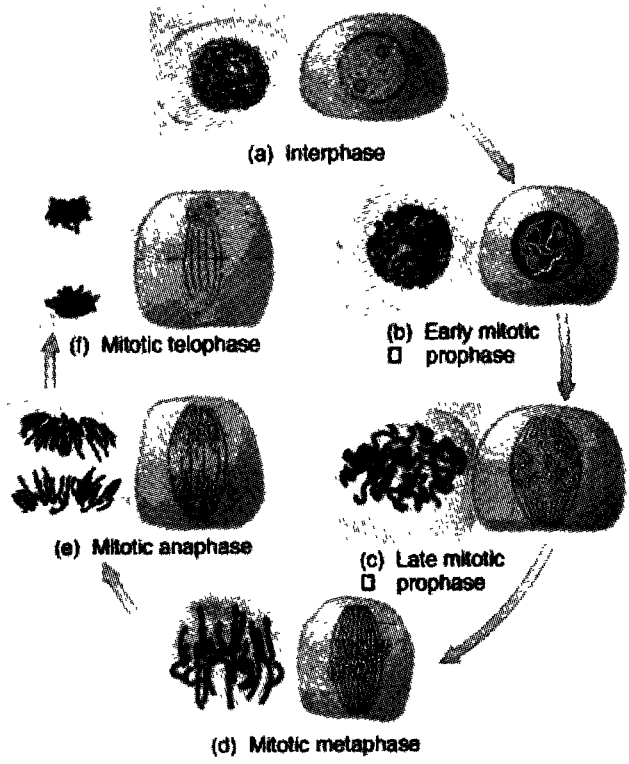



**Fig.4: Diagramatic representation of various stage of mitosis.**

6. **Cytokinesis (Division of cytoplasm):** Cytokinesis starts form cell plate formation. In the end of telophase in the middle of cytoplasm spindle fibers make fragmoplasts. Several fragmoplasts join and form a plate. Length of this plate increases and joins with cell wall. The plate is called cell plate. Over this cell plate pectin deposition starts. Afterwards this cell plate is known as middle lamella. From both side of middle lamella secretion of some compounds (ploysacharide) takes place. Afterwards this wall is called primary wall. Again deposition of cellulose, hemicellulose and polysacharide take place. Afterwards the wall is called secondary wall. This is the end of cylokinesis and the cell gets divided into two cells.

In animals cytoikenesis take place. in different way . It takes place by furrowing of the cytoplasm.

**Significance of mitosis:**

An important significance of mitosis is that the chromosomes of all the produced cell are identical to the mother cell. Chromosome number and their characters are just the same of the mother cell. Due to increase in mitotic cell division the organism achieves growth. Mitosis is very important phenomina for vegetative propagation.

**Meiosis:**

Meiosis cell division is also known as reduction division and heterotypic cell division. Gamete formations are due to meiosis only. Meiotic cell division results in reduction of chromosome number to half.

Meiosis occurs in two phases ie. two times prophase and two times metaphase, two times anaphase and two times telophase. Therefore stages of first meiosis are called prophase-1 metaphase-1, anaphase-1 and telophase-1.With telophase-1 first meiotic cell division completes. The second meiotic cell division starts. The stages are prophase-II, metaphase-II, anaphase-II and telophase -II. With telophase-II the nucleus divided two times and the meiosis (Fig-5) finishes. Meiosis is discussed in detail below.

**First meiosis:**

1. **Prophase-1:** During prophase-1 various changes in chromosomes can be described in five stages as given below.

*a.* ***Leptotene or Leptonema:*** Cell size increases, chromosomes are thin and long. Presence of chromomere is clear.

*b.* ***Zygotene or Zygonema:*** At this stage pairing or synopsis in chromosomes takes place. Every living organism has one haploid chromosome set from father (sperm or pollen) and one haploid chromosome set from mother (egg). Chromosomes obtained from father matches from chromosomes obtained from mother except to sex chromosome. Therefore in every diploid cell there are pairs of chromosomes (resembling to each other) obtained from father and mother, are called homologous chromosomes. At zygotene stage of meiosis these homologous chromosomes come together and form pairs. This process is called synopsis or pairing. In this process centromeres of homologous chromosomes come opposite to each other. Pairs of homologous chromosomes are called bivalents. Therefore if number of chromosomes is 2 at leptotene then at zygotene it appears as one bivalent.

*c.* ***Pachytene or Pachynema:*** At pachytene every chromosome of bivalent, splits longitudinally into two chromatids. But the chromatids are united at centromere. Chromatids do not split. Therefore each bivalent appears to have four strands at pachytene. Nucleolus is visible at this stage.

*d.* ***Diplotene or Diplonema:*** At diplotene stage homologous chromosome start movement from each other. But the homologous chromosomes are not completely separated i.e. those are connected at one or more places. If connected at one place then the bivalent appears to be a cross. If connected at two places then bivalent has one loop. If connected at more places then the bivalent has more loops. Each place of connection in a bivalent is called chiasma (plural chiasmata). Number of chiasma and its location depends on length of chromosome and the type of species. Even the smallest bivalent has one chiasma. Depending on location chiasm is of two types.

(i) Terminal chiasma: It is located at the end of chromosome.

(ii) Interstitial chiasma: Instead at end, the chiasma is located any where else on the chromosome. With the separation of homologous chromosomes interstitial chiasmata can get converted into terminal chiasma. The process is simple. Due

to movement in centromeres the homologous chromosomes moves apart. So interstitial chiasma moves towards end of the chromosome. Thus ultimately it becomes terminal chiasma. This movement of chiasma is known as terminalization. At diplotene stage length of chromosomes gets reduced but their thickness increases.

e. ***Diakinesis:*** At diakinesis chromosome appears short and thick, Nucleolus starts disappearance and completely disappears by the and of diakinesis. Most of bivalents specialy smaller ones because of terminalization assumes nail or similar shapes,

**2. Metaphase:**

Like metaphase stage of mitosis, in meiosis also at metaphase -1 the nuclear membrane disappears.Spindle fibres appears. Some cytologists give the name prometaphase to the phases between disappearance of nuclear membrane and formation of spindle. All bivalents come on the equator.

There is a difference between metaphase of mitosis and metaphase-1 of meiosis During metaphase of mitosis every chromosome has a divided centromere and all the chromosomes lie on equator. Where as in metaphase-1 of meiosis each bivalent has two undivided centromers and bivalents do not just lie on equator but their centromeres appears to be diverted towards poles.

**3. Anaphase-1:**

At ananphase-1 of meiosis bivalents do not have divided centromere, due to which homologous chromosomes along with two chromatids moves towards their poles in contrast to mitotic anaphase where centromeres are divided and chromatids goes towards poles The result is that at end of anaphase-1 on the poles there is only haploid number of chromosome, In other words only half the number of chromosomes at the poles.

**4. Telophase-1:**

Telophase -1 is the last stage of first phase of meiosis. It is for a short time only. Nuclear membrane appears. Spindle fibers disappear. These nuclei are called dyad. Dyad contains only half the number of chromosome in the organism. In certain organism such as Trillium the chromosomes after reaching the poles without centering telophase-1 attains second phase of meiosis.

**Second Meiosis:**

All stage of second meiosis is similar to mitosis except the differences mentioned below.

a. In comparisom to mitosis, second meiosis has only half the number of chromosomes.

b. Chromatids remain at some distance from each other. Hence there is no relational coiling.

c. In mitosis there is no change in the genetic sequence, where as during first meiosis due to chiasma (plural chiasmata ) there is a change in genetic sequence at second meiosis.The degree of changes depend on number of chiasma formed.

Stages of second meiosis are mentioned below:

a. **Prophase-II:** It is a small stage. Nuclear membrane appears and chromosomes appear as a net work.

b. **Metaphase-II:** At this stage nuclear membrane disappears. Spindle fibres appear. Chromosomes come on the equator and their centromers get connected with the spindle fibres.

c. **Anaphase-II:** Centromers get divided. Both the chromatids of chromosomes start movement towards respective poles.

d. **Telophase-II:** At this stage chromatids reach to the respective poles and form net like structure there. Nuclear membrane gradually appears. Cell plate forms and due to this four cells are formed. Each cell has a nucleus. Number of chromosomesis only half (n) in all the four nuclei.

**Cytokinesis in meiotic cells:**

Cytokinesis (division of cytoplasm) mechanism varies in different organism. In some organism during first meiotic division a wall is formed in middle of cell division, dividing the cell into two cells. In second meiotic division another wall get made at right angle to the first wall. In this way the meiotic cell gives rise to four cells. In some other organism during first meiotic division cytokinesis do not take place. In the end of second meiotic division two walls get made in the cell dividing it into four cells. Each cell have only half the number of chromosomes.

**Significance of meiosis:**

1. Meiosis helps in continuation of chromosome number in organisms: Due to meiosis the chromosome number gets reduced to half in gametes. when male and female gametes (with half number of chromosomes) unite and fertilize then the zygote gets the complete number of chromosomes. In this way the chromosome number remain constant
2. Helps to know breeding behaviour of organism. Investigations of meiosis helps to understand breeding behaviour of the organism.

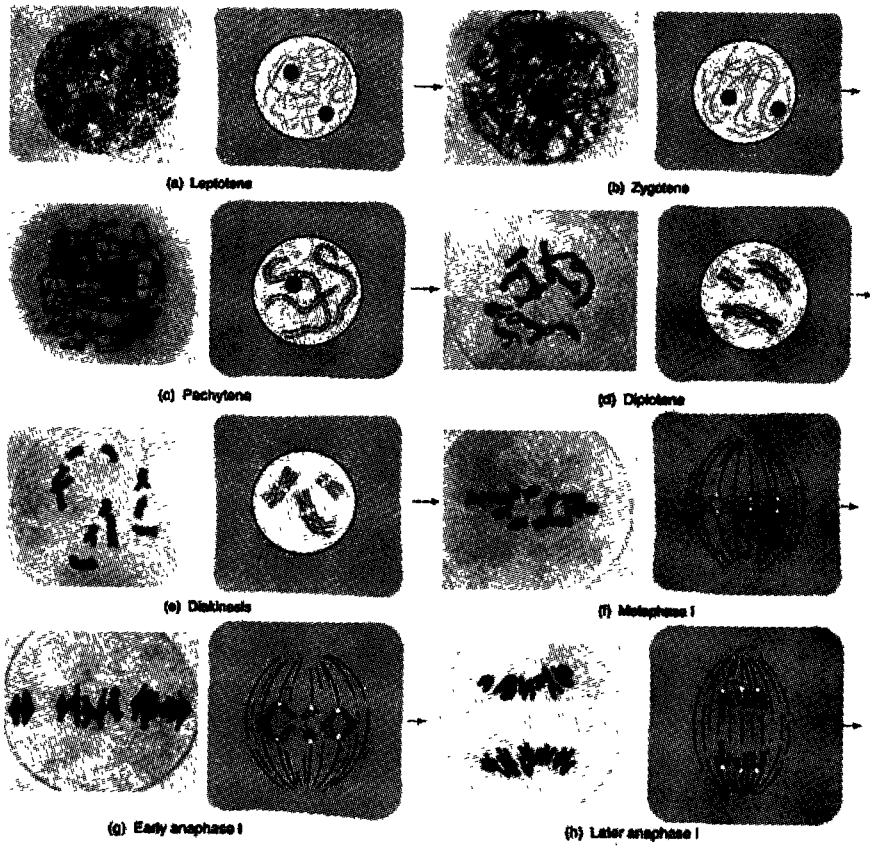



**Fig. 5: Diagrammatic representation of various stages of meiosis.**

**Differences in mitosis and meiosis are given below:**

**Diffrences in mitosis and meiosis**

| Mitosis | Meiosis |
|---|---|
| 1.Nucleus divides once | 1. Nucleus divides twice. |
| 2. Prophase is short duration. There is no substage | 2. Prophase has long duration there are substages such as leptotene, zygotene, pachytene, diplotene and diakinesis, |
| 3. There is no pairing in homologous chromosomes | 3. Homologous chromosomes pair and form bivalents |
| 4. Chiasma formation is absent | 2. Chiasma formation occurs therefore chromatids exchange some parts. |
| 5. At anaphase the chromosomes along with centromere divide & move towards opposite poles and maintain 2 number of chromosomes. | 5. At anaphase -1 centromere do not divide. In each bivalent the two chromosomes move towards the opposite poles along with their chromatids at the poles. Chromosome number is reduced to half. |
| 6. At telophase both the daughter cells have 2 n chromosome number | 6. At telophase-1 both the daughter cells have only n chromosome number. |
| 7. There is no second division of nucleus. | 7. There is second division of nucleus. In the second division centromere also gets divided. Thus second division is matching to mitosis. But instead of 2n there is only n number of chromosomes. |
| 8. In end of mitosis neither the number of chromosomes nor the characters of chromosomes change. | 8. In end of meiosis number and character of chromosomes are changed. |
| 9. Two cells are formed. | 9. Four cells are formed . |
| 10. Mitosis occurs in only somatic cell. | 10. Meiosis occurs in only reproductive cells. |

**Spermatogenesis and Oogenesis**

Spermatogenesis result in sperm formation and oogenesis result in ova formation in animals. Therefore this knowledge is also essential to students of genetics. There is similarity in these two processes. But in some matter they differ.

These processes occur in animals when they achieve sexual maturity. In certain mammals such as human being once this process starts it continue till he or she becomes sexually inert. In other animals these

processes occurs only in their breeding season.

Testes of animals have numerous tubules which are united with germinal epithelium. Every cell of this epithelium acts as spermatogonium. Similarly every cell in epithelium of ovary acts as Oogonium. Spermatogonium and oogonium grow by mitosis, each have somatic chromosome number. On reaching sexual maturity all spermagoniums produce primary spermatocytes. But in ovary at one time only one oogonium results primary oocyte which is much bigger as compared to primary spermatocyte. First meiosis occurs in primary spermatocyte and primary oocyte. Due to this action primary spermatocyte gives rise to two secondary spermatocyte.

Incase of primary oocyte there is uneven division resulting one big secondary oocyte and one small polocyte or polar body.

Second meiosis occurs in secondary sepermatocyte and secondary oocyte. Secondary spermatocytes result four spermatids. Secondary oocyte gives rise to ootid and polocyte. Each spermatid undergoes changes then it becomes sperm.

(a) Nucleus combines with some cytoplasm and forms head.

(b) Golgi bodies accumulate in anterior tip and form acrosome which help in penetration of ovum.

(c) Mitochondria accumulate in middle piece which gives strength to sperm

(d) Remaining cytoplasm takes the shape of flagellum or tail which helps in movement of sperm.

In contrast to spermatids ootid straight way develops in ovum. Almost full cytoplasm of primary oocyte comes in ovum. Polycyte disappear.

Number of chromosomes is only half (n) in sperm and ovum. Differences in spermatogenesis and oogenesis are mentioned below:

**Differences in spermatogenesis and oogenesis:**

| Spermatogenesis | Oogenesis |
|---|---|
| 1. Several spermatogonia divide and forms sperm | 1. At one time only one oogonium divides to from ovum (egg.) |
| 2. One spermatogo-nium produces four sperms. | 2. One oogonium produces only one ovum (egg). |
| 3. Sperm is minute. | 3. Ovum (egg) is quite bigger than sperm. |

Chapter 6

# Laws of Inheritance

To understand genetics properly, we have to understand the laws of inheritance.These laws are based on breeding experiment on pea done by the famous scientist John Gregor Mendel (native of Austria) who lived from 1822 to 1884. By this time it was clearly understood that transfer of characters from one generation to other generation can occur only through reproductive cells (gametes). One more thing was clearly understood that characters achieved by environment or artificial means can not be transferred from one generation to other. Due to these facts John Gregor Mendel and other scientists have expressed that phenotypic appearance of any organism depends on its genetic constitution (genotype). One thing more, change in phenotype does not have any effect on its genetic structure

Based on above mentioned facts John Gregor Mendel and other scientists did several experiments on plants and animals. Before John Gregor Mendel several scientists have done experiments but were unsuccessful because of adopting faulty methodology. Therefore seeing all this John Gregor Mendel very carefully adopted the right method and the material. Those precautions are detailed below.

1. The organism selected for the experiment should be homozygous. If it is not homozygous then after several generations of selfing it should be made homozygous.
2. For crossing only those parents should be selected who possess contrasting characters.
3. At one time only one character should be investigated.
4. For the experiment the organism should be available in plenty and it should have short life-cycle.
5. Record of observations should be clear and complete.

John Gregor Mendel discovered the principles of genetics in 1867 but it came to light after a long period. Later on De Veries in Holland, Correns in Germany and Tschermark in Austria have worked on this aspect, then in 1900 they have brought out the discovery of John Gregor mendel .

**Material and method of John Gregor Mendel:**

Keeping in mind all the above mentioned precautions John Gergor Mendel preferred pea (*Pisum sativum* ) for his research work . Pea has all the desired attributes because:

1. Varieties of peas have contrasting characters. Following 7 characters were studied by John Gregor Mendel.

| | Character | Details of character |
|---|---|---|
| 1. | Seed shape..... | Smooth & Wrinkle |
| 2. | Cotyledon colour..... | Yellow & Green |
| 3. | Seed coat colour ..... | Coloured & White |
| 4. | Pod type ..... | Hard & Soft |
| 5. | Pod colour ..... | Yellow & Green |
| 6. | Flower position..... | Axillary & Terminal |
| 7. | Stature ..... | Tall & dwarf |

On intensive studies of these characters Gregor Mendel has observed that characters written first in column 3 are dominant over characters written later in same column. Various experiments of John Gregor Mendel have revealed some important phenomenon which became well known as principles of heredity. These principles are followings.

1. Law of dominance.
2. Law of segregation.
3. Law of independent assortment. These laws are discussed below:

**Law of dominance:**

On performance of breeding experiments on peas and other organisms John Gregor Mendel has observed that in F1 offspring characters of only one parent appear. On Studies of contrasting characters these observations were confirmed. Therefore the law of dominance was put forth. When homozygous chromosomes having contrasting characters are crossed then in the F1 hybrid the appearing character is called dominant and the character not appearing in F1 hybrid is known as recessive. Based on results of breeding experiments on seven contrasting characters of pea Gregor Mendel has listed dominant and recessive characters as given below.

| Dominant | Recessive |
|---|---|
| 1. Smoothness of seed | Wrinkleness of seed |
| 2. Coloured seed coat | White seed coat |
| 3. Yellow cotyledon | Green cotyledon |
| 4. Hard pods | Soft pods |
| 5. Green pods | Yellow pods |
| 6. Axillary position of flowers | Terminal position of flowers |
| 7. Tall plants | Short plants. |

After proving the law of dominance in monohybrid crosses Gregor Mendel did several dihybrid crosses and again found the truthness of law of dominance. This can be explained by following example.

**Examples to explain Law of Dominance:**

1. **Monohybrid crosses (Cross between one pair of contrasting character):** Homozygous purple flowered pea plants were crossed with homozygous white flowered pea plants (pollen from one plants was transferred on stigma of other plant). The seeds obtained were sown & cultivated. These plants yielded pink flowers. So the pink character was expressed as dominant and white character was expressed as recessive (Fig-6).
2. Another example to prove law of dominance is a monohybrid cross between white cow and red cow. The F1 generation has only roan colour showing dominant red over white colour rat. (Fig- 7)

**Cytological explanation of the law of dominance:**

It has been clear that in every cell (Somatic or Gonad) there are homologous chromosomes in the nucleus, every chromosome has numerous genes. At reproductive phase one of the chromosomes from pairs gets transfered to gametes. The process is explained below.

In black rat the genes responsible for black colour in the homologous chromosomes can be designated as BB In Brown rat in the homologues chromosome the genes responsible for brown colour can be designated as bb At reproductive phase from BB by meiosis one of the B comes to sperm. In the same way from bb by meiosis one of the b comes to ovum (egg) of the rat, at the time of crossing the sperm with B unite & fertilize with ovum having b and forms the zygote Bb.

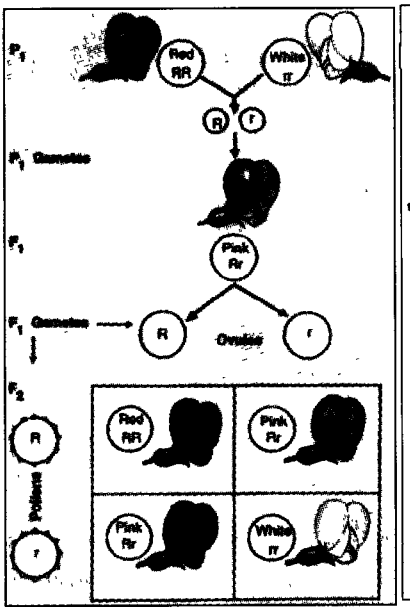



**Fig.6: Monohybrid cross between pea flowers showing dominance of character in F1.**

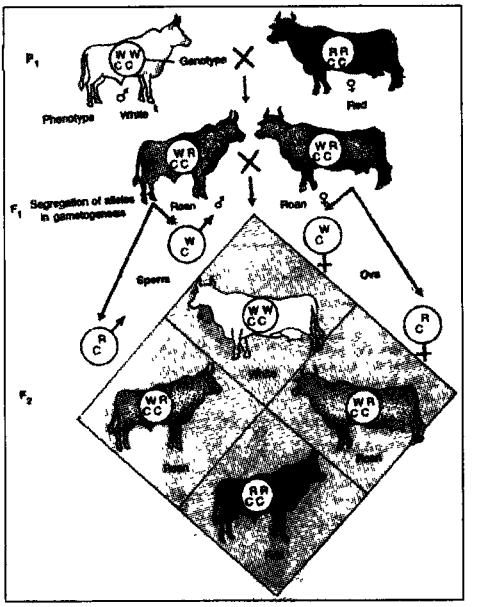



**Fig. 7: Monohybrid cross between white cow and red cow showing Law of dominance in F1.**

Because black is dominant therefore B express the black colour though b coexist.

**Incomplete dominance:**

For testing the law of dominance several scientists have done many experiments on several plants and animals. They observed that law of dominance is not always true. In some case dominance is incomplete. The result is that characters of F1 generation do not match with any parent (Fig-8). Examples of such phenomenon are found in 4 o'clock (*Mirabilis jalapa*), snap dragon and australian fawl.

In case of 4 O'clock plant (*Mirabilis jalapa*) when red flower type was crossed with white flower type then in the F1 hybrids neither red nor white flowers were present. Instead all the plants expressed light red flowers only. When light red flowered plants were selfed and F2 generation was raised then the F2 generation have revealed three colours - red, light red and white in the ratio of 1: 2: 1. Where as, as per the law of dominance only red and white flowers should have

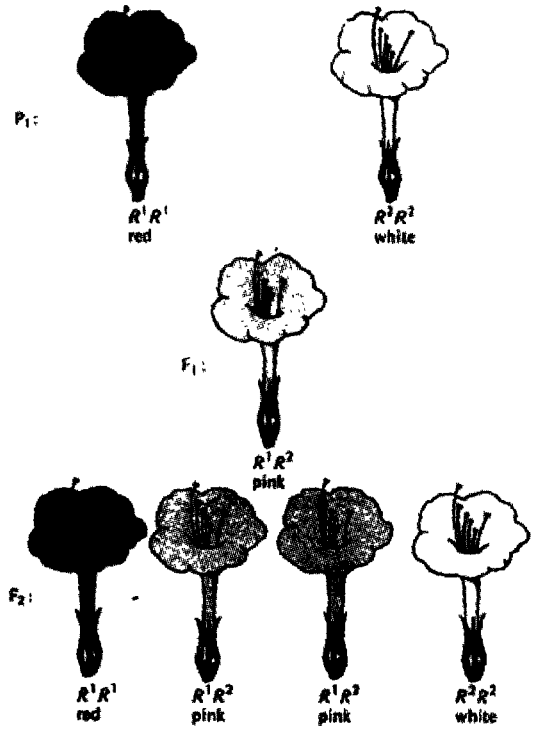



**Fig.8: Incomplete dominance in 4 O'clock plant (*Mirabilis jalapa*)**

appeared in ratio of 3:1. Therefore the above mentioned phenomenon was called incomplete dominance.

**Law of Segregation:**

John Gregor Mendel has selfed F1 generation and rose F2 generation. He observed that most of the F2 generation expressed the character of one dominant parent or the character expressed by F1 generation and the character of one recessive parent. In the F2 generation these characters always expressed in ratio of 3:1 i.e. 3 dominant and 1 recessive. Based on these observations Gregor Mendel has put forth the law of segregation. It is explained below:

When two alleles of contrasting characters (one is dominant and the other recessive) are brought together by hybridization in F1 generation then those contrasting alleles do not blend, interact or affect each other. When F1 forms gametes then these alleles (characters) separate freely and go freely in gametes. Afterwards

they reach freely in next generation. This law has been expressed as law of segregation. In one gamete allele for either pink colour or for white colour can go. Both these characters can not go in same gamete.

**Explanation for law of segregation:**

1. Law of segregation can be understood well by hybridization in pea (*Pisum sativum* ). Homologous pink flowered variety was crossed with homologous white flowered variety. In the F1 all the plants expressed pink colour, showing it is a dominant character. When the F1 was selfed and raised to F2 generation then F2 generation flowered in the ratio of 3 purple : 1 white according to law of segregation (Fig-9)

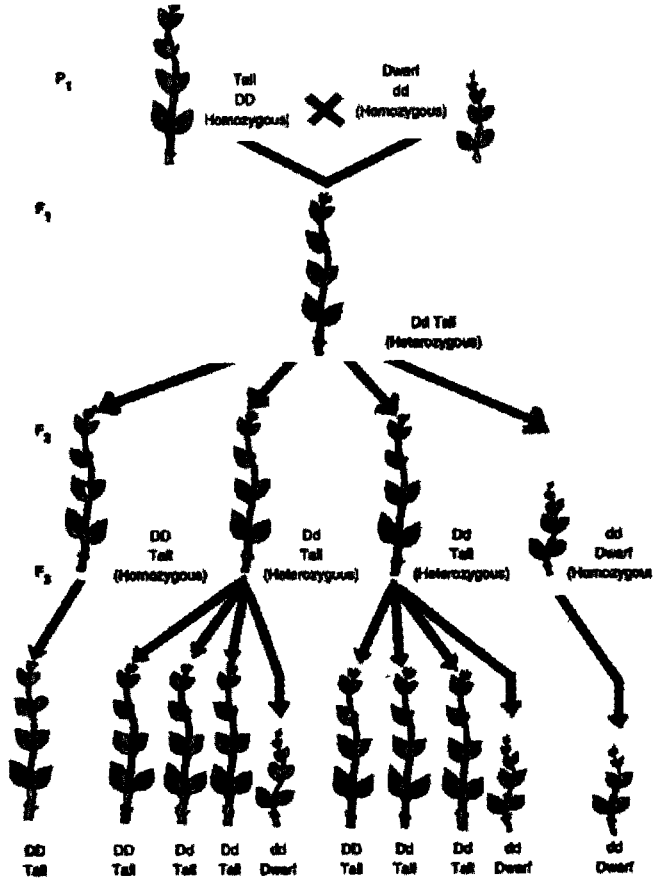



**Fig.9: Hybridization between pink and white flower pea showing the law of segregation in F1 and F2 generation.**

**Law of independent assortment:**

On achieving success in experiments on monohybrid crosses John Gregor Mendel experimented on dihybrid crosses i.e. on two contrasting characters. In case of pea (*Pisum sativum*) out of seven contrasting pairs he chosen two contrasting homozygous characters, for example round & yellow and wrinkled & yellow seeds. On hybridization of these two contrasting characters as expected Gregor Mendel in F1 found all hybrid plants bearing round and yellow seeds only because these two are dominant characters. When this F1 generation was self crossed and F2 was produced then the phenotypic ratio was not 3:1 but it was 9:3:3:1 - seeds were of four types. Gregor Mendel observed that out of four types two types were totally new types. Based on these facts he has formulated a new law - the law of independent assortment.

Mendel explained that various characters are totally free from each other. The character form of seed is totally free from colour of seed. Any form can be with any colour. Any colour can be with any form. Segregation at F2 therefore is fully free.

The law of independent assortment can be explained cytologicaly. Two or more than two contrasting contractors are totally free from each other because in every cell there are separate pairs of chromosomes. In every chromosome there are several genes (each gene is responsible for one character) each pair of chromosome is totally free. Therefore at the time of gamete formation during meiosis every pair of chromosome is totally free and during metaphase-1 get attached to spindle as per their wish. If suppose there are two pairs of heterozygous chromosomes then at meiosis those can get arranged in four types on the spindle. If there are three pairs of heterozygous chromosomes then at meiosis those can get arranged in eight types on the spindle (Fig-10)

Other example with cytological explanation of law of independent assortment

Law of independent assortment can be explained by experiment on pea having two contrasting characters. Suppose the characters are smoothness & yellow seeds and wrinklness & green seeds. Smoothness & yellow is dominant over wrinklness & green. For smoothness the responsible genes are SS. For yellow seeds the responsible genes are YY. Genes responsible for wrinkleless are ss and genes responsible for green seeds are yy (Fig- 11).

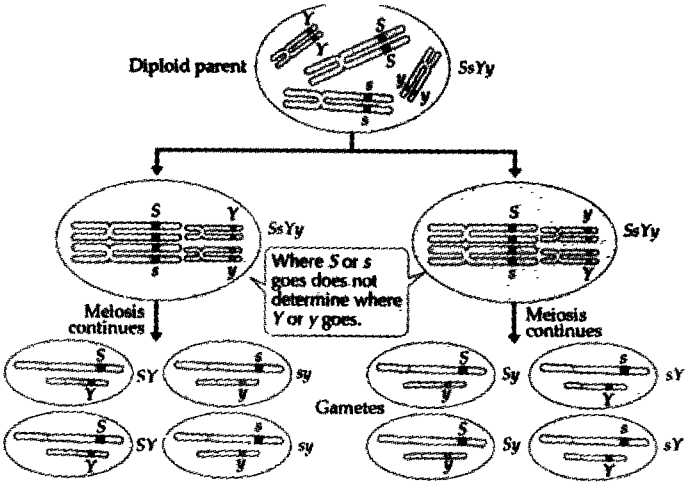



**Fig.10: Process of assortment of chromatids during gamete formation.**

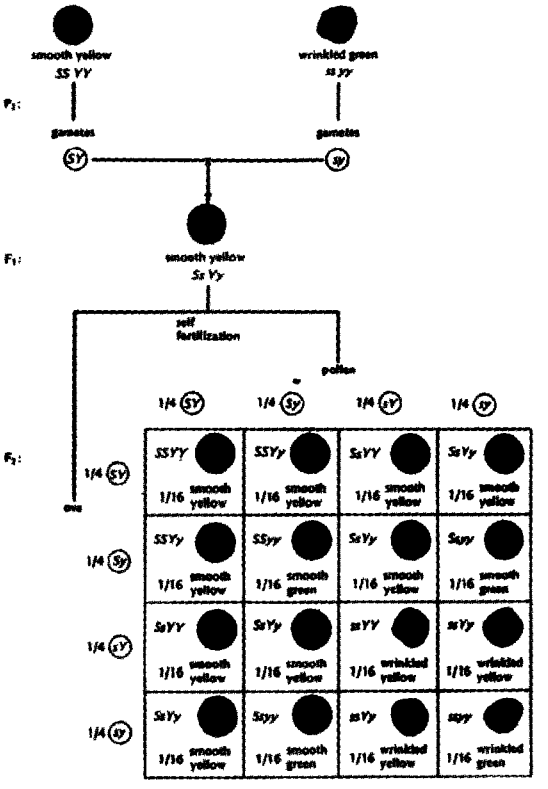



**Fig. 11: Dihybrid cross (seed shape and colour) of pea seeds showing impendent assortment.**

If pea plant with SSYY genome is crossed with ssyy genome, then in F1 generation all the plants will have SsYy genome Therefore their seeds will be round and yellow. The reason was that by meiosis in the plant with SSYY genome SY reached in one gamete and SY reached to other gamete. Similarly in the plant having ssyy genotype by meiosis sy went to one gamete and sy went to other gamete. For fertilization any SY gamete can unite with any sy gamete. Therefore SsYy genomed heterozygous F1 is produced whose seeds are round and yellow. In the F1 during reproduction stage in male and female gametes four type of genetic combination may take place. First possibility is that S combines with Y and form SY. Second possibility is that S combines with y and form Sy . Third possibility is s combines with Y and form sY. Fourth possibility is that s combines with y and form the gamete with sy genetic combination. Similar genetic combination will happen in female gametes.

Therefore if the hybrid plant with SsYy genome is self fertilized then pollen of four kinds are produced -SY,Sy,sY and sy. Similarly ovules of four types are produced i.e.-SY Sy, sY and sy. These pollen and ovules can combine in sixteen ways. These can be mentioned below:

1. SSYY (smooth & yellow)

2. SsYy, SsYY, SsYy, SSYy,RrYy, SsYY, SsYy, SsYy (all smooth & yellow) 9

3. SS yy (smooth & green)

4. Ssyy, Ssyy (all smooth & green) 3

5. ssyy (wrinkled & green) 1

6. ssYY (wrinkled & yellow)

7. ssYy, ssYy (wrinkled & yellow) 3

In this way 9 smooth & yellow seeded, 3 smooth & green seeded, 3 wrinkled & yellow seeded and 1 wrinkled & green seeded pea plant were obtained. It is the law of independent assortment.

**Study of dihybrid cross by checkerboard**

Above explained dihybrid cross can be also understood very well by checker board (Fig-12.) On the horizontal side various types of male gametes are written. On vertical side various types of female gamete are written. Afterwards every male gamete is fertilized with every female gamete. The results of fertilization are written in the corresponding squares. All the squares are filled up, and then the phenotypes are separated based on following principles:

1. Dominant gene is written in capital letter. Wherever it is presented (double or single) it expresses phenotypically.
2. Recessive gene is expressed in small letter. This gene expresses phenotypically when present in double. For example in the genome Rryy letter y mean green seed, But the seed is green only if there are two y genes. In other words in case of Yy the letter y is unable to expresses itself because of the presence of only one y gene.

| | RY | Ry | Ry | Ry |
|---|---|---|---|---|
| RY | RRYY | RRYy | RrYY | RryY |
| Ry | RrYy | Rryy | Rr Yy | Rryy |
| rY | RrYY | RrYy | Rr YY | RrYy |
| ry | RrYy | Rryy | rrYy | rryy |

RRYY (round & yellow)

RRYy, RrYy, RrYy, RRyY, RryY, RryY. RrYy, RryY, (round & yellow)9

Rryy, Rryy, Rryy (round & green) 3

RrYy, rryY, rryY (wrinkled & Yellow) 3

rrYY ( wrinkled & green ) 1

Phenotype ratio 9:3:3:1

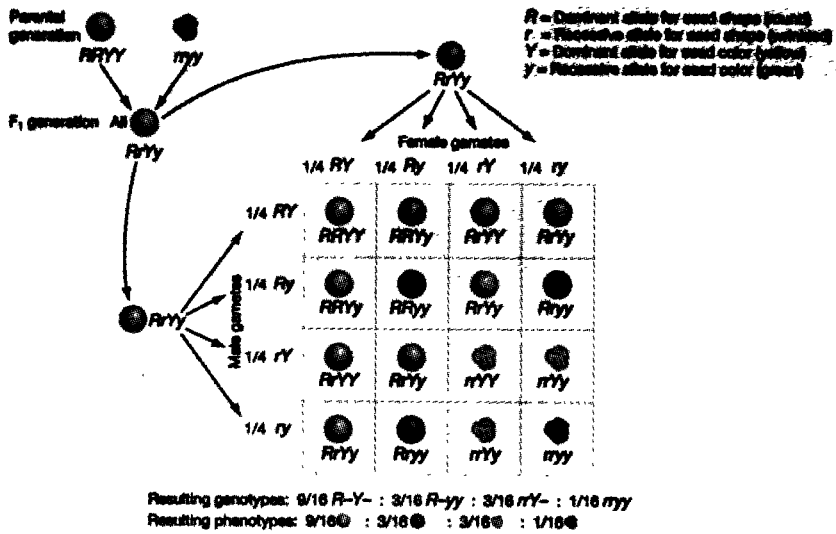



**Fig. 12: Dihybrid cross (seed shape and colour) of pea seeds showing impendent assortment.**

**Exceptions to the Law of independent assortment :**

After John Gregor Mendel several scientists have observed that in some organisms two or more genes pairs do not show the law of independent assortment. Among such scientist some important ones are Bateson, Punnet and Shull. These and others have recorded following observations.

**Exception 1 (Duplicate factor):**

In shephered purse plants when triangular seeded and round seeded varieties were crossed in F1 generation all plants revealed triangular seeds. This F1 was self-crossed to produce F2 generation. In F2 generation triangular and round seeded plants were recorded in the ratio of 15:1. Scientists have explained that this ratio was because of duplicate factor ( gene) . Duplicate factor can be defined as these are such factors (genes ) which express parallel affect. It can be understood by following cross.

Triangular seed x Round seed

TT RR ttrr

Tt Rr – – – – – F1

Triangular seeds.

Selfed

9TR 3Tr 3tR 1tr – F2

| Triangular Seed | Triangular Seed | Triangular Seed | Round Seed |
|---|---|---|---|

It can be also written as:

Triangular seed=9+3+3=15

Round seed= 1

Hence phenotypic ratio =15:1

Genotypic ratio =9:3:3:1

**Exception 2 (Inhibitory factor):**

In poultry when coloured feathers were crossed with white feathered poultry, in the F1 generation all poultry revealed white feathers. After selfing the F1 generation the F2 generation was produced. F2 revealed 13 white feathered poultry and 3 coloured feather poultry. So the ratio was 13: 3 . This ratio was the result of inhibitory factor (gene).

Inhibitory factor ( gene) is that gene which does not express its effects but inhibits the affect of other gene. This phenomenon has been expressed by following experiment.

White feathered fowl x coloured feather fowl.

WWcc wwCC

WwCc

White feathered fowls

Selfed

| 9WC | 3Wc | 3wC | 1wc |
|---|---|---|---|
| white feather | white feathers | coloured feathers | white fethers |

It can be also written as:

white feathers : 9+3+1=13

coloured feathers: 3

Hence, phenotypic ratio=13:3

Genotypic ratio=9:3:3:1

In white feathered fowls there is W gene but this gene independently does not produce any white colour in feathers. Moreover this W gene (factor) inhibits the expression of coloured feathers by the gene C.

**Exception 3 (complimentary factor)**

When two white flowered varieties of pea were crossed then in the F1 generation all the plants had coloured flowers. On selfing the F1 generation F2 was produced . In the F2 9 coloured flower plants and 7 white flower plants were obtained. This change was the result of a new phenomenon - complimentary factor (gene). It is that gene which singly express one character but when exits together then a new character is developed. It will be easily understood by following cross.

White flowered pea x White flowered pea .

AAbb aaBB

Aa Bb----------F2

Coloured flowered pea

Selfing

| 9 AB | 3Ab | 3aB | 1ab |
|---|---|---|---|
| Red flowered | White flowered | white flowered | white Flowered |

Red flowered pea =9

White flowered pea 3+3+1 =7

Therefore phenotipic ratio = 9:7

Genotipic ratio = 9:3:3:1

In this cross when A and B are present separately then on hybridization only white flowered pea is produced. But when A and B come together then it produces red flowered pea.

**Exception 4 (supplementary factor ) :**

In *Sorghum vulgare* there are two main types e.g.(1) blackish- purple glumes and (2) brown glumes. When blackish - purple glumes type is hybridized with brown glume type then in F1 generation plants the colour of glumes was quite different - it was reddish purple. On Self-fertilization of F1 plants F2 was produced. In F2 plant 9 had reddish purple glumes, 3 had blackish - purple glumes , and 4 had brown purple glumes. Hence the phenotypic ratio was 9:3:4 This ratio happened because of supplementry factor (gene).It is those genes (factors) which separately express different characters but when they are together then they express new character . This can also be understood by following cross.

| | | |
|---|---|---|
| Blakish purple gulme | x | Brown glume |
| Sorglum vulgare | | Sorghum vulgare |
| AAbb | | aaBB |

AaBb

Reddish brown

Self fertilization

---

| | | | |
|---|---|---|---|
| 9AB | 3Ab | 3aB | 1ab Reddish |
| purple | Blakish purple | Brown | Brown |

It can be mentioned like following:

Reddish purple = 9

Blakish purple = 3

Brown = 4

Hence Phenotypic ratio = 9:3:4

Genotypic ratio = 9:3:3:1

As mentioned in the cross in case of brown glumes there is a gene B which does not has any expression but has some connection with brown colour. But when this B unite with other gene A (which express blackish purple color) then a new color reddish purple is produced. Hence B is supplement to A. Therefore on uniting B with A only reddish purple color develop.

**Exception 5 (Polymorphism factor or additive gene action):**

In some varieties of wheat (*Triticum aesativum*) colour of seed is deep red and in some varieties it is whitish. When deep red wheat is hybridized with whitish wheat then the F1 generation expressed deep red coloured wheat. F1 was self fertilized to produce F2 generation. F2 revealed 9 deep red, 6 light red and 1 white seeded plants. So the phenotypic ratio was 9:6:1. This change happened due to polymorphism factor ( gene), inotherwords it is additive gene action. It can be understood by following cross:

Deep red seeded wheat x Whitish seeded wheat

AABB aaBB

AaBb

Deep red seeded wheat

Self fertilization

| 9AB | 3Ab | 3aB | 1ab |
|---|---|---|---|
| Deep red | Light red | Light red | Whitish |

It can also be written as:

Deep red = 9

Light red = 6

Whitish = 1

Hence, Phenotypic ratio = 9:6:1

Genotypic ratio - 9:3:3:1

In this example genes A and B separately produce light colored seed. But when come together they intensify the wheat color of deep red, such genes are called polymorphism gene (factor) or additive gene (factor)

**Exception 6 (Epistasis)**

In *Sorghum vulgare* ( Jowar) when red nonpearly grained variety was hybridized with white pearly varicty then in the F1 generation all the plants expressed red non pearly grain. F1 were self fertilized to produce F2. It was found that twelve plants expressed red non pearly grains, three plants expressed white pearly seeds and one plant expressed white chalky (without pearly character). This behaviour happened because of epistasis gene (factor). Masking effect is called epistasis . So the gene (factor) which masks the effect of other gene is called epistasic gene (factor), and that gene whose expression is masked is called hypostatic factor (gene). This phenomenon can be understood by the following cross.

Red nonpearly seeded jowar x White perly seeded jowar

AAbb aaBB

AaBb

Red nonpearly seeded jowar

Self fertilised

---

| 9AB | 3Ab | 3aB | 1ab |
|---|---|---|---|
| Red nonpearly | Red nonpearly | White pearly | White chalky |

It can also be written as:

Red non pearly = 12

White pearly = 3

White chalky = 1

Hence, Phenotypic ratio = 12:3:1

Genotypic ratio = 9:3:3:1

In this cross in the red grain variety there is a gene for pearliness but it has been masked by A gene (epistatic gene or factor).

**Causes of these exceptions.**

Experiments of later on have suggested that the causes of these exception are mutation, crossing over and gene interactions. These phenomenons are explained lateron.

**Method to know genotype of an organism**

Whether a character is homozygous or heterozygous, to know it John

Gregor Mendel has explained a method - known as test cross. Its details are given below.

**Test Cross:**

The organism to be examined is crossed with homozygous recessive parent. By study of F1 the genotype of the organism is found out as mentioned below:

1. If in F1 all the organisms are showing recessive character then the organism is homozygous recessive.
2. If in F1 all the organisms are showing dominant character then the organism is homozygous dominant.
3. If in F1 some organisms exhibit dominant character and some organisms exhibit recessive character then the organism has heterozygous genotype.

**Explanation for test cross:**

The above mentioned facts are based on a truth that heterozygous monohybrids produce two types of gametes. Whereas homozygous monohybrid produced only one kind of gamete. Therefore on crossing

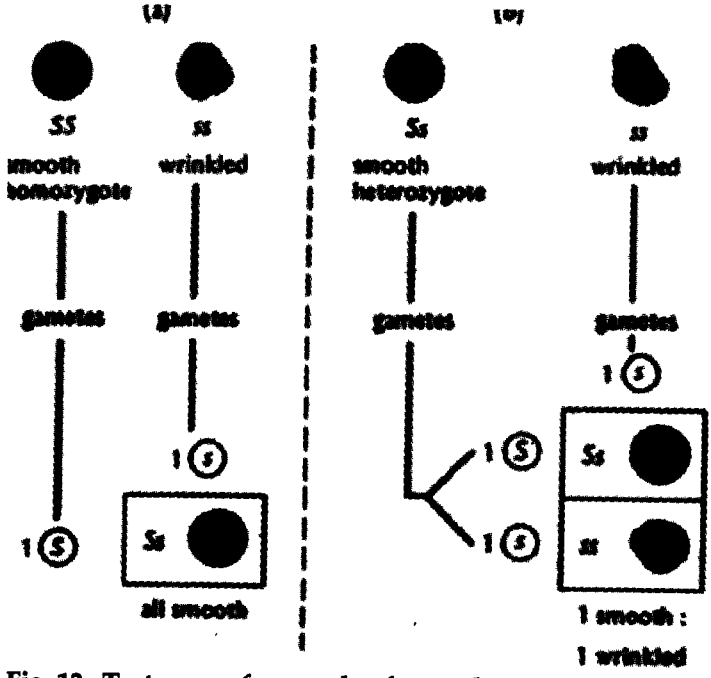



**Fig. 13: Test cross of a sample of smooth & wrinkled pea seeds**

of heterozygous recessive with homozygous recessive parent (test cross) two types of offsprings are produced. Whereas homozygous dominant when crossed with homozygous recessive only one type of offspring is produced.

To explain above the example of summer squash plant be taken. In two varieties white & disc shaped fruits and yellow & spherical fruits are produced. We have to find out the genotype of 3 samples of white & disc fruits. Plants produced from these seeds were crossed with yellow & spherical fruited plants (because it is homozygous recessive).

(A) If one sample is crossed with yellow & spherical and the F1 generation reveals white & disc fruits, then the sample is homozygous dominant ( Fig-13).

Assume smooth seed are controlled by the gene (factor) S and wrinkled shape is controlled by the gene s.

**Back cross**

Back cross is that breeding experiment where F1 generation is crossed with homozygous parent. If the F1 is crossed with homozygous recessive then it is called test cross and also back cross. But if the F1 is crossed with only dominant homozygous parent then it is called only back cross.

On crossing F1 with homozygous dominant parent all the resultant plants resemble to dominant parent but their genotypic ratio is 3 heterozygous dominant and I homozygous dominant

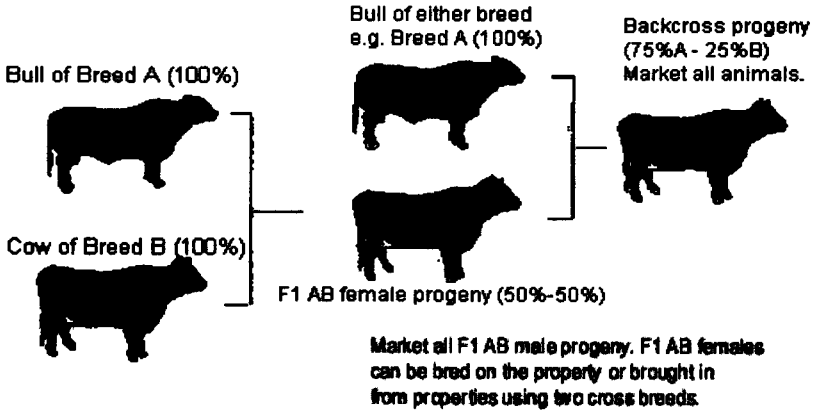



**Fig.14: Back cross.**

If F1 is back crossed to homozygous recessive parent then half of the resultant population has dominant character and other half population has recessive characters (Fig-14).

**Trihybrid Cross:**

If a parent having three dominant homozygous parents is hybridized with the parent having three recessive homozygous then such a hybridisation (cross) is called trihybrid cross and such a hybrid is called trihybrid. This difficult cross can be well understood by Gregor Mendels law of independent assortment.

Yellow, round seeded & red flowered plants of pea was crossed with green, wrinkled seeded & red flowered pea. Genotypes of these parents are RRYYCC and rryycc . From these parents the gametes will be RYC and ryc. On hybridization the F1 hybrid will have the genotype RrYyCc and the phenotype round, yellow seeds with red flowers. This F1 will form eight types of male and female gametes such as RYC, RYc, RyC, Ryc, rYC, rYc, ryC & ryc . Such male and female gametes will fertilize in 64 combinations. Phenotypic ratio of F2 will be 27 round yellow red ; 9 round yellow white ; 9 round green red ; 9 wrinkled yellow red ; 3 wrinkled green red ; 3 wrinkled yellow white ; 3 round green white & 1 wrinkled, green white. So there are 8 types of phenotypes. Genotypes are of 27 types.

**Polyhybrid cross:**

A cross between parents having more than three contrasting characters is known as polyhybrid cross. In polyhybrid cross the number of genotype and phenotype become quite high. Following table gives number of contrasting characters (genes), number of genotypes formed in F1, number of genotypes formed in F1, number of phenotypes in F2 and possible number of combinations in F2 are given.

**Table: Relation between number of contrasting characters, types of gametes formed for F1, number of phenotypes in F1 and number of possible combination in F2**

| No.of contrasting characters | Type of gametes for F1 | Type of phenotypes in F1 | Possible combination in F2 |
|---|---|---|---|
| 1 | 2 | 2 | 4 |
| 2 | 4 | 4 | 16 |
| 3 | 8 | 8 | 64 |
| 4 | 16 | 16 | 256 |
| 5 | 32 | 32 | 1024 |
| n | 2n | 2n | 4n |

**Inheritable characters in human beings:**

In human beings following characters are inherited:

1. **Albinism:** Whitening ( fully or partial) on skin, hairs or eyes is a recessive character.
2. **Blue colour and brown colour of eye balls:** Blue colour of eye ball is recessive character. brown colour is dominant character.
3. **Inefficiency to taste thiocarbamide:** Thiocarbamide taste bitter but because of recessiveness human being is unable to taste it.
4. **Baldness:** Too much automatic removal of hair from head is a dominant character.
5. **Skin spotting:** Premature whitening of hairs on front side of head is a dominant character.

**Numericals in genetics:**

**Exercise-1**

**Question 1.** In sweetpea the purple flower colour results as an interaction of two genes C and P, either of which alone produces only white flower. What will be the flower colour in hybrids obtained by following cross:

A. CcPp x ccPp
B. CcPp x CcPP
C. ccPp x CCpp
D. Ccpp x ccPp
E. CCPP x CcPp
F. CcPp x CcPp

**Answer:**

**Summary**

According to the question:

C=Independently produced white flowers.

P=Independently produced white flowers.

CP=On interaction produces purple flowers.

C=Produces white flowers.

P=Produces white flowers.

(a) CcPp x ccPp
purple white

In the first parent C and P on interaction produces purple flowers. In the second parent P is single so it produces white flowers.The first parent (CcPp) produces 4 types of gametes i.e. CP, Cp, cP, cp. The second parent (ccPp) produces only two types of gametes i.e. cP and cp. To form F1 generation these gametes will combine as mentioned in following checkerboard:

| | CP | Cp | cP | cp |
|---|---|---|---|---|
| cP | CcPP<br>+ | CcPp<br>+ | ccPp<br>o | ccPp<br>o |
| cp | CcPp<br>+ | Ccpp<br>- | ccPp<br>o | ccpp<br>= |

| | | | | |
|---|---|---|---|---|
| + | 3 CP | = Purple flowers | | : 3 purple flowers |
| - | 1 Cp | = White flowers | | |
| o | 3 cP | = White flowers | | |
| = | 1 cp | = White flowers | | : 5 white flowers |

So, the phenotypic ratio is 3:5 because of following reasons:

+ there are two dominant genes.

- there is one dominant gene.

= both genes are recessive.

(b) CcPp x CcPP

purple purple

First parent (CcPp) is purple because of interaction of two dominant genes. Same thing is true with the other parent. First parent will produce four types of gametes viz. CP,Cp,cP,cp. The other parent (CcPP) produce two types of gametes viz. CP and cP. To make F1 these gametes will combine as mentioned in following checkerboard:

| | CP | Cp | cP | cp |
|---|---|---|---|---|
| CP | CCPP<br>o | CCPp<br>o | CcPP<br>o | CcPp<br>o |
| cP | CcPP<br>o | CcPp<br>o | ccPP<br>x | ccPp<br>x |

| | | | |
|---|---|---|---|
| o | 6 | CP | = purple flowers |
| x | 2 | cP | = white flowers |

So phenotypic ratio is 6:2 or 3:1 because of following reasons:

o = two dominant genes.

x = it has one dominant gene.

(c) ccPp x CCpp

white white

Both the parents have white flowers because of presence of only one dominant gene. First parent (ccPp) produce only two types of gametes viz. cP and cp.Second parent ( CCpp) forms only one type of gamete viz' Cp. To form F1 these gametes will combine as mentioned below:

| | cP | cp |
|---|---|---|
| Cp | CcPp | Ccpp |
| o | | + |

o 1 cP = purple flowers

\+ 1 Cp = white flowers

Hence, the phenotypic ratio is 1:1 because of following reasons:

o = 2 dominant genes.

\+ = 1 dominant gene.

(d) Ccpp x ccPp

white white

Both parents have white flowers because of presence of only one dominant gene. The first parent (Ccpp) will produce two types of gametes viz. Cp and cp. The second parent (ccPp) will also form two types of gametes cP and cp. To form F1 these gametes will combine as demonstrated in following checkerboard:

| | Cp | cp |
|---|---|---|
| cP | CcPp | ccPp |
| o | + | |
| cp | Ccpp | ccpp |
| | = | - |

| | | | | |
|---|---|---|---|---|
| o | CP | = | purple flowered | = 1 |
| + | cP | = | white flowered | |
| = | cp | = | white flowered | |
| - | cp | = | white flowered | = 3 |

Hence, phenotypic ratio = 1:3 because of following reasons :

O = 2 dominant genes

+ = 1 dominant gene

= = 1 dominant gene

- = No gene is dominant

(e) CCPP x CcPp

purple purple

Both the parents have C and P two dominant genes therefore both have purple flowers. First parent (CCPP) forms only one type of gamete CP. Second parent forms four types of gametes i.e. CP, Cp, cP, cp. To form F1 these gametes will combine as demonstrated below:

| | CP | Cp | cP | cp |
|---|---|---|---|---|
| CP | CCPP<br>o | CCPp<br>o | CcPP<br>o | CcPp<br>o |

o = 4 CP = coloured flowers because in every case two dominant genes are present.

(f) CcPp x CcPp
purple purple

Both the parents have purple flowers because of presence of two dominant genes C and P. The first parent (CcPp) will form four types of gametes viz. CP, Cp, cP, cp. The other parent (CcPp) will also form four types of gametes viz. CP, Cp, cP, cp. To form F1 these gametes will combine as demonstrated in following checkerboard:

| | CP | Cp | cP | cp |
|---|---|---|---|---|
| CP | CCPP<br>o | CCPp<br>o | CcPP<br>o | CcPp<br>o |
| Cp | CCPp<br>o | CCpp<br>x | CcPp<br>o | Ccpp<br>x |
| cP | CcPP<br>o | CcPp<br>o | ccPP<br>- | ccPp<br>- |
| cp | CcPp<br>o | Ccpp<br>x | ccPp<br>- | ccpp<br>= |

In other words,

0 = 9CP = coloured flowers = 9

x = 3Cp = white flowers

- = 3 cP = white flowers

= = 1 cp = white flowers = 7

Hence the phenotypic ratio = 9:7 because of following reasons :

o = two dominant genes

x = one dominant gene

- = one dominant gene

= = all recessive genes

**Exercise 2**

In summer squash plant gene ( factor) W expresses white colour of fruits. It is also epistatic over Y factor which is responsible for yellow colour of fruits. When only recessive genes ( factors) are present (wy) the fruit colour is green. What will be the genotype and phenotype of the ofsprings ( progeny) of following crosses:

(i) wwYY x Wwyy

(ii) WwYy x WwYy

(iii) WwYy x wwyy

(iv) Wwyy x wwYy

(v) WWyy x wwYY

Answer:

Summary: As mentioned in the exercise -

W = Independently produces white. It is also epistatic over Y.

Y = In absence of W it produces yellow fruits.

WY = Produce white fruits.

wy = Produce green fruits.

(i) wwYY x Wwyy

yellow white

First parent ( wwYY) will form one type of gamete i.e. wY. The second parent ( Wwyy) will form two types of gametes i.e. Wy and wy. To form F1 these gametes will combine as demonstrated below:

| | Wy | wy |
|---|---|---|
| wY | WwYy | wwYy |
| | o | + |

Inother words,

o = 1 WY = white fruited

+ = 1 wwYy = yellow fruited

So, the phenotypic ratio = 1:1 because of following reasons:

o = W works as epistatic gene.

+ = Y is free to express yellow colour.

(ii) WwYy x WwYy

white fruits white fruits

First parent will form 4 types of gametes viz.WY,Wy, wY,wy. Similarly the second parent will also 4 types of gametes such as WY, Wy, wY,wy. To form F1 these gametes will fertilize as mentioned below:

| | WY | Wy | wY | wy |
|---|---|---|---|---|
| WY | WWYY | WWYy | WwYY | WwYy |
| | o | o | o | o |
| Wy | WWYy | WWyy | WwYy | Wwyy |
| | o | x | o | x |
| wY | WwYY | WwYy | wwYY | wwYy |
| | o | o | - | - |
| wy | WwYy | Wwyy | wwYy | wwyy |
| | o | x | - | = |

In other words, o = 9 WY = white fruited plants

x = 3 Wy = white fruited plants = 12

- = 3 wY = yellow fruited plants = 3

= = 1 wy = green fruited plants = 1

Hence, phenotypic ratio is 12:3:1 because of following reasons:

o = W is epistatic to Y

x = W is free to act

- = Y is free to act

= = wy both are recessive genes

(iii) WwYy x wwyy

white green

First parent ( WwYy ) forms four types of gametes viz. WY, Wy, wY,wy.Second parent ( wwyy ) will form only one type of gamete i.e.wy. To form F1 these gametes will fertilize as indicated below:

| | WY | Wy | wY | wy |
|---|---|---|---|---|
| wy | WwYy | Wwyy | wwYy | wwyy |
| | o | x | - | + |

In other words, o = 1 WY = white fruits

x = 1 Wy = white fruits = 2

- = 1 wY = yellow fruits = 1

+ =1 wy = green fruits = 1

Hence, phenotypic ratio = 2:1:1 because of following :

o = W is working as epistatic gene

x = W is free in action

- = Y is free in action

= = wy both are recessive genes

(iv) Wwyy x wwYy

white yellow

The first parent will form 2 types of gametes i.e. Wy and wy . The other parent will form two types of gametes wY and wy. TO form F1 these gametes will fertilize as mentioned below:

| | Wy | wy |
|---|---|---|
| wY | WwYy<br>o | wwYy<br>- |
| wy | Wwyy<br>+ | wwyy<br>x |

In other words, o = 1 WY = white fruit
+ = 1 Wy = white fruit = 2
- = 1 wY = yellow fruit = 1
x = 1 wy = green fruit = 1

So the phenotypic ratio is 2:1:1 because of following reasons:

O = W is acting as epistatic gene.

+ = W is free to act.

- = Y is free to act.

x = wy both genes are recessive.

(v) WWyy x wwYY
white yellow

First parent ( WWyy ) will form one type of gamete i.e. Wy. Other parent will form one type of gamete i.e. wY. To form F1 the gametes will fertilize as given below:

| | Wy |
|---|---|
| wY | WwYy |

Because in every case W is acting as epistatic over Y. Therefore all the F1 generation will express white fruits.

**Exercise 3**

A purple flowered variety in summersquash plant gives rise to 3/8 purple and 5/8 white in F1 when crossed with another white flower variety. Find out genotype of both the parents.

**Answer:**

Suppose A and B genes ( factors ) independently produce white fruits but when these genes come together then produce purple fruits due to gene interaction. In that case genotype of both the parents will be :

Purple parent = AaBb

White parent = Aabb or aa Bb

It can be proved by following checkerboard. Purple parent will form 4 gametes viz. AB, Ab, aB ,ab.White parent will form Ab, ab or aB, ab. Various gametes will combine in following ways to form F1.

| (a) | AB | Ab | aB | ab |
|---|---|---|---|---|
| Ab | AABb<br>o | AAbb<br>- | AaBb<br>o | A a b b<br>- |
| ab | AaBb<br>- + | Aabb<br>x | aaBb | aabb |
| o | | | | |

In other words, o = 3 AB = purple because both are dominant genes.

- = 3 Ab = white because A is free in action.

+ = 1 aB = white because B is free in action.

x = 1 ab = white because both genes are recessive.

So the phenotypic ratio is 5 white : 3 purple

| (b) | AB | Ab | aB | ab |
|---|---|---|---|---|
| AaBb | Aabb<br>o | aaBb<br>- | aabb<br>+ | ab<br>x |
| aB | AaBB<br>o | AaBb<br>o | aaBB<br>+ | aaBb<br>+ |

In other words, o = 3 AB = purple because both are dominant genes.

- = 1 Ab = white because A is alone.

+ = 3 aB = white because B is alone.

x = 1 ab = white both are recessive genes.

Therefore phenotypic ratio is 3 purple : 5 white.

Hence, genotype of purple = AaBb

Genotype of white = Aabb or aaBb

**Questions for practice**

(1) In following crosses, in which genotype of parents are given, what are the gametes produced by each parent and what will be the flower colour of the offsprings from each cross : Rr x RR, rr x Rr, RR x rr, Rr x Rr ?

(2) What will be the appearance of the offsprings of following crosses in which genotypes of parents are given:

(a) TTGgRr x ttGgrr

(b) TtGGRr x TtGgRr

(c) ttggRr x TtGgrr

(d) Ttggrr x TtGgRr

(3) If the homozygous dwarf, green and wrinkled pea plant is crossed with a homozygous tall, yellow and round one, what will be the appearance of the F1 ? What gametes does the F1 form ? What is the appearance of the offspring of a cross of the F1 with its dwarf green & wrinkled parent and with its tall , yellow and round parent?

(4) In snapdragons tall (T) is dominant to dwarf (t) and red flower (R) are incompletely dominant to white (r), the hybrid being pink. A pure tall white is crossed to a pure dwarf red. The F1 is self fertilized. Give the F2 phenotypic ratio.

(5) In 4 oclock plant ( *Mirabilis jalapa* ) a plant which is heterozygous for red flower (R) and white (r) is pink. What will be the flower colour of the F1 produced and in the F2?

(6) Purple flowered x white flowered gives 50 per cent purple and 50 per cent white. Give the genotypes of the parents.

(7) A green maize plant when selfed produces about 15/16 green and 1/16 white (lethal) seedlings . Find out the genotype of the plant and the mode of inheritance of the pigment.

(8) Wheat plant has 21 pairs of chromosomes. State the number of chromosomes present in the (a) stem tip (b) nucleus (c) pollen type nucleus (d) embryosac (e) seed embryo (f) endosperm.

(9) In fowl the dominant gene R gives rosecomb and the dominant gene P gives peacomb.When P and R are present together the comb form is walnut. The homozygous recessive of P and R produce single comb. Determine the comb form of the offspring of following crosses.

a. RrPp x RrPp
b. rrPP x RrPp
c. rrPp x RRPp
d. Rrpp x RrPp
e. Rrpp x Rrpp
f. rrPp x Rrpp

(10) Both parents of a blue eyed man are brown eyed. He marries a brown eyed women who had a brown eyed mother, a blue eyed father and a blue eyed brother. The man and women in question have a brown eyed child. Give the genotypes of (i) parents of man and women (ii) man and women (iii) their child.

Chapter 7

# Heterosis or Hybrid Vigor

It is a general observation that when dissimilar varieties of plants and animals are crossed then the F1 hybrid is more robust as compared to the parents (Fig-15). This quality of hybrid is called hybrid vigor or heterosis. It can express in several ways e.g. increased size, resistance to diseases or pests, easy propagation by vegetative means, more yield etc.

First report of hybrid vigor was available from Kolereuter in 1763 while experimenting on tobacco. Afterwards several scientists reported this phenomina in several plants and animals. A good example in animal is mule which is a hybrid between crossing of ass with mare.

A special feature of hybrid vigor is that it get reduced in F2 and further reduced in future generations.

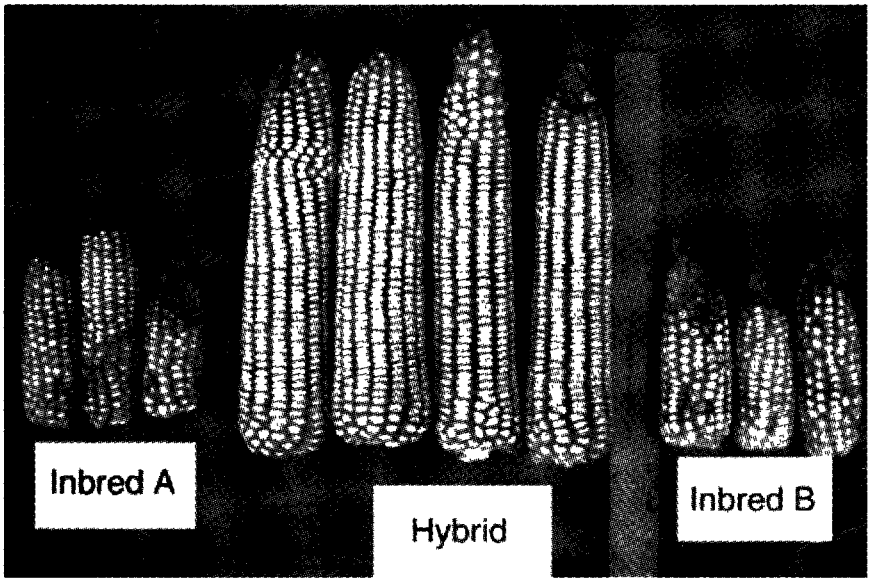



**Fig. 15: Hybrid vigour in maize. Outer plants are parents of the middle plants.**

Occurrence of hybrid vigour ( heterosis) in plants and animals

Hybrid vigour ( heterosis ) has been reported in several plants and animals. Such plants are maize, jowar, barly, pearl millet, cucumber, onion, brinjal, tomato, forage crops, sunflower, sugarcane, coconut, tobacco, penicillium fungus etc. Examples in animals are mule, rats, cattles, silkworms etc.

**Causes of heterosis (hybrid vigour)**

Several scientists from time to time have proposed several causes. But none has been found to be fully satisfactory. Various causes have been explained as below:

**(1) Heterozygosity induces physiological stimulus:**

This idea was proposed by East and Hays in 1912. They expressed that hybrid vigour is due to heterozygosity in hybrids. Union of dissimilar genes induces physiological stimulations which lead to hybrid vigour.

**(2) Greater initial capital hypothesis:**

This hypothesis was proposed by Ash in 1930. According to him in such hybrids there is increase in embryo size and capacity which leads to hybrid vigour.

**(3) Dominant gene (factor) hypothesis:**

The scientist Bruce proposed this hypothesis.. According to him number of dominant genes is more in hybrids compared to their parents. This fact results hybrid vigour.

**(4) Linked favorable dominant gene hypothesis:**

This hypothesis was proposed by Jones in 1917. According to him from the parents growth favorable linked dominant genes come in the hybrid which causes hybrid vigor.

**(5) Cytoplasmic nuclear hypothesis:**

This hypothesis was proposed by the scientists Michaelis, Shull and Lewis. They expressed that hybrid vigor is an induction due to interaction between nucleus and cytoplasm.

**Effects of heterosis**

**A. Morphological effects:**

(1) **Root:** Increase in diameter, length and number of branches.

(2) **Stem:** Increase in thickness, wrinkleless, length and number of branches.

(3) **Leaves:** Increase in number, size and intensity of green color.

(4) **Flowers:** Increase in number, size and beauty.

(5) **Fruit:** Increase in size and quality.

**B. Physiological effects:**

1) Increase in germination.

2) Increase in cell division and cell size.

3) Early flowering.

4) Resistance to drought, diseases and pests.

5) Increased life.

6) In animals increase in tolerance and working capacity.

**Role of heterosis in increasing production:**

Productivity is the power of producing or multiplying which is the result of several biological and environmental factors accumulated as effective stable system. In agricultural context productivity is yield. Yield is a complex parameter reflecting the influence of a number of systems on the final product. The influences can be identified as seed, soil, water, climate, management etc. These can further be subdivided into several components. For example the seed factor can be a good high yielding genotype or a local nondescriptive genotype. Among high yielding genotype it can be an improved variety or synthetic or composit or a F1 hybrid seed which is expected to be heterozygous for most of loci. Higher production than parents is known as heterosis

Commercial F1 hybrids available in India are limited to maize , sorghum, bajara, cotton etc in which substantial output through heterosis is being utilised for increasing food and fibre production. In next order comes the crops like castor, arhar, melons, mango etc. where heterosis was identified and exploited on limited scale.

In crops like rice, wheat & pulses substantial success was achieved through conventional breeding, polyploidy, mutation breeding etc. in developing superior genotypes. Extensive use of dwarfing genes (discovered by Vogal in USA & Borlaug in Mexico) particularly in

wheat has brought out redistribution of dry matter for higher harvest index and resistance to lodging under high fertility conditions.Indian wheat varieties have harvest index of upto 49 per cent. In case of rice the dwarfing gene discovered by Taiwan scientists lead to restructuring of rice types like IR 8 to IR 26 Apart from these Indian scientists have developed several varieties for high productivity that are suitable to different regions. Sorghum, maize, bajara etc. occupy a large part of cultivated area. Those are main food crops of dry land farmers. The basic scientific approach for improvement of these crops has been heterosis breeding. India's high yielding hybrids of sorghum and pearlmillet are considered one of the best in world.

New arhar varieties of less than 5 months duration can be grown in rotation with wheat crop. The short duration varieties of moong maturing in 60 -70 days can be planted during spring and summer months when normaly the land is kept fallow.

**Achievements by heterosis in various crops:**

**(a) Sorghum hybrid varieties:** CSH-1 to CSH-10

**(b) Pearlmillet hybrid varieties:** HB-1 to HB-6

BJ-104 and BK-559 are resistant to downy mildeu

(c) **Maize hybrid varieties:** DHM-101 & 103 and Histarch.

(d) **Cotton hybrid varieties:** H-4, Varalaxmi, DCH-32, JKH-1 etc.

**(e) Castor hybrid varieties:** CCH-1, CCH-3 etc.

**(f) Sunflower hybrid varieties:** BSH-1,

**(g) Groundnut hybrid varieties:** J-24, Kadiri-3

**(h) Mango hybrid varieties:** Amrapali and

**(i) Cattle hybrid varieties:**

Sahiwal x Holstein - Friesian

Ongole x Jersey

Haryana Zebu x Brown Swiss

Effect of breeding on increase of milk yield has been estimated to be 38.7% apart from the effect of feeding and management (Ram and Chowdhary, 1984)

Chapter 8

# Mutation

Usually genes get distributed without any change from one cell to another by mitosis or from one generation to other generation by gemetes thorough meiosis. But sometimes due to some special circumstances genes get chemical change due to which new varieties develop. Such changes may also happen in structure of chromosomes and sequence of genes. Such changes in gene or chromosome are called mutation. Changes in gene are called gene mutation. Changes in chromosome may be called chromosome mutation. Genetic or chromosomal changes in somatic cell are called somatic mutation. The mutations in germinal cells (gametes) are important in evolution. These mutations develop new varieties of organism.

**Definitions of mutation:**

Sudden changes in hereditary material are mutation (Poelman). It may happen is following ways:

(i) change in constitution of gene.

(ii) Change in sequence of genes of chromosome.

(iii) Duplication or loss of segment of chromosome.

**Types of mutation:**

Mutation may be of two types viz.

a) **Spontaneous mutation:** It is developed by nature

b) **Induced mutation:** These are produced by physical or chemical mutagens.

According to nature of tissues mutation may be two types.

a) **Somatic mutation:** It occurs in somatic cells so it may not be heritable.

b) **Germinal mutation:** This mutation happens in germ cells (gonads, anthers and ovary or ovum). It is of following types:

   (i) **Biochemical mutation:** These mutation cause chemical or physiological effect.

(ii) **Spurious mutation:** Due to this mutation recessive character gets expression.

(iii) **Point or gene mutation:** It is caused by change is structure of gene.

(iv) **Chromosomal or anamozygous mutation:** This mutation causes change in chromosome number or change in arrangement in segment or gene. This mutation can be of following types.

**Mutation leading to change in number of chromosome:**

It is two types:

(1) **Genomatic mutation:** Reduction in number of chromosome pairs may be haploidy. Increase in numbers of chromosome pair may be polyploidy

**Heteroploidy i.e.** increase or decrease in number of chromosome in chromosome pair.

Mutation leading to change in arrangement of segment or gene. It is of following types.

(i) **Intrachromosomal segmental rearrangement:** It is also called chromosomal inversion.

(ii) **Inter chromosomal segmental rearrangement:** It is also called translocation.

(iii) **Delection or duplication :** It is either decrease in chromosome segment or gene (deletion) or increase in chromosome segment or gene (duplication).

Now a days the world mutation is used only for gene or point mutation only. Mutation may be dominant or recessive. Dominant mutation is expressed in F1 generation. However recessive mutation may not be expressed for several generations unless it attains homozygosity.

**Stages where mutations may occur**

Through mutation may happen at any stage or life. But its occurrence is more likely in following conditions:

(i) Mutation may happen in mature gamete. So where this gamete goes only that offspring gets this mutation.

(ii) Mutation may occur before meiosis in primary gametocyte. Therefore half of the gametes which are produced from this gametocyte develop mutation.

(iii) After fertilization, at anaphase of mitosis daughter chromatids form, Mutation may occur in one of the daughter chromatid. So it may effect half of the body.

(iv) It item 3 both side chromatid get mutation then the whole body may get affected.

(v) Mutation may occur in sex chromosomes X or Y.

(vi) So either male or female generation gets affected.

(vii) During growth of the body mutation may occur only in one cell. So from that mutated cell the developed organ may get affected.

**Size of mutation:**

Size of mutation is studied from difference in parent and mutated gene. If the difference is clear then such mutation is called **prominent or conspicuous mutation.** If the difference is vary small then such mutation may be called as **inconspicuous mutation.**

**Frequency of Mutation:**

Possibility of frequency of mutation may be less in a gene. But plant or organism are made of million of cells.Therefore the chance increases for more frequency of mutation. Some scientists have found out frequency of mutation in some organisms as mentioned below.

1. In *Drosophila melanogaster* fly mutation frequency in x sex chromosomes is 0.15 percent per generation. In the second chromosome it is 0.5 percent and in the third chromosome the mutation frequency is 1.2 percent
2. In maize (*Zea mays*) in R gene the frequency of mutation is 492 per million. In C gene the frequency is 105 per million. In the gene Pr the mutation frequency is 11 per million
3. In human beings out of 24 thousand gametes only in one gamete mutation frequently has been found out.

**Effect of mutation:**

Mutation maybe harmful or usefull or lethal in effect. Most of the mutations have harmfull effect. Some times mutation have useful effect for example seedless grape, naval oranges and disease resistant varieties of maize and wheat.

Due to harmful effect of lethal mutation developing embyo are killed. Lethal mutation may appear in autosomes and sex chromosome. In maize because of lethal mutation development of chlorophyll stops due to which the seedling gets killed.

**Properties of mutation:**

Mutation has following special properties:

(1) Not gradually but suddenly mutations appear .

(2) Mutation is heritable.

(3) Mutation appear in one or very few organism.

(4) Effect of mutation may be morphological, physiological or biochemical. In *Drosophila melanogaster* due to mutations antennae changes into legs, balancers converts in second pair of wings and sucking mouth parts are changed to mouth parts of inferior quality.In Oenothera plant mutation has developed several varieties which have different types of sizes, leaves and flowers.

(5) All mutants either developed from prominent or inconspiquous mutations match more or less with ancestors.

**Mutagen:**

Mutagen means the device to induce mutation. Mutagens are of two types i.e. **physical mutagen** and **chemical mutagen.** Muller was first to find out physical mutagen. In 1927 Muller irradiated *Drosophila melanogaster* with X ray which produced mutation. Since then scientists are using high energy penetrating ionizing radiations for example x rays, gama rays, alfa rays, beta rays, neutron, cosmic rays, radioactive isotopes, ultraviolet rays etc. as physical mutagen to produce mutation in various plants and animals.

In addition to physical mutagens several chemical mutagen are also being used such as mustard gas, ethyl methane sulphonate, ethyl urethane, magnous salt, high temperature, formaldehyde, eosin, enthrosine etc.

**Method to induce mutation:**

For induction of mutation plants, seeds, male & female gametes or whole body are treated with suitable doses of physical or chemical mutagen. After treatment with mutagen M1,M2,M3,M4, etc. generations are grown. Selection for desirable character is made in each generation. After a variety is developed it is subjected to field testing in replicated trials. Afterwards the improved variety is finaly released.

**Problems of induction of mutation in fruit trees and ways to tackle**

a) Problem of diplontic or intra-somatic selection in irradiated tissue which necessitates an effective screening technique for recovering wholly mutated tissue from an initially induced chimeric situation.

b) Long juvenile phase of fruit trees rendering the evaluation of radiation effects, a delayed and long range programme.

c) The question of land space & technical know how for proper evaluation of large number of population.

d) Primary and secondary ploidy nature of fruit trees limits the chance of detectable mutation, though total mutational events might have been more.

e) Threshold value of mutagenic and lethal dose is comparatively narrow with acute radiation.

Nakajima (1965) from Japan reported a gradual cutting back technique towards the lower buds to recover a large number of somatic mutants in chronically irradiated roses. Das (1969) by nipping off the undesirable buds and forcing up buds only from charged axil could recover totally mutated twigs in irradiated Hibiscus.

**Biological effect of irradiation on mutation:**

Radiations are of two types -long waves (wave length 2000 to 2950 °A) and short waves (wave length 0.06 to 1000 °A). Long wave rays (e.g. ultra violet radiation) do not express ionization and have less capacity for penetration. But short wave rays such as x rays and gamma rays (wave length 9.01 to 1.4 °A) express ionization and have more capacity for penetration. Both of these radiations can damage cells. These radiations are used for disinfection or for induction of mutation. In case of short wave radiations very less dose is used for mutation induction in plants and animals which are used for human welfare. To explain ionizing radiation geneticist have given following theories:

(1) **Target theory:** According to this theory the ionizing radiat energy hits certain targets such as enzyme molecule, gene molecule, nucleotide or muton. Its use in increased dose causes more effects to the cell.

(2) **Free radical theory:** This theory explains that with the effect of radiation non- specific material or water gets broken down due to which many free radicals such as H2 O2 are formed. After some reactions it forms organic peroxidase. These cause several

oxidation - reduction reactions resulting changes in chromosomes.

Ionizing radiations are more sensitive to nucleic acid (DNA and RNA) as compared to cytoplasmic structure (example enzyme) . Ionizing radiation reduces cell division. In addition to it chromosomes also gets affected. Chromosomes get breakage or there happen change in genes. In this way all this results genetic mutations. Its genetic effect is irreversible and permanent.

Certain organisms are sensitive to high temperature treatment for development of mutations. In contrast to it, certain organism responds to low temperature treatment for production of mutants. We can take the example of human eye disease Aniridia. In eyes, for development of iris only one gene is responsible. Due to certain reasons this gene gets changed (mutated) and become unable to form iris. If this gene present in gonad, gets mutated then it gets transferred to zygote. Through mitotic cell division this mutated gene reaches to every cell of the body. But it expresses its effect at proper time only. Due to this mutated gene, development of iris do not occur which causes the disease Aniridea. It is due to the dominant mutation. The parent has this mutated gene in his/ her gonads. At sexual maturity half of the gametes get this mutated gene. Due to which half of the offsprings inherit this eye disease Aniridea

**Methods to find out mutation:**

**(A) Mutation in sex chromosome:** To find out mutation muller has developed a good method knwn as CIB Method. This method suits to those organisms in whose x chromosomes mutations are produced artificially. In this method such female *Drosophila melanogaster* in whose x chromosomes there is a cross over suppressor gene C, a recessive lethal gene l and a dominant gene B responsible for Bar eye, that female Drosophila fly is irradiated with x ray or other suitable mutagen and then hybridized with male *Drosophila melanogaster.* In F1 Bar eyed daughters CIB chromatid comes from mother and x chromatid comes from father. The Bar eyed daughter fly was crossed with normal male. In F2 all such sons died which received CIB chromatids from mother. In other sons recieving x chromatid, if those x chromatid get lethal mutation then those sons also die. But in F2 all the daughters were alive because those received normal x chromatid from father ( Fig-16)

Hence the above experiment reveal that in F1 the CIB female produce

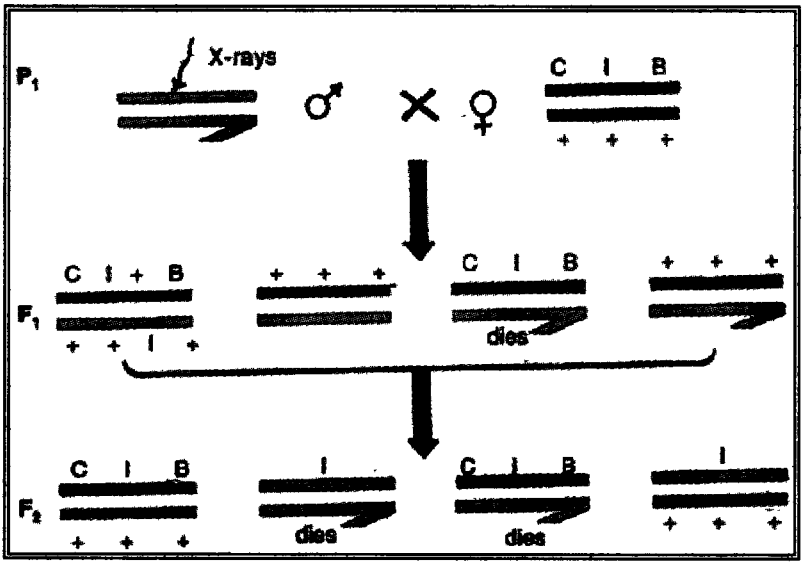



**Fig. 16: Diagram showing the CIB method of detecting lethal mutation.**

only daughters and no son at all. This means that lethal gene from father came to sons and so all sons died. Now to know that whether that lethal gene was developed by itself or by treatment we have to perform two experiment together.

In one experiment mutagen treated male was hybridized with CIB female of F1. In other experiment normal male was hybridized with CIB female of F1. If in the experiment with mutagen treated male most of the offsprings died because of lethal gene then one conclusion could be drawn that the lethal gene was developed by mutation treatment.

Second conclusion is that if in the mutagen treated x chromosome there is any prominent (visible) mutation then that appear in the sons of F2 generation.

**(B) Mutation in autosomes:**

CyLpm method of finding out mutation is popular for autosomes. In *Drosophila melanogaster* fly second chromosome's one sister chromatid had gene Cy for curled wings and gene L for lobed eye. The second chromatid had gene pm for brown colour. The three genes (Cy, L, pm) are lethal if present in homozygous condition.

*Drosophila melanogaster* fly with Cy L pm genome is crossed with x ray irradiated Drosophila fly. The result is that in F1 generation the gene

Cy, L or pm comes from one parent and x ray treated chromatid comes from other parent.

Now from F1 any type of fly which had either (a) Cy, L and x ray treated chromatid, (b) had the gene pm and x ray treated chromatid. Such fly was crossed with similar type of Drosophila fly. In the F2 three types of offsprings appear in 1:2:1 ratio given below:

1 = Cy L/ Cy L or pm / pm double chromatid

2 = Double treated chromatid

3 = One chromatid with Cy L or pm genes and the other x ray treated chromatid.

In this experiment the first type of *Drosophila* dies. Third type of *Drosophila* survive. Second type of Drosophila either survive, but dies only if lethal mutation appeared in the treated x chromosome.

**Importance of mutation in evolution:**

Evolution of any organism is possible only by inherent variations developed by mutation. Mutation just appears not for any purpose. If it can be useful then that can be helpful in evolution. Generally inconspicuous mutations are more useful for evolution. Importance of mutation is given below:

**(a) Importance of gene mutation in evolution:**

For evolution gene mutations are comparatively more useful. But Deveries was of the opinion that only conspicuous mutations are useful in evolution. However on further researches this opinion of Deveries was found baseless. Inconspicuous mutations accumulate to develop sufficient evolutionary changes. After Watson and Crick explained the structure of gene since then scientists have the opinion that mutation happen because of changes in genetic code.

**(b) Importance of chromosomal mutation in evolution:**

Under this duplication, inversion and translocation have much importance. Deficiency is not important because it is lethal for organisms. Duplication results increase in number of genes resulting new characters, so it is of importance in evolution. Translocation and inversion generally bring about varietal difference and species differentiation. For example, two varieties of *Drosophila melanogaster* differ because of inverted segment. In *Datura stramonium* varieties have developed spontaneously due to occurrence of reciprocal translocation.

Chapter 9

# Polyploidy

If more than two homologous chromosomes sets are found in cell or organism then that is called polyploid. Polyploidy is more found in plants as compared to animals. The reason for less polyploidy in animals is their sex balance. In plants sex balance is weak. In animals if number of chromosome becomes more than 2n they become intersex and loose the capacity for reproduction. So it is very rare in animals such as in *Artemia salina.*

**Origin of polyploidy:**

Polyploids are produced in several ways. Some time meiosis happens in anamolous way due to which number of chromosome is not halved but the number remain unchanged and it forms gametes. Two such gametes fertilize and form tetraploid.

During mitotic cell division at anaphase chromatids may not move to the poles. Another situation may happen where cell wall does not form. Results of both these situations are somatic doubling i.e. duplication of the somatic chromosome number. In other words formation of a tetraploid cell takes place.

On fertilization of a tetraploid plant with a diploid plant birth of a triploid plant takes place. Somatic doubling of a triploid will lead to development of hexaploid plant. On fertilization of a hexaploid plant with a tetraploid plant, a pentaploid develops.

**Types of polyploidy:**

Differences in chromosome number results two types of polyploidy i.e. (a) **Euploidy** and (b) **Aneuploidy.**

**(a) Euploid:** Euploid is the exact multiplication of chromosome set. Euploid is also known as true polyploidy. Euploid is of following two types:

(i) Autopolyploid or Autoploid

(ii) Allopolyploid or Alloploid

## 1. Autopolyploid or Autoploid:

If chromosome number of diploid due to any circumstances becomes double then polyploidy formed in this way is called autopolyploid or autoploid (Fig- 17). For example AA genome if becomes AAAA then it is autotetraploid. For development of new varieties or species autopolyploidy has great role.

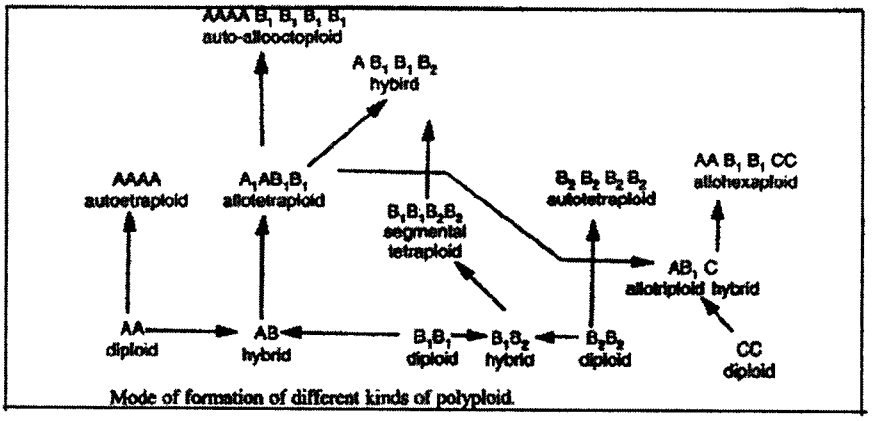



**Fig. 17: Origin of auto and allopolyploids.**

## 2. Allopolyploid

On hybridization of different diploid species, hybrid get developed. If somehow chromosome number of hybrid gets doubled. Then the polyploidy developed in this way is known as allopolyploid or alloploid. This is important for development of varieties and species. For example, a diploid species with AA genome gets hybridized with a different BB diploid. Then the hybrid will have AB genome. This

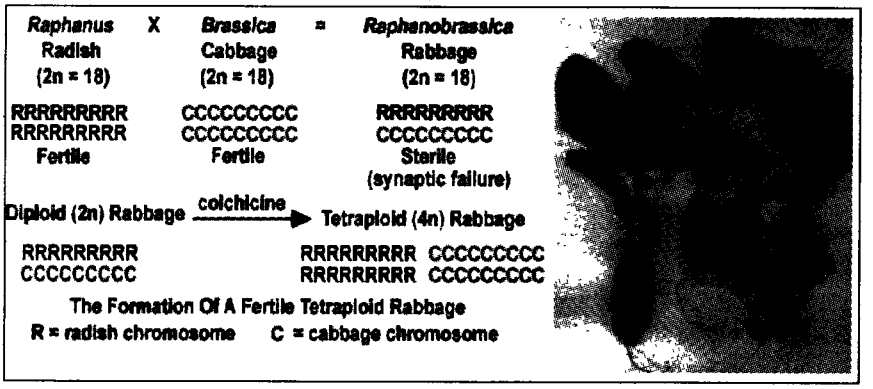



**Fig.18: Cross of radish (*Raphanus*) with cabbage (*Brassica*) leading to formation of allopolyploid *Raphno brassica*.**

genome is sterile to some extent. On duplication of its chromosome number the tetraploid formed is known as allotetraploid. The sterility disappears by the allotetraploidy. Such allopolyploid are known as amphidiploid. It is fertile like diploid. Some examples of amphidiploid are *Primula kewensis, Crepis rosea, Iris versicolar, Serpentina townsendii* and *Raphno brassica* etc.( Fig-18).

**(b) Aneuploidy:**

Sometimes in nucleus there is no exact multiplication of chromosome set such as 2x + n or 2x- n. Such a situation of polyploidy is known as aneuploidy. Due to aneuploidy the genic balance gets disturbed leading to sterility.

Aneuploidy is of several kinds for example monosomic ( 2x-1) example tobacco and *Drosophila melanogaster* ; trisomic ( 2x+1) example datura, maize & tomato ; tetrasomic ( 2x+2) example wheat and double trisomic ( 2x+1+1) example maize.

Aneuploid if increases the number of chromosome for example 2x+1 then this situation is known as hyperploid. If aneuploid bring down the number of chromosome then that phenomenon is called hypoploid. Aneuploidy has great role in evolution.

**Polyploid series:**

While study of various genus of plants it has been observed that the basic chromosome number get multiplied in form of series. This type of series is called polyploidy series. Some examples of polyploidy series are given in the following table:

| Plant/ Genus number | Polyploid series | Types of polyploidy | basic |
|---|---|---|---|
| 1 *Triticum* wheat | 14, 28, 42 | Diploid, Tetraploid, Hexaploid | 7 |
| 2 *Oryza* Rice | 24, 28 | Diploid, Tetraploid | 12 |
| 3 *Rosa* Rose | 14, 21, 28, 35, 42, 56 | Diploid, Triploid, Tetraploid, Pentaploid, Hezaploid, Octaploid | 7 |
| 4 *Solanum* Potato | 12, 24, 36, 48, 60, 72, 108, 144 | Diploid, Tetraploid, Hexaploid, Octaploid, Decaploid, 12 ploid, 18 ploid, 24 ploid | 6 |
| 5 *Psidium* Guava | 22, 33, 44,55 | Diploid, Triploid, Tetraploid, Pentaploid | 11 |

**Effect of polyploidy**

Polyploid organisms (mostly plants) as compared to normal organism are different. This difference is morphological or physiological or genetical as described below.

**(A) Morphological effect of polyploidy:**

1. Due to polyploidy stem become thicker and stouter.
2. Polyloid leaves are larger, thicker and deeper green.
3. Polyploid leaf hairs are thicker and longer.
4. Polyploid plants are of larger size.
5. Polyploid flowers are larger. Its sepals , petals , stamens and ovary become large and attractive.
6. Pollen of polyploid plants are larger.
7. Generally due to polyploidy fruit size and quality increases. So their economic value increases.
8. Sometimes due to polyploidy fruits become irregular so their economic value gets reduced. Example triploid guava.
9. Cotyledon size increases in polyploidy.
10. Polyploidy reduces reproductive power. The reason is that due to multivalent formation at anaphase distribution of chromosomes is not proper. This leads to genetic unbalance resulting into sterility.
11. In polyploids number of flower gets reduced.
12. Due to polyploidy cell size of stomata and xylem increases.
13. Polyploidy increases size of cell and nucleus.
14. Polyploidy increases resistance to diseases.

**(B) Physiological effects of polyploidy:**

1. Generally polyploidy reduces growth rate.
2. In certain cases polyploidy changes annual habit to perennial habit , example autotetraploid maize.
3. Polyploidy delays flowering.
4. In certain plants e.g. tobacco polyploidy increases the content of alkaloid nicotin.
5. Polyploidy increases ascorbic acid content in certain plants such as tomato, cabage, guava etc.
6. Due to polyploidy in tobacco ( *Nicotiana tobacum*) there is less

carbohydrate and sulphure but there is increase in content of nitrogen, calcium, potassium and magnesium.

7. Polyploidy enhances vitamin content.
8. Polyploidy increases osmatic concentration.
   Polyploidy increases water content in plants.

**(C) Genetical effect of polyploidy:**

1. Polyploidy increases the content of allelomorphic genes.
2. Compared to diploid the autopolyploids are more stable genetically.
3. Polyploidy increases the rate of mutation.

**Artificial production of polyploidy:**

Because of economic importance of polyploids and looking its breeding possibilities following techniques may be tried to produce chromosome doubling at somatic cell division of zygote. To achieve it ionizing radiation, non ionizing radiation, temperature etc. can be tried.

**Doubling of chromosome number in somatic cell:**

It may be by following treatments -

(a) Irradiation with ionizing radiation: Irradiation with x ray at seedling stage mostly result in doubling of chromosome number. Other radiations such as gamma rays also can be tried.

(b) Heat treatment: Treatment of roots with high or low temperature are used for chromosome doubling.

(c) Treatment by callus formation: On decapitation of stem or branches, afterwards callus formation takes place. One of this callus cells may happen to be polyploidy. It may grow a polyploid branch. By this technique Winkler produced tetraploid in *Solanum nigrum* .

(d) By chemical treatment: Several chemicals may be tried to induce polyploidy. Some examples are colchicine, chloroform, alchohol, lactic acid, chloral hydrate, nichotine sulphate and phytoharmones e.g. alfa NAA, beta NAA, gibberelic acid, beta IAA and IBA etc.

(e) Hybridization: New polyploids are formed by hybridization between various ploidy levels.

**Use of abnormal meiosis for production of polyploidy:**

For production of polyploidy to induce abnormal meiosis ionizing radiations, chemicals and hybridization techniques are used. Above mentioned methods may be used for induction of polyploidy. However colchicine is most commonly used.

**Colchicine treatment for polyploidy induction:**

Now a days colchicine treatment is most popular technique to induce polyploidy. Colchicine is an alkaloid. Colchicine is extracted from root and corm of two plants of Liliaceae family- *Colchicum autumnale* and *Colchicum luteum* . Indian scientist Parthasarthy reported colchicines from the plant *gloriosa superba* of the family Liliaceae. Some scientists from USSR haye reported that a chemical Granoson is also effective like colchicine for polyploidy induction.

For use of colchicines there are several techniques used in plants. It should be used only when the mitotic cell division is maximum in the plant. The techniques are given below:

(a) Young and developing twigs are immersed in weak solution of colchicine for suitable time, at time of maximum mitotic cell division.

(b) Weak solution of colchicine may be dropped slowly for sometime at maximum mitosis.

(c) Spray the weak solution of colchicines at timely intervals on growing twigs at times of maximum mitosis.

(d) Injection of weak solution of colchicine in growing twigs at times of maximum mitosis.

(e) Seed treatment with colchicines for suitable timings, washed and then sown.

(f) In agar or lanoline paste colchicine in suitable concentration is mixed and applied on growing tissue of plant to induce polyploidy.

The techniques of colchicines application for induction of polyploidy was invented by Blackslee, Avery and Nebel in 1937. Afterwards several scientists such as Derman ( 1940, 1953),Luong (1951), Pope and Love (1952), Brewbacker ( 1952), Hutton ( 1953 ), Hunter ( 1954) and Evans ( 1955) have induced polyploidy by colchicine. Several Indian scientists such as A.K. Sharma, R.P. Roy, A. Abraham, M.S. Swaminathan and many others have developed several useful polyploids in several crops such as cotton, sugarcane, gram, tobacco. rye, rice, barley, oat, wheat, oil seeds etc.

**Importance of polyploidy in evolution:**

Maximum amount of polyploidy have been reported in angiosperm and perennial herbs by Stebbins in 1938. Medium amount of polyploids are found in animals. Least amount of polyploids are found in woody plants. Muntzing ( 1936) suggested that polyploidy may change annual plant into a perennial plant.

From point of view of evolution polyploidy is important. In evolution of polyploidy a time comes when it behave like diploid. Polyploids adjust to changed circumstances by modification in physiological, morphological and genetical characters. This fact is important in evolution.

By synthesis of allopolyploids new genera and species may get formed. It may play great role in evolution. Karpechenko (1928) hybridized radish (*Raphanus sativus* ) with cabbage ( *Brassica oleracea*). These plants belong to different genus but have same number of chromosomes. The hybrid had 18 chromosomes but these chromosomes from father and mother were non homologous. So during they failed to pair. Therefore they produced sterile gametes. Due to some reasons meiotic cell division failed so the number of chromosomes could not be reduced - it remained 18 only. In due course of time such male and female having 18 chromosomes could fertilize and produce a hybrid with 18 chromosomes. On doubling this 18 chromosome number a allotetraploid with 36 chromosomes was formed . It was fully fertile because of full pairing of chromosomes. This was named as *Raphano brassica* by Karpechenko. Compared to parents this hybrid was larger. Its pods were larger and had more seeds.

**Role of polyploidy in plant breeding:**

Objective of plant breeding is to develop superior varieties with more yield, disease resistance, better quality etc. Generally polyploids have most of these features.

But in certain cases polyploidy has negative effect such as less yield, poor quality, small size, slow growth and lower fertility. This fact means that there is a saturation point beyond which increase in ploidy brings about negative effect. Similar ideas were presented by Wettstein (1927), Levan (1942), Stebbins (1952) etc. Therefore there is an optimum level of polyploidy for every type of plant. It is called optimum level of polyploidy.

For improvement of plant, hybridization is a good technique. But in certain cases F1 hybrids are sterile. To make these fertile polyploidy breeding is a boon.

In plant breeding as compared to autopolyploidy the allopolyploidy is more helpful because in autopolyploids due to more multivalent formation the genic balance is disturbed. So they are sterile.

# Chapter 10

# Linkage

In the chapter on Laws of Inheritance it has been explained that there are some exceptions to Gregor Mendel's law of independent assortment. In F2 segregation ratio of characters is not always 9:3:3:1. The differences are more in case of trihybrid and polyhybrid crosses. These types of exceptions were first reported by Batson in England and Morgan in USA.

Law of segregation and law of independent assortment are infact based on locations of various pairs of segregating genes on non homologous chromosomes. Hence larger the size of chromosomes number of genes are there. Therefore at times of independent assortment more combinations will be expected. Keeping these facts in mind if we examine the research work of John Gregor Mendel, we find that success of this scientist was because of the material pea (Pisum sativum). Because the seven characters (genes) are located separately on separate chromosomes.

On chromosomes there is large number of genes. For example in Drosophila melanogaster on 8th chromosome there are more than 100 genes, in human being on 46th chromosome according to Stern ( 1949) there are about one lakh genes. This fact reveals that in every pair of homologous chromatid there are many genes. It also happens that two or more genes responsible for a character are located on chromatid of pair. Such genes are linked in such a way that all such genes together reach to gamete through meiosis. Such relationship between genes is known as linkage.In homologous chromatid presence of total number of linked genes is called linkage group.

Discovery of linkage was done in 1906 by Bateson and Punnet, but they could not explain it. In 1910 Morgan did similar experiment in Drosophila melanogaster. Results of Morgan were similar to results of Bateson and Punnet. Morgan explained the reason as linkage. He explained that alleles (genes) from homozygous parent reach together in same gamete, but if same alleles (genes) come from heterozygous parent then they reach separately in different gametes. The

phenomenon of existing of genes together in a gamete has been named as coupling by Morgan. The phenomenon of dispersing genes independently in different gametes has been named as repulsion.

Morgan expressed that coupling and repulsion are two phases of linkage process. Explaining the reason of coupling Morgan told that the reason for linked genes to be together is they are annexed on one chromatid only. Explaining repulsion Morgan told that because the alleles (genes) exist on separate chromosomes therefore they go to different gametes. Morgan also told that quantity of linkage is inversely proportionate to distance of linked gene. After the discovery of Morgan scientists could feel that genes are arranged in line on chromosomes. Based on this theory of Morgan it could be possible to prepare linkage map (genetic map), by its help location of genes on chromosomes can be understood.

**Explanation of linkage through experiment:**

Phenomenon of linkage can be well understood by breeding experiment on maize (*Zea mays*) and Drosophila flies (*Drosophila melanogaster*) because in their offsprings characters can be understood well. Moreover they produce offsprings in large numbers. Therefore it is easy to make ratio. Details of experiments are given below:

**Experiment with maize:**

Two contrasting characters of maize are coloured & full seeds and colorless & shrunken seeds. These characters can be expressed like this:

Colored = gene C  Full seed = gene S  Colorless = c
Shrunken seed = s

Therefore genotype of coloured & full seed is CCSS and genotype of colourless & shrunken seed is ccss. These two when produce genotype produce the gametes CS and cs. On crossing these the hybrids obtained has the genotype Cc/ Ss (linked genes are written above and below the line). The F1 will produce coloured and full seeds. As per the Gregor Mendels law of independent assortment the F1 will produce 4 types of gametes viz. CS, Cs, cS, cs in equal numbers. But when this hybrid was test crossed (crossed with recessive parent) then in F2 the ratio of 1:1:1:1 could not be obtained, which should have happened. The obtained ratio was different i.e. 29 coloured & full: 1 coloured & shrunken: 1 colourless & full : 29 colourless & shrunken ( 29: 1: 1: 29)

In another experiment when coloured & shrunken seeded (Cc ss) maize plant was crossed with colourless and full seeded plant. Then in F1 the offsprings were of four types in the ratio 33:1 :1:33 ( 33 coloured & shrunken = Ccss : 1 coloured & full seed = CcSs : 1 colourless & shrunken = ccss : 33 colourless & full seed = ccSs ).

Both above mentioned experiments reveal that colored & full and colourless & shrunken in the experiment A and coloured & shrunken and colourless & full in experiment B are more popular. This happens because of following causes.

(1) Two pairs pf genes i.e. Cc and Ss could not assort independenly.

(2) During meiosis these pairs of genes are arranged in parental combination and recombination.

(3) In homozygous (CCSS) as well as in heterozygous (CS /cs)

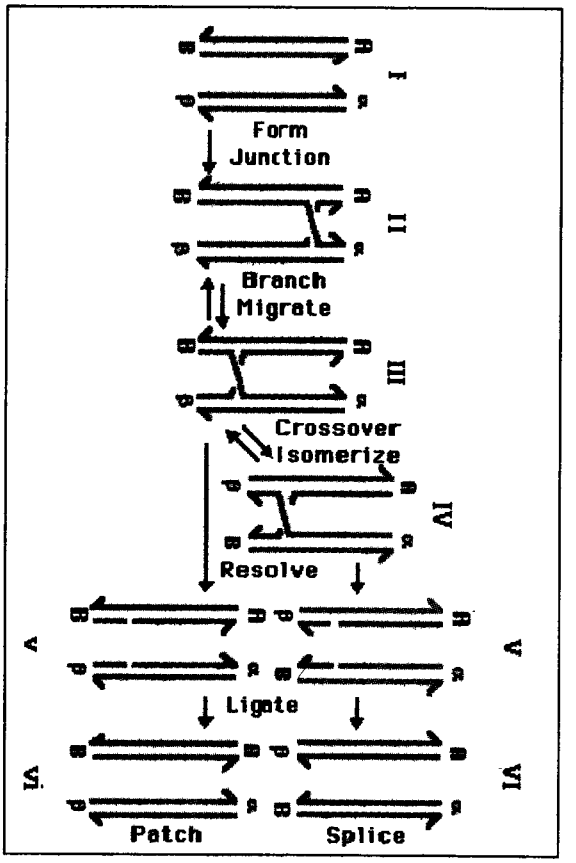



**Fig. 19: Recombination of genes.**

parental combinations are much more i.e. 90 to 95 per cent. The reason may be that parent genes are closely linked.

(4) In F2 the new characters such as coloured & shrunken (Cs / cs) and colourless & full ( cS/ cs) are visible due to recombination in experiment A. Similarly coloured & full (cS/cs) and colourless & shrunken ( cs/cs) are visible due to recombination in experiment B.

Mechanism of recombination is shown in ( Fig-19).

**Complete linkage:**

If two or more characters are inherited together and continue to be inherited together in next 2 or more generations then those characters express complete linkage.

Complete linkage is very rarely found. Example is male *Drosophila melanogaster* (female do not show complete linkage). Another example is female silk worm. An other example of complete linkage is *Sorghum vulgare*. The phenomenon of complete linkage is being explained by following examples.

**Example to explain complete linkage:**

Complete linkage can be well understood by a breeding experiment *in Drosophila melanogaster*. If normal grey bodied & long winged female (genotype GGLL) is hybridized with black bodied & vstigeal winged male (genotype ggll). In F1 all the offsprings will be grey bodied and long winged (genotype GgLl). If this F1 (GgLl) is crossed with black bodied & vestigial wing homozygous recessive (genotype ggll). Then in F2 generation grey bodied & long long winged and black bodied & vestigial wing will be produced in equal numbers (1:1 ratio).

**Explanation of the experimental results:**

Results of the above experiment can be explained with cytological background. It is clear that male of F1 produces only two types of sperms - GL and gl in equal numbers. But the homozygous double recessive female produce only one type of gamete - gl.

Therefore production of two types of gametes (GL and gl) by heterozygous male of F1, are infact contrasting characters and are closely linked with each other. The reason is that G and L are situated on same chromatid. Similarly g and l are situated on other same chromatid.

Hence two types of sperms - GL and gl and one type of egg - gl when fertilize produces only two types of offsprings which resemble only to their parents. Because these characters have complete linkage, therefore new recombinations were not be produced.

Instead of homozygous dominant parent ( GL/GL) when homozygous recessive parent black bodied & long winged ( gl/gl) was chosen for crossing with grey bodied & vestigial winged ( Gl/ gl) flies, then in the F1 these two types of flies are produced in equal numbers ( 1:1).

**Incomplete linkage:**

If two or more characters come together in offspring but due to recombination produce new character in such case those characters are said to express incomplete dominance. As compared to complete linkage the incomplete linkage is more common in plants. From the following example incomplete linkage can be understood well.

**Examples to explain incomplete linkage:**

In the above mentioned experiment on Drosophila melanogaster in F1 if grey bodied long winged heterozygous female fly ( genotype GL/ gl) is crossed with double recessive ( gl/gl) black bodied wing male then the F2 will produce four types of offsprings . Out of this, two parent type combinations will be more in number. Other two types of combinations will be of new types . The four types of offsprings will be 41% grey bodied & long winged, 9% grey bodied & vestigeal winged, 41 % black bodied & vestigeal winged and 9% black bodied & long winged.

Instead of homozygous parent if mutant parents - black bodied & long winged (genotype gL/gL) are crossed with grey bodied & vestigial wing ( genotype Gl/Gl ), then the F1 generation will be grey bodied & long winged with a genotype Gl/gL.

If F1 female with genotype Gl/gl crossed with male of the genotype gl/gl then in F2 three types of offsprings are found i.e. 41% grey bodied & vestigial winged 9 % black bodied & vestigial winged 41 % black bodied & long winged.

**Causes of complete and incomplete linkage:**

(1) One main reason for complete linkage is that in F1 offsprings of normal heterozygous or mutant heterozygous parents, the alignment of gene without any change in original condition

reaches to gametes. The result is that chromatid of homozygous parents having the genes Gl & gL and the chromatid of mutant parent having the genes Gl and gL without any change goes to their gametes during F1. Therefore heterozygous F1 male produces two types of gametes. Due to which in F2 two kinds of offsprings are produced in equal number.

(2) Second reason according to Morgan is the exchange of one segment of a chromatid with other segment of chromatid. Such exchange takes place in first prophase of meiosis. Generally it happens in non homologous chromatids. Because in this activity there is exchange of genes from one chromatid to another, therefore this activity is called crossing over. Therefore one of the main reason for incomplete linkage therefore is crossing over.

**Strength of linkage:**

Strength of gene linkage depends on distance between genes on chromatid. In other words what is the frequency of crossing over there. For example if two genes Aa and Bb are a distance of 10 units and other gene pair Cc and Dd are at a distance of 4 units. In that case the linkage in Cc and Dd will be stronger than linkage in Aa and Bb. In other words it can be said that the stronger linkage leads to lesser crossing over. Similarly weaker linkage leads to possibility of more crossing over. Therefore it can be said that strength of linkage depends on possibilities of crossing over.

**Factors affecting strength of linkage:**

As described above linkage strength depends upon distance between genes located on chromosomes. Beside it there are following physiological and environmental factors affecting strength of linkage.

(1) Age: Strength of linkage increases with increase of age.

(2) Temperature: Increase in temperature results reduction in strength of linkage.

(3) Ionizing radiation: Increase in dosage of x rays, gamma rays, etc. reduces of strength of linkage.

(4) Chemicals in food: Presence of chemicals in food for very long time may weaken the strength of linkage.

**Measurement of linkage strength :**

To measure the strength of linkage, the organisms having two or more linked genes are hybridized. In the F1 heterozygotes are

obtained. This heterozygote is crossed with homozygous double recessive parent. F2 has offsprings with parental combinations. These are counted separately and the percentage is calculated. Percentage of new recombination is also found out- this suggests the strength of linked genes. More the percentage of new recombination lesser the strength of linkage.

**Importance of linkage:**

(1) The discovery of linkage by T.H. Morgan has important place in genetics.

(2) Based on linkage some new genetical concepts were generated and developed.

(3) For improvement of crops, vegetable, fruits etc. to plan out the programme, understanding of linkage is very helpful.

# Chapter 11

# Crossing Over

In earlier chapter it has been explained that during first prophase of meiosis two chromatids of homologous chromosomes pair with each other at several places. It results in exchange of segments. This type of pairing of chromatids is called crossing over. During pairing chromatids gets broken where linkage is weak. Broken parts of a chromatid join with broken part of other chromatid. Therefore crossing over can be defined as: at pachytene stage of meiosis, the inner chromatids of homologous chromosomes pair with each other and get broken at pairing place. Recombination of broken places is called crossing over.

**Somatic & germinal crossing over:**

Depending on type of cell crossing over is of two types. If crossing over occurs in somatic body cells then it is known as somatic crossing over. If crossing over occurs during gamete formation, thenit is called germinal crossing over. It is very important in heredity. Therefore it is discussed in this chapter. Details of somatic crossing over are described below.

**Somatic crossing over :**

In development of embryo during mitosis pairing between chromatid leading to crossing over is termed somatic crossing over. It occurs very rarely. In such tissues there is a mosaic pattern of cross over and non crossover. Somatic crossover can not be inherited so it will not be discussed here.

**Germinal crossing over:**

At the time of gametogenesis during meiosis germinal crossing over takes place. It happen at pachytene stage. Germinal crossing over is common in organisms. It occurs more in heterozygous chromatids as compared to homozygous chromatids.

**Mechanism of crossing over:**

You have already studied that germinal crossing over happens in early prophase of meiosis. At this time two homologous chromosomes come near each other and do pairing (or synapsis). Afterwards both the paired chromosomes replicate to form two chromatids separately. Hence the chromosome pair now has four chromatids. At this stage the inner two chromatids twist on each other. The place where the two chromatids lie on each other is called chiasmata. Mostly on one place the pairing chromatics breaks. Afterwards one broken part joins with other broken part. This results that new segments appear in the chromatids. Afterwards these chromatids move away from each other. In last all the four chromatids move to four gametes. Out of 4 gametes, in two gametes there are normal (non cross over) chromosomes and in rest two gametes there are recombined (crossover) chromosomes. Therefore these gametes are also known as non crossover gametes and cross over gametes ( Fig-20).

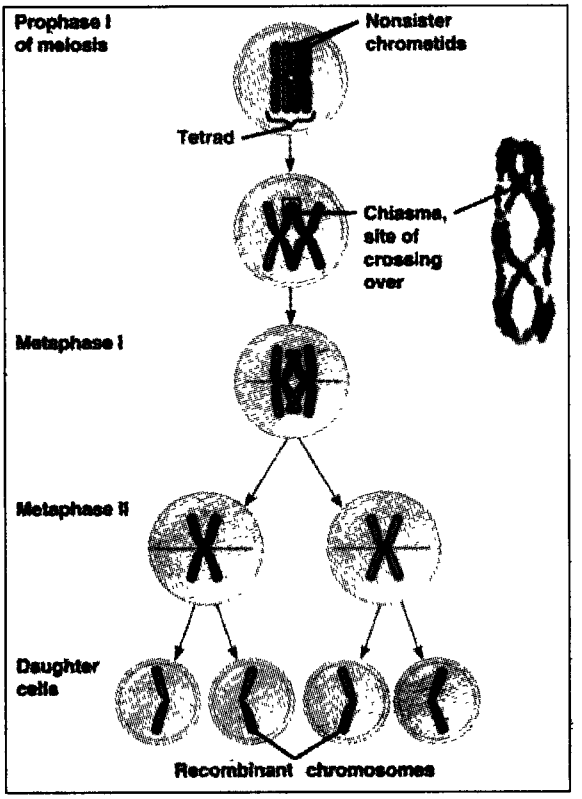



**Fig. 20: Mechanism of crossing over.**

**Cytological explanation of crossing over:**

Cytological explanation first of all wasgiven by C. Stern on *Drosophila melanogaster* and B. M. Clintoch on *Zea mays* (maize)

C. Stern observed red and round eye in ordinary Drosophila melanogaster. However in case of mutant eye of *Drosophila* there was different colour and shape. Deep red eye colour is controlled by recessive mutant and bar eyed is controlled by dominant mutant gene. Both these mutant genes are present on x chromosome. This X chromosome in *Drosophila melanogaster* is rod shaped. In female fly there is a pair of X chromosome. In his experiment Stern observed

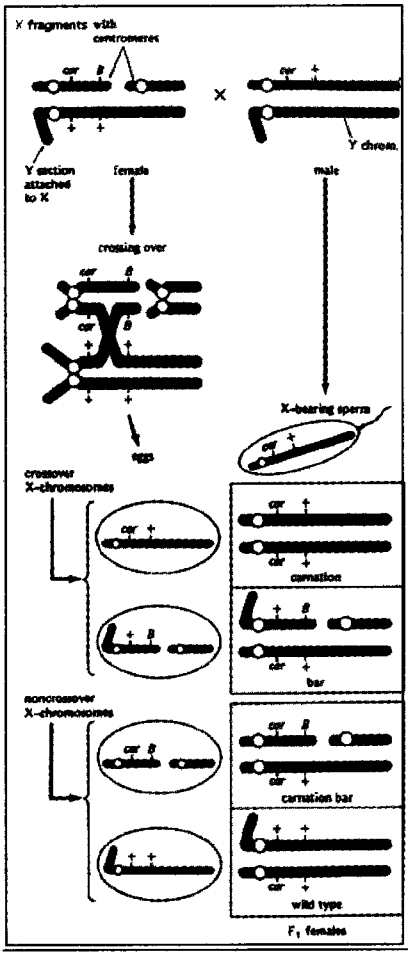



**Fig. 21: Cytological proof of crossing over.**

such a fly in which one x chromosome was divided in two parts. Broken parts had mutated genes Carnation and Bar. These were located on broken points (Fig. 29). On the end of another X chromosome there is attached a fragment of Y chromosome. These abnormalities happened because of sudden chromosome breakage. Y fragment attached X chromosome has normal allele of Carnation and Bar genes. Crossing over happened in both this x chromosomes during meiosis. With the result Y fragment of X chromosome gets transferred to broken x chromosome. Hence crossover chromosome and non crossover chromosomes differ in their morphology which can be observed under microscope. An example is described in Fig-21.

**Theories of crossing over :**

Though crossing over is a precise process but it is not clear that what the reasons for crossing over are. To explain this phenomenon several theories were put forth, but following theories are most popular:

**1) Contact first theory:**

It was proposed by Serebrovsky According to this theory the inner chromatids touch each other at one or many places and do the process of crossover. At places of touch the chromatids have breakages. Broken segments of the chromatids join with each other and form new combinations (Fig- 22)

**2) Breakage first theory:**

This theory of crossingover was given by Muller. According to this theory chromatids break without doing crossing over. Afterwards broken segments of the chromatids join with each other to yield new combinations. Now a days this theory is accepted by many scientists (Fig-23).

**3) Strain theory:**

Strain theory of crossing over was given by Darlington. This theory explains that the reason for breakage is the tension due to pairing. Both the chromatids of homologous pair have spiral coiling on each other. It results tension. Due to which at point of contact chromatid breaks. Afterwards these broken ends recombine leading to recombination of genes.

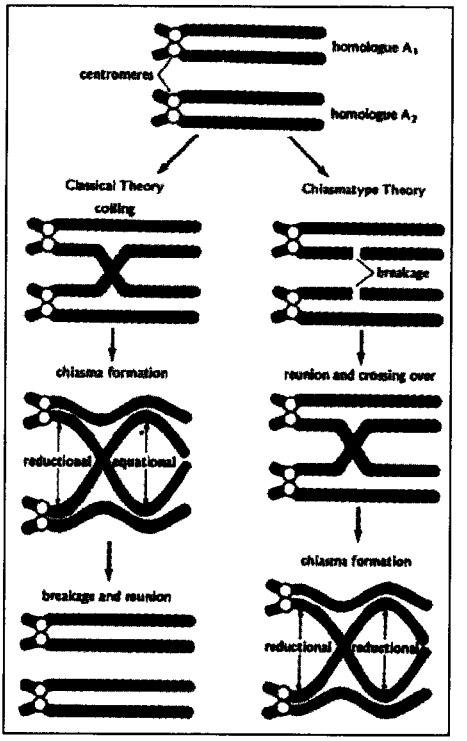



**Fig. 22: Contact theory of Crossing over.**

**Fig. 23: Breakage theory of Crossing over.**

**Factors affecting crossover:**

Following factors affect crossing over:

**1) Internal factors:**

**(A)** ***Sexual difference*:** Amount of crossing over differs in male and female sexes. For example males of *Drosophila melanogaster* do not have crossing over because it has complete linkage. In females of silkworm moth (*Bombyx mori*) there is no crossing over. It has been observed that if in organisms both the sexes exhibit crossing over then it happens in equal numbers e.g. peas (*Pisium sativum*). In some females crossing over happens more than males example mouse. Sometimes reverse is true i.e. crossing over happens morein males as compaired to females example pigeon. Haldaneons (1921) reported that wherever crossing over is different in different sexes then heterozygous sex express less crossing over.

(B) ***Modification in chromosomes:*** Change in structure of chromosome results in reduction of crossing over. Change in chromosome happen due to inversion, translocation, duplication, deletion etc.

(C) ***Mutation:*** Occurrence of mutation results in reduction of crossing over.

(D) ***Age:*** With increase in age there is reduction in crossing over.

**(2) External factors:**

(A) ***Temperature:*** Reduction in temperature leads to increase in crossing over. Medium temperature reduces this increase in crossing over. At high temperature crossing over does not occur or is negligible.

(B) ***Ionizing radiation and chemicals:*** Ionizing radiation such as treatment with X ray, gamma rays etc. or treatment with chemicals increases frequency of crossing over.

**Importance of crossing over:**

Crossing over happens in bacteria, yeast, fungi, higher plants and animals. Crossing over has following importance:

(i) Because of exchange of segments crossing over results recombination of characters.

(ii) Studies of crossing over reveals that genes are arranged in length of chromosomes.

(iii) Chromosome maps are made based on frequency of crossing over.

(iv) Crossing over helps to understand nature and mechanism of genes.

(v) Crossing over has lot of importance in plant breeding.

**Importance of crossing over in breeding:**

(i) Crossing over is very important in breeding of plants and animals. Breeder gets recombination because of crossing over in hybridization.

(ii) It is necessary to remove undesirable or uneconomical characters which may occur in hybridization, but due to occurrence of linkage the removal of undesirable characters become not possible because those are linked with desirable characters. Therefore because of this close linkage recombination can not occur. Therefore to over come this problem treatment with ionizing radiation (X- ray, gamma ray etc.), heat, chemicals etc. are tried.

# Chapter 12

# Chromosome Map

Before the knowledge of linkage and crossing over it was only known that chromosome contains genes. But nothing could be known about location of genes. Morgan, Bridge and Sturtevant based on their experiments have given following conclusions:

(1) In chromosomes genes are located in linear fashion.

(2) Linked genes do not show recombination in normal circumstances.

(3) Frequency of recombination and strength of linkage depends on distance between genes.

(4) Every gene is located on locus (plural loci) situated on chromosomes.

(5) In linked gene recombination occurs to the extent of 1% to 50 %.

Based on the above mentioned facts geneticists have tried to draw chromosome map. In this map based on calculations of crossing over percentage, distance between genes are located on a straight line on a paper. Therefore chromosome map can be defined as chromosome map is a line on which location of gene is decided based on crossover percentage.

**Example to draw chromosome map:**

In a trihybrid F1 the heterozygous AaBbCc was back crossed to the homozygote aabbcc parent. Resulting progenies were the followings:

| Genotype | Number |
|---|---|
| AaBbCc | 79 |
| AabbCc | 18 |
| AaBbcc | 82 |
| Aabbcc | 21 |
| aaBbCc | 19 |
| aaBbcc | 23 |
| aabbCc | 80 |
| aabbcc | 78 |
| | 400 |

Prepare a chromosome map showing the order of genes and their distance.

**Method to prepare chromosome map:**

Parental types are selected from all the progenies mentioned in the question. For selection the easy way is the progenies having maximum number are the parental types. Therefore in this example the parentals are mentioned below:

| Genotype | Number |
|---|---|
| ABc | 82 |
| abC | 80 |

Afterwards recombination percentage obtained from two genes is found out. To find it the test cross used is known as two point test.

**Calculation of recombination percentage from A and B genes**

Because both genes come from same chromosome, therefore they express coupling phase. Given below the side two combinations are parental types and the central two combinations are recombination types:

| AB | Ab | aB | ab |
|---|---|---|---|
| 79 | 21 | 19 | 80 |
| 82 | 18 | 23 | 78 |
| 161 | 39 | 42 | 158 |

Two recombination types

Recombination = 39+42 = 81

Total population = 400

Recombination percentage = $\frac{100 \times 81}{400} = 20.25\%$

**Calculation of recombination percentage from B and C genes:**

Because B and C genes comes from different genes, therefore they express repulsion phase. Therefore in the combinations given below

the side two are recombinations and the central two are parental type combination.

| BC | Bc | bC | bc |
|---|---|---|---|
| 79 | | | 78 |
| 19 | | | 21 |
| 98 | | | 99 |

Parental recombination

Recombination = 98+99 = 197

Total population = 400

Recombination percentage = $\frac{197 \times 100}{400}$ = 49.25 %

Calculation of recombination percentage from A and C genes

The genes A and C are from different chromosomes, therefore they express repulsion phase. In the following combination the two types of corners are parental types and the central two types are recombination types.

| AC | Ac | aC | ac |
|---|---|---|---|
| 79 | | | 78 |
| 18 | | | 23 |
| 97 | | | 101 |

Parental types

Recombinations = 97 + 101 = 198

Total population = 400

Recombination percentage = $\frac{198 \times 100}{400}$ = 49.5 %

AB = 20.25 % recombination

BC = 49.25 % recombination

AC = 49.5 % recombination

49.5 %

A 20.25 % B 49.25 % C

49.5 %

A 20.25 % b 49.25 % c

From the above mentioned two point tests, sequence of genes on chromosomes is known, but distance is not known between genes. The reason is that between A and B & B and C there exist double crossing over value. On subtracting double crossover value from the two values the distance between A and B & B and C can be known.

To find out double crossover value we have to follow three point test described below:

Non crossover ABc = 82

Or parental type abc = 80

162 divided by 4 = 40.5 %

**Cross over between A and B**

aBc = 23

AbC = 18

41 divided by 4 = 10.25 %

**Cross over between B and C**

ABC = 79

Abc = 78

157 divided by 4 = 39.25 %

**Crossing over between A and C or double cross over value** ( Fig-24)

aBc = 19

Abc = 21

40 x 10/400 = 10 %

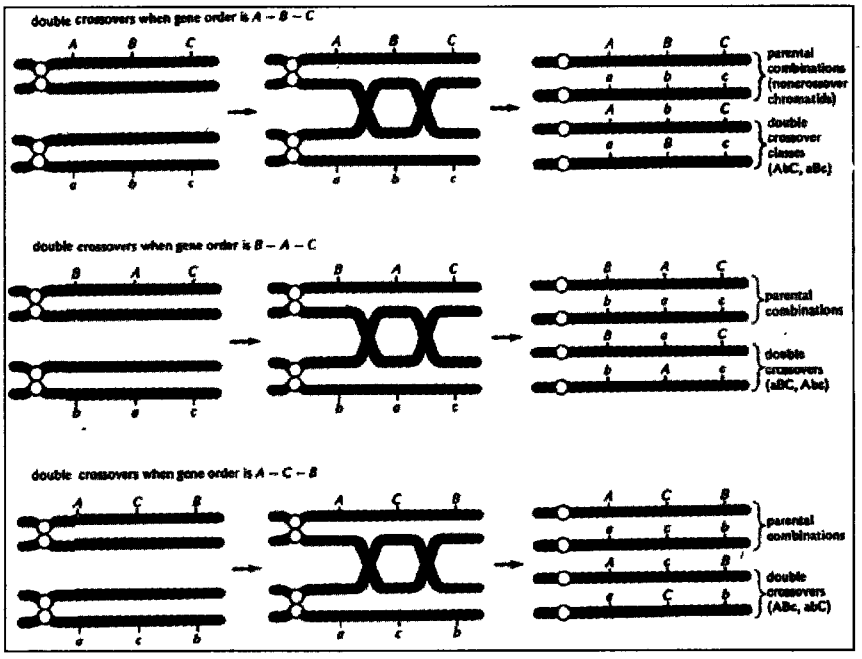



**Fig. 24: Crossing over between A and C or double cross over.**

On subtracting double cross over percentage between A and C i.e. 10% from the cross over percentage calculated by two point test distance between A & B and B & C can be known.

Note: Percentage value has been calculated from total population (i.e. 400).

**According to three point test:**

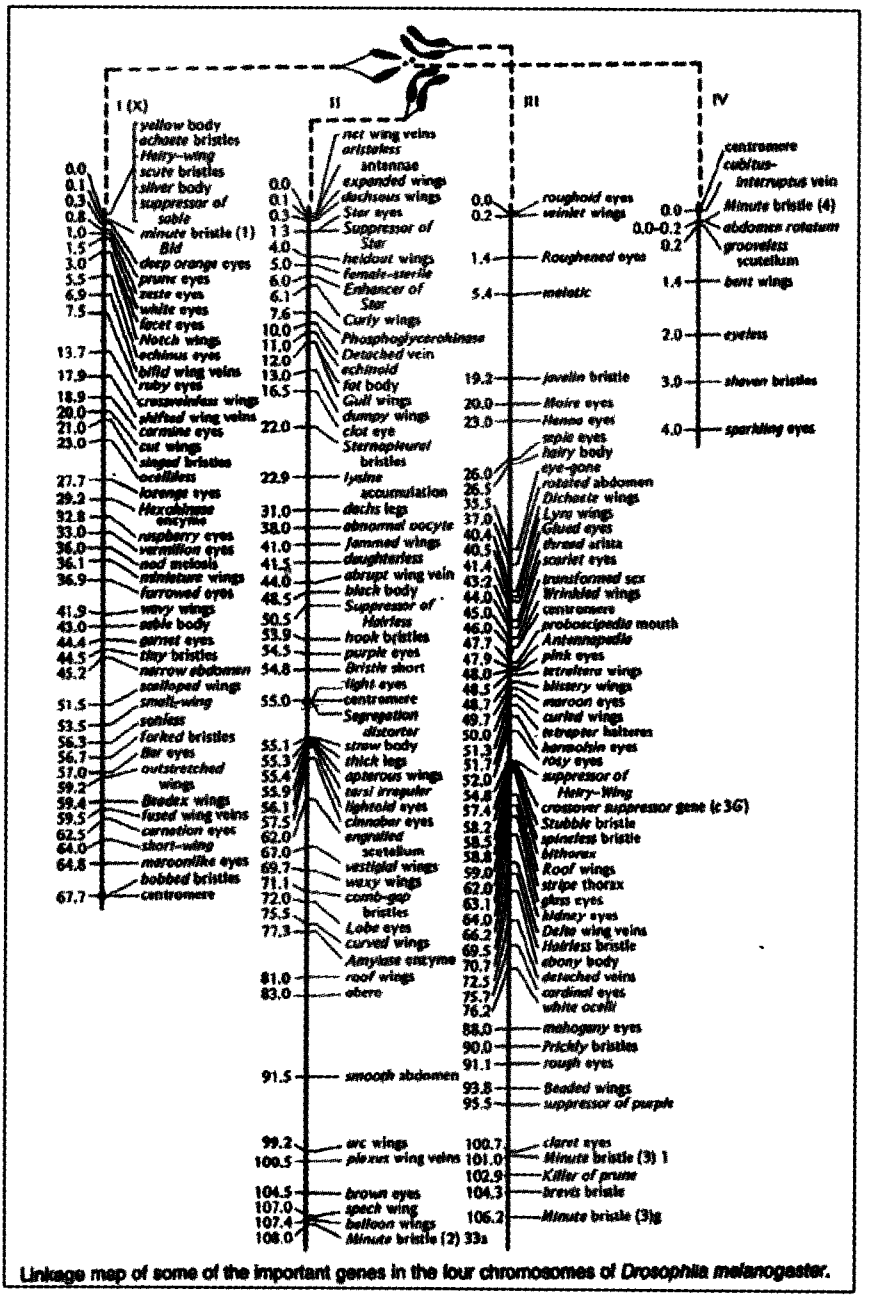
AB = 10.25

BC = 39.25

AC = 39.25 + 10.25 = 49.5%

Now after deciding scale, chromosome map can be made as decribed below:

Scale : 10% = 1 centimeter




Linkage map of some of the important genes in the four chromosomes of *Drosophila melanogaster*.

**Fig. 25: Chromosome map of four chromosomes of *Drosophila melanogaster*.**

| | | |
|---|---|---|
| 10.25% | 39.25% | 49.5% |
| A | B | C |
| 10.25% | 39.25% | 49.5% |
| a | b | c |

Therefore it is the desired chromosome map which shows sequence of genes and distance between the genes.

Geneticists have made chromosome map of several organisms. For example chromosome map of *Drosophila melanogaster* in Fig-25.

**Importance of chromosome map:**

Chromosome map is important because of following reasons:

(1) Chromosome map gives details about exact location of genes, arrangement of genes and combination of genes.

(2) By study of chromosome map it is possible to guess results of hybridization.

(3) Chromosome map can be helpful as probability table.

(4) Chromosome map helps in breeding work because it gives details of genetic combination.

Chapter 13

# Chromosomal Aberrations

**Structural changes in chromosomes (Chromosomal aberrations):**

Any deviation in normal chromosomes architecture or ploidy level is known as chromosomal aberration. Chromosomal aberrations are of two types - (1) structural aberration, (2) numerical aberration. Both aberrations can occur during any stage of cell division due to certain circumstances.

According to Morgan's theory of chromosome, in every organism there are fixed number and structure of chromosomes. Genes are arranged there in linear order. These chromosomes are inherited to their offspring in definite number and structure. But because of certain internal and external factors chromosomes gets some changes. It results in changes of characters. These changes are important in development of new types. It may give evolutionary benefit occasionally of new genotypes.

**How chromosomal aberrations or structural changes are produced:**

A small broken part of chromosome is called fragment. A fragment without centromere is unable to move to poles. Such fragment without centromere is called acentric fragment. It is well known that centromere is an important part of chromosome because it helps in movement of chromosomes to poles during cell division. If due to certain circumstances chromosomes gets broken and are joined again. It may result following modifications in chromosomes:

(1) Any broken segment can join with a centric segment.

(2) Any acentric segment can join with centric segment.

(3) Two acentric segments can join with each other.

Out of 3 situations described above only the second one survive. Rest both situations can join with each other and perish.

**Types of chromosome aberrations:**

Chromosome aberrations are of following types:

(1) Deletion or deficiency
(2) Duplication.
(3) Simple translocation.
(4) Reciprocal translocation.
(5) Inversion.

These are described below.

**(1) Deletion or deficiency:** Either of two ends of chromosome due to certain cause separate out and forms two parts. One part is monocentric i.e. have one centromere and other part without any centromere or in other words acentric. This situation is called delition. It is a simple chromosomal aberration. In this only one chromosome is affected. Such change is less harmful for the organism. Characters of offspring are less affected by deletion. Deletion plays a great role in evolution. It is of following two types.

**(a) Terminal deletion:** If any terminal segment gets broken and destroyed then it is called terminal deletion. If a chromosome consists of abcdefgh and the ab gets separated then only cdefgh remain left over. In the ab portion there is no centromere. So it can not move to either pole. Hence it gets ultimately lost. (Fig-26)

**(b) Intercallary deletion:**

From in between chromosome, segment may break off. Afterwards the broken chromosome again joins. This situation is known as intercalary deletion. As for example from abcdefgh chromosome cd segment breaks away. So the ends b and e join together. The segment cd does not have centromere, so it can not move to any pole and therefore get destroyed. (Fig-26)

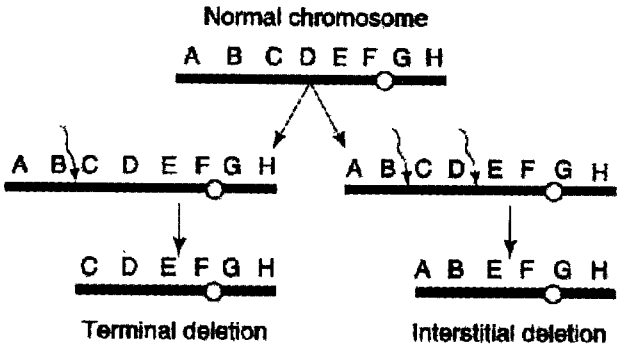



**Fig. 26: Different types of deletions.**

**(2) Duplication:** In chromosome if a segment gets replicated then this phenomenon is called duplication. Number of genes increases (gene amplification) because of duplication. This situation sometimes results lethal effect on the offsprings. Duplication may be of two types i.e. terminal or intercalary.

**(3) . Simple translocation:** In simple translocation atleast two chromosomes are affected. From one chromosome a segment breaks off and joins with other chromosome. This translocation affects the inheritable characters. In evolution it has great importance. Simple traslocation can be understood well with the help of Fig-27. From the wxyz chromosome yz segment breaks off and joins with the abcd chromosome giving it a new genotype abcdyz.

**(4) Reciprocal translocation:** It differs from simple translocation. In case of reciprocal translocation some portion (segment) of both the chromosomes are broken off and then joined to each other chromosome. These change the structure of both the chromosomes and results in change of characters. It is important in evolution. It can be well understood by Fig-27. From abcdef chromosome ab segment is broken. Similarly from vwxyz chromosome vw segment gets broken. Afterwards these broken segments get joined with each other chromosome making the new genotypes as abxyz example Philadelphia chromosome.

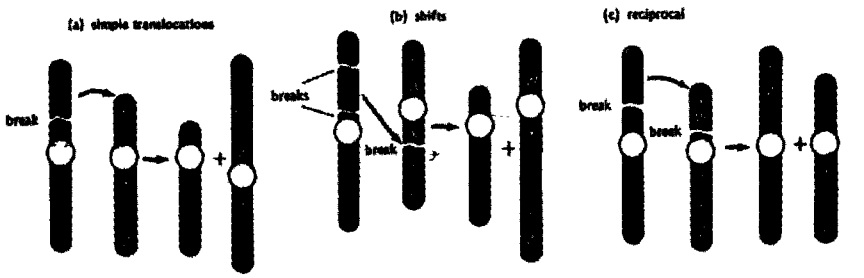



**Fig. 27: Different types of translocations.**

**Differences between reciprocal translocation and crossing over**

There are following three differences:

(1) Main difference between reciprocal traslocation and crossing over is that in crossing over transfer of chromosome segments occurs only in between inner chromatids of a chromosome where as reciprocal traslocation occur in whole chromosome. It is not essential that the chromosomes are homologous.

(2) Another difference is that in crossing over formation of chiasma occurs where as in reciprocal translocation chiasma do not occur.

(3) Crossing over is an essential process but reciprocal translocation is an accidental and unplanned process.

**(5) Inversion:** In chromosomal inversion only one chromosome is affected. In the chromosome, sequence of genes is changed. Its affect is not lethal. Hence inversion has great scope in evolution.

There are following two differences in inversion and translocation:

(i) In case of translocation structure of chromosomes gets changed, but in case of inversion structure does not change at all. Only there is a change in sequence of genes.

(ii) In translocation at least two chromosomes are involved but in case of inversion only one chromosome is involved.

**Types of inversions:**

Inversion is of following two types:

**(i) Terminal inversion**: Sequence of genes is changed at end of chromosome. The sequence of genes get changed to abedc from abcde.

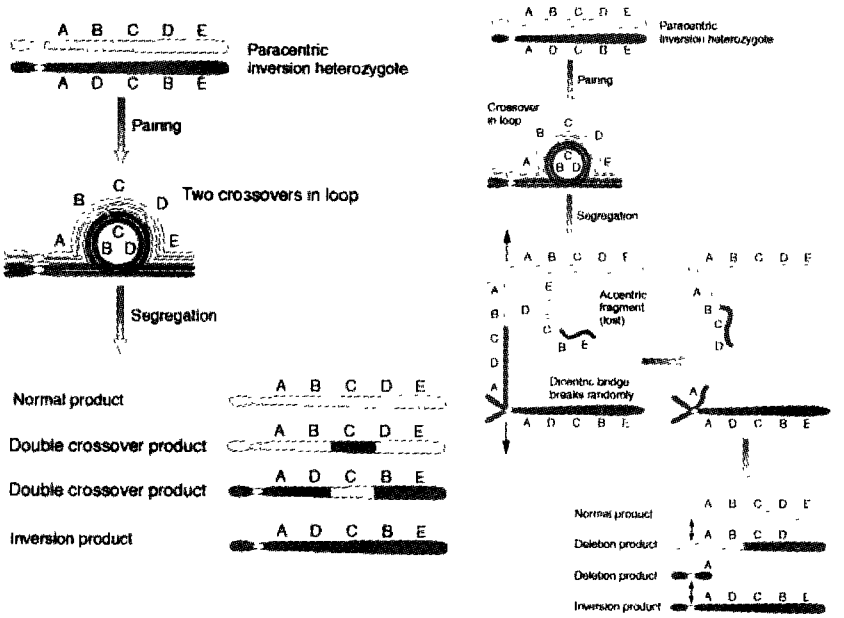



**Fig. 28: Types of inversion and inversion loop.**

(ii) **Intercallary inversion**: In this case instead of at end, the sequence of genes changes in between the chromosome. The sequence of genes instead of abcde it changes to acbde. If inversion occurs in one chromosome out of two homologous chromosomes then at time of pairing it forms inverted chromosome loop as shown in Fig-28.

**Evolutionary significance of chromosomal aberration**

(1) Due to chromosomal aberration there is a change in genotype. Therefore phenotype gets changed.

(2) If any organism has homologous deletion, then it has lethal or fatal effect . But due to duplication this harmful effect is neutralised.

(3) Usually duplication results increase in chromosome number which has evolutionary significance.

(4) Inversion and translocation play a great role in evolution because it results semi - male sterility or it changes the character.

Chapter 14

# Cytoplasmic Inheritance

Now a days this type of inheritance, governed by non nuclear factors are refered as extrachromosomal inheritance, somatal inheritance, extranuclear inheritance, maternal inheritance, uniparental mode of transmission or transovarian transmission.

By definition, it is the inheritance controlled by the extra chromosomal i.e. cytoplasmic factors that is transmitted to the succeeding generation through the egg of female organism.

It does not follow Mendelian inheritance but have following characters.

(a) It does not follow Mendelian segregation in crosses and reciprocal crosses.

(b) A particular set of characters are controlled by a set of cytoplasmic factors (genes) producing dissimilar hybrid.

(c) Follows the maternal line i.e. uniparellel mode of transmission

These cytoplasmic factors are contained by:

(i) mitochondria ( mt DNA)

(ii) chloroplast ( cp DNA)

(iii) symbionts ( bacteria and virus)

It has been explained earlier that inheritance happens due to genes. Genes are located on chromosome located in nucleus. Now a question may be raised that is there any method of inheritance that does not involve genes? The answer is yes. In modern times it has been proved that certain gene like particles present in cytoplasm are responsible for inheritance of some characters. These particles are called plasma gene. Therefore the mechanism where plasma genes effect inheritance is called cytoplasmic inheritance. Plasma genes and nuclear genes express relationship.

**Maternal effect:**

It is well known fact that the amount of cytoplasm in female gamete is much more than male gamete. Therefore by cytoplasm the female

gamete inherit more characters. The female gamete is responsible for transfer of cytoplasm to zygote. Therefore it can be said that female gamete express maternal effect for example ephertia moth.

**Extranuclear inheritance:**

There is enough experimental evidence to show that for inheritance not only nuclear genes but extranuclear genes are also responsible. This phenomenon is known as extranuclear inheritance or cytoplasmic inheritance. The examples are mitochondria, chloroplast inheritance. These items exist outside nucleus and are inherited independently from generation to generation and also called organelle inheritance.

**Examples of cytoplasmic inheritance:**

**(1) Cytoplasmic inheritance in plants:**

**(a) Plastid inheritance:** Good example of this phenomenon is variegated *Mirabilis jalapa*. In this plant some branches have variegated leaves. The variegation of leaves is controlled by some plasma genes present in cytoplasm. It is not concerned with chromosome. Similar inheritance of plastid is reported in beans, maize etc.

**(b) Pollen sterility in maize:** Working on maize Rhoades in 1933 reported a mutant race. This mutant race produced sterile pollen grains. When this mutant race was hybridized with normal fertile race then it could be known that the genes responsible for pollen sterility were inherited by cytoplasm present in egg cell.

**(c) Inheritance in rusts:** Rust is caused by parasitic fungus which causes disease in wheat and other crops. Inheritance of the fungus takes place by chromosome. Sometimes its inheritance is controlled by plasma genes.

**(d) Inheritance in Epilobium:** On hybridization of *Epilobium luteum* as female with *Epilobium hirsutum* as male. The F1 offsprings are fertile. But when the position of female and male was reversed (reciprocal cross), then the F1 produced sterile pollen. The experiment reveals that the pollen sterility gets inherited by cytoplasm of *Epilobium hirsutum.*

**(e) Cytoplasm and hybrid vigour:** Dhavan, Bhatt and Jagi (1965) have reported that in maize the cytoplasm controls hybrid vigour.

**(2) Cytoplasmic inheritance in animals:**

**(a) Cytoplasmic inheritance (transmission) in Drosophila:** Heritier and Teissier (1951) have reported that ordinary Drosophila (Drosophila *melanogaster* ) is resistant to carbon dioxide, but one particular race is very sensitive to carbon dioxide. The reason is presence of plasma genes. Carbon dioxide sensitive Drosophila was crossed with cabon dioxide resistant Drosophila. The female offsprings were hybridized with ordinary Drosophila. On repetition of this hybridization the offsprings were sensitive to carbon dioxide. This character is inherited by the cytoplasm of carbon dioxide sensitive fly.

**(b) Cytoplasmic inheritance in Paramecium:** Sonneborn (1949) reported a killer race of *Paramecium aurelia.* It produces toxic substances so kills other race of *Paramecium aurelia* . This killing character of *Paramecium aurelia* is controlled by Kappa Particles gene like material present in cytoplasm. Ordinary race of *Paramecium aurelia do* not have Kappa particles.

Genetic examinations have revealed that kappa particles are related to gene present in chromosome. In absence of this gene Kappa particles do not grow.

**Role of cytoplasmic inheritance in evolution:**

Darlington and Write have expressed that cytoplasmic inheritance has secondary role in evolution. However, Lamprecht (1944) has reported lot of importance of cytoplasmic inheritance because it may develop lethality and sterility. Moreover plasma genes, like nuclear genes, express mutation. Therefore plasmagenes are as important as nuclear genes in evolution.

# Chapter 15

# Sex Determination

Male and female two sexes are most popular. These can be identified by morphological, anatomical and physiological characters. These characters can be primary and secondary as described below.

(1) **Primary sex structures:** Primary sex structures are directly related to gonads, ovaries and testes. In higher female animals it is in form of vagina, uterus and oviduct. In males it is in form of penis, vas deferens etc.

(2) **Secondary sex structure:** Under secondary sex structures we consider accessory sex organs for example human females have more growth of breast. In male breast growth is less. In addition to it males have beards and mustache where as female females do not have these.

In humans and animals male and female sexes are present separately. The same phenomenon is also observed in some plants. But many plants are hermaphrodite.

**Sex determination:**

Sex determination differs from sex differentiation. According to Darlington and Mathur (1949) sex determination is a process by which male and female gametes give rise to male and female organisms. Sex differentiation is the anatomical, morphological and physiological changes during development of organism.

Before the knowledge of inheritance several biologists have attempted to know sex determination. Before 1900 in this connection some hypothesis were also put forth. But attention was more to external environment than inheritance by reproductive cells. Some scientists thought that sex of a child depended on relative vigour of their father and mother. If father is more vigorous then male child and if mother is more vigorous then female child is borned. Some scientists expressed that sex of a child was dependent on relative age of father and mother. Some scientists said that sex of a child depends on nutrition given to the mother. Some scientists opined that time of

copulation of parent had effect on sex determination of child. Some scientists have expressed opinion that sperm and egg producing organs (testes and ovary) have effect of sex determination of child. On discussion none of the ideas expressed above proved successful.

**Time of sex determination:**

As mentioned below sex determination can be possible in three times:

(1) Progamic: Before fertilization of egg cell.

(2) Syngamic: At fertilization.

(3) Epigamic: After zygote formation.

**Theories of sex determination:**

For sex determination two types of theories have been proposed:

(1) Genetic theories: Bateson, Emersion, Correns and other scientists have accepted the genetic theory.

(2) Physiological theories: Riddle and Goldschmat are the main scientist who accepted the physiological theory.

The above mentioned theories are enumerated below:

**(1) Genetic theories:**

(a) Alternate dominance theory.

(b) Heterogamosis theory or chromosome theory.

(c) Genic balance theory.

**(2) Physiological theories:**

(a) Metabolic differentiation theory.

(b) Quantitative theory.

These theories are discussed below in some detail:

**(1) Genetic theories:**

**(1 a) Alternate dominance theory:**

Alternate dominance theory was suggested by Castle. According to it in fertilization there is union of male and female gametes. From one type of gamete fertilization will not be possible. Hence for sex every gamete acts heterozygous. Castle also stated that dominance is also expressed in sex characters and for sex determination there are some determiners During fertilization if male gamete determiner is dominant then offsprings are male. In case if the female gamete

determiner is dominant then the offsprings will be of female sex. In modern time this idea is not accepted.

**(1 b) Heterogamosis or chromosome theory:**

This theory was presented by Correns. He pointed out that in fertilization out of the gametes uniting is heterozygous for one sex and homozygous for the other sex. Correns has proved this theory by cytological and genetical experiments.

**Sex determination by chromosomal mechanism:**

Sex determination by chromosomal mechanism was proposed first of all by Mc Clung (1902). Working on cytology of grass hopper Mc Clung has seen that in body cells of female there are all pairs of chromosomes but in male there is only one chromosome alone i.e. without any pair. In other words number of chromosomes is even in female but uneven in male. On this observation Mc Clung suggested that because in males number of chromosomes is odd, therefore during meiosis two types of gametes should be formed. In half number of gametes chromosome number should be odd and in other the chromosome number should be even. Mc Clung stated that in case of female grasshopper all the gametes should have even number of chromosomes. Moreover after fertilization with one type of male gametes the offsprings should have one type of sex. Similarly after fertilization with other type of male gamete the offspring should have other sex. The ratio between sexes should be 1:1. Therefore Mc Clung told that odd chromosome number is the sex determiner.

In 1905 E. V. Wilson and N.M. Stevens proposed some proofs to confirm chromosome theory of sex determination. He designated heterochromosome as X chromosome. He expressed that in male only one X chromosome is present but in female two X chromosome are present. Naming the absent X chromosome as O Wilson expressed the XO system. (Fig-29)

After few years Wilson again did experiments on sex determination on other insects. He told that though male and female have same number of chromosomes. However in female there are two x chromosomes but in male there is only one X chromosome. The other pair is very small and is called Y chromosome. Therefore on this basis the female has xx chromosome and the male has XY chromosome.

So from both angles the male has heterogametic or heterozygous gametes. Sex determination of any organism is done at fertilization.

If X chromosome of male fertilize the egg cell then it produces female sex. But if Y chromosome of male fertilize the egg cell then male sex is produced (Fig-29)

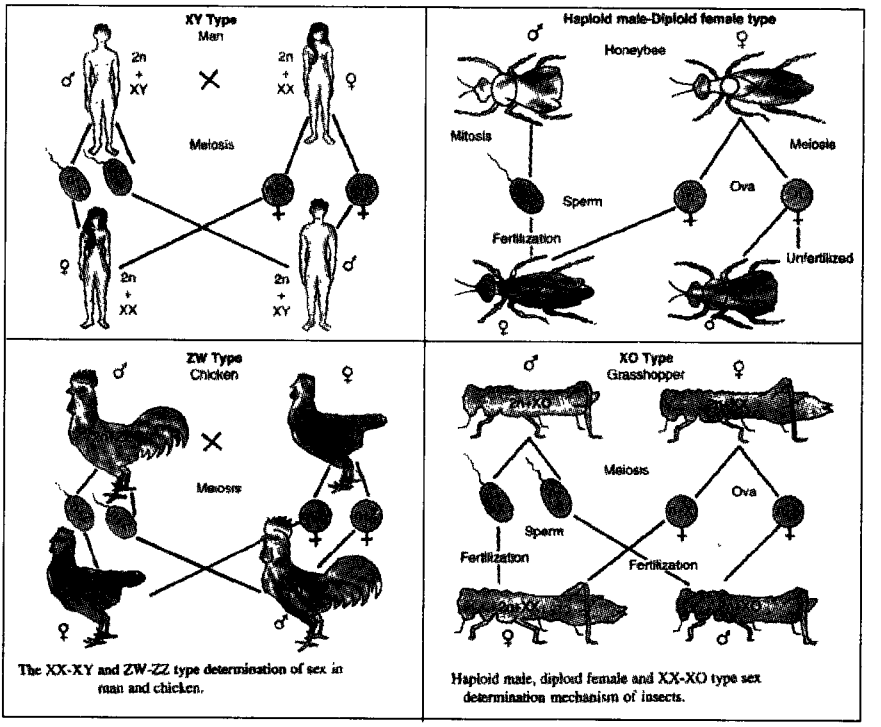



**Fig. 29: Sex determination systems**

Though in all organisms sex determination depends on one principal, but construction of chromosome is different in different animals. In human, animals and Drosophila and other insects female have XX sex chromosomes and male have XY sex hromosomes. In some animals Y chromosome is absent. On the hand in birds and moths female is heteroxygous and male is homozygous. In other words female has XY sex chromosome and male has XX sex chromosome.

In some insects, female has only one X chromosome and Y is absent. Therefore the female has XO chromosomes and male has XX chromosomes as shown in the following table

Types of sex chromosomes in various organisms

| Organism | Heteroxygous Sex | Gametes Sperm | Zygote Egg |
|---|---|---|---|
| Human, animals & drosophila etc | Male | XY | XY |
| Grasshopper & Parotiner bug | Male | XO | XX |
| Birds & moths | Female | XX | XY |
| Fumea moth | Female | XX | XO |

**Sexual functions of X and Y chromosomes:** X and Y chromosomes though are called sexual chromosomes but they not only do the work of sex determination because there are also genes which are unconcerned with sex. In XY type of sex determination it is understood that on Y chromosome there is no active gene for sex. Morgan on the basis of researches on *Drosophila melanogaster* concluded that X chromosome is free from gene. Bridges (1916) has seen a male in *Drosophila melanogaster* where Y chromosome was absent. Despite its size, shape and behaviour such fly was perfectly normal. But these were sterile. Not only this, a female has been seen in Drosophila where in addition to xx chromosomes there was one Y chromosome. But such a fly was perfectly normal in size, shape, behaviour and fertility. Therefore from these facts the conclusion can be drawn that in *Drosophila melanogaster* with presence of one X chromosome males develop and with presence of xx chromosomes female flies develop.

### (1 c) Genic balance theory:

Genic balance theory of sex determination was presented by Bridges in 1921. He explained that in any sex determination sex chromosome and body (somatic) chromosomes i.e. autosomes both works. X chromosome is tempted to produce female. Whereas autosomes is tempted to produce male. But all female determiners are not present on X chromosomes. Similarly all determiners for male are not present on Y chromosome. Both the type of determiners are infact present on X as well as autosome chromosomes. However female determiners are in dominant phase on X chromosome and male determiners are in dominant phase on autosomes. Therefore if compared to autosome the quantum of X chromosomes increases then sex determination goes to femaleness. Similarly if quantum of autosome is more than X chromosome then in that case sex determination is towards maleness. Therefore it is the balance between X and autosome which determine sex. In other words sex determination is dependent on ratio of X/A. It has been explained in (Fig-30).

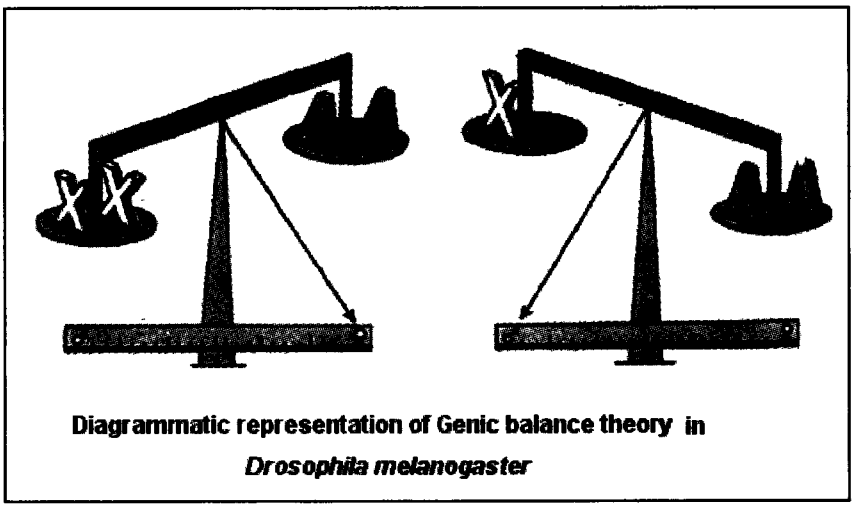



**Fig. 30: Genic balance theory.**

**(2) Physiological theories:**

**(2 a) Metabolic differentiation theory:**

Metabolic differentiation theory of sex differentiation was proposed by Riddle. He expressed that male or female sex is dependent on metabolic condition. According to him sex determination is not dependent on chromosome differentiation. To prove it he gave examples of sex reversal from male or female or vice versa.

**(2 b) Quantitative theory of sex determination:**

This theory was proposed by Goldsmith. Based on experiments he stated that quantity of certain harmone determine sex of organism. Quantity of harmone control metabolic condition. Sex determination depends on metabolic condition. Hence determination of sex depends on quantity of harmone.

**Sex reversion:**

In human beings, animals, birds etc. sex reversion have been reported. Explaining it Sharp (1934) mentioned that sex determination is not dependent only on sex chromosome. According to him sex chromosome is only a part of the system of sex determination. Like other inheritable characters sex development is also controlled by interaction of gene and environment.

The geneticist Emersion stated that in dioecious plant and some animals

genic balance is very delicate. Due to change in environment that genic balance get disturbed resulting in sex reversal. Shaffner working on *Crotolaria juncea* reported that growth of the plant in shallow soil and deficient light result in sex reversion. He also found out that by treatment of *Crotolaria juncea* plant with smoke of tobacco, sex reversion takes place. In tomato (*Lycopersicum esculentum*) deficiency in carbohydrate results no development of male sex and deficiency in nitrogen result no development of female sex. In case of castor (*Ricinus comunis*) too much pruning results more development of female sex organ. Edmonds ( 1930) reported thatin cucumber exposer of the plants for sharp light for long duration increases growth of male sex. On the other hand in reverse condition female sex grows more.

**Intersex and supersex in *Drosophila*:**

Bridges (1922) working on *Drosophila melanogaster* reported such females in which x chromosome and somatic chromosomes occurred in triplicate. Such triploid female fly resembled to diploid female in all respects including fertility.When this female was hybridized with diploid male, then 8 type of offsprings were produced.

(1) **Triploid female:** It has 3X chromosomes and 3 sets of autosomes.

(2) **Normal diploid female:** It has 2 X chromosomes and 2 sets of autosomes.

   a) Xxy female.

   b) Intersex: It has 2 X chromosomes and 3 sets of autosomes (Fig-31, 32).

   c) Intersex having 2 Xchromosomes, 1 Y chromosome and 3 sets of autosomes.

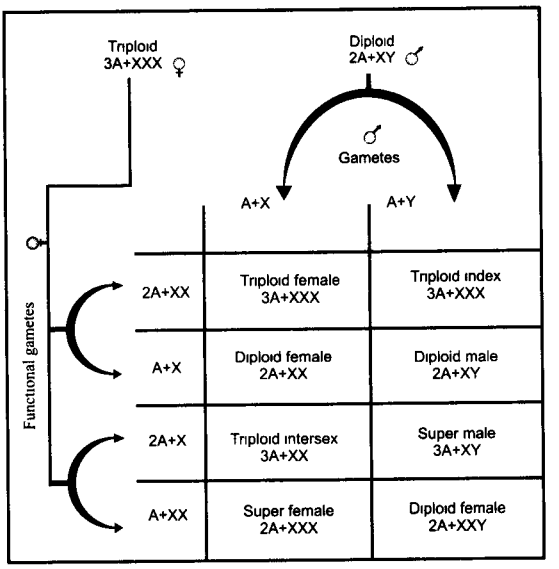
   d) Normal male: It has 1 X chromosome, 1 Y chromosome and 2 sets of autosomes.

   e) Super male: It has 1X chromosome, 1 Y chromosome and 3 sets of autosomes.

   f) Super female: It has 3 X chromosomes and 2 sets of autosomes.

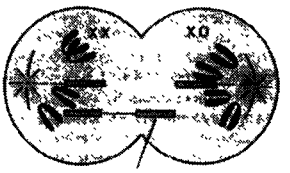
**Gynandromorph or Gynander:**

Sometimes in animals some parts of body is male and other part is female. Such animals are called Gynandromorph or Gynander. Gynandromorph has been observed in *Drosophila*, silk worm and




Results obtained from 2 Bridge's classical cross of a triploid (3A+XXX) female fly and a diploid (2A+XY) male fly *(Drosophila)*

**Fig. 31: Effect on sex of the balance between X chromosomes and autosomes in *Drosophila melanogaster* as observed by Bridges (1922).**




The loss of an X chromosome during mitosis in a 2A+XX cell and formation of two types of cells —XX and XO

| Phenotypes | | Number of chromosomes | Number of autosomes (A sets) | Sex index= No. X's / No. A sets |
|---|---|---|---|---|
| Super female | | 3 | 2 | 1 5 |
| Normal female | tetraploid | 4 | 4 | 1 0 |
| | triploid | 3 | 3 | 1.0 |
| | diploid | 2 | 2 | 1.0 |
| | haploid | 1 | 1 | 1 0 |
| Intersex | | 2 | 3 | 0 67 |
| Normal male | | 1 | 2 | 0 50 |
| Super male | | 1 | 3 | 0 33 |

Different doses of X-chromosomes and autosome sets and their effect on sex determination in *Drosophila*

**Fig. 32: Intersex in *Drosophila melanogaster.***

honey bees. Morgan and Bridges (1919) on the basis of chromosomal theory described gynandromorphy and told that these are produced by zygote having two x chromosomes in *Drosophila*. Individual with 2X chromosomes are female, therefore gynandromorphy develop first as female. But sometimes due to irregular cell division X chromosome gets destroyed. Therefore all the resultant cells have only one sex chromosome. Organism with one x chromosome are male. Organism with two x chromosomes are female. During occurrence of misdivision

of zygote one X chromosome disappear. Therefore half part of gynandromorph is male and other half part is female (Fig-33). But it is not necessary that division of zygote is misdivision. It is also possible that after the individual is half grown if at that time misdivision of cell occurs then ¼ part of the body become male and ¾ of the body becomes female. It is also possible that when growth of the organism is almost complete then misdivision of cell may happen deleting one X chromosome. In that case only a small part of the body shows male like symptom.

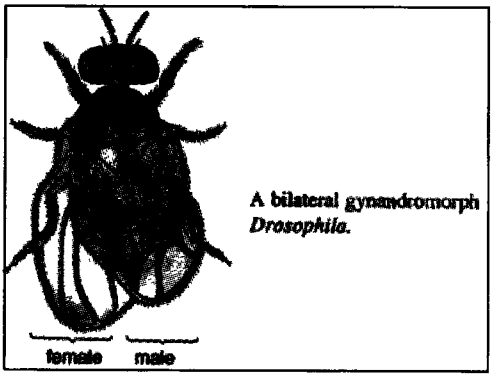



**Fig. 33: A gynandromorph of *Drosophila melanogaster* - the half side of the fly is female and other side is male.**

Goldsmidt and Katsuki working on sex determination of silkworm found out another mechanism of development of gynandromorph. They could observe such egg cell which had two nucleus. If one nucleus is fertilized with a sperm having X chromosome and the other nucleus is fertilized with a sperm having y chromosome. In that case the zygote with xx chromosomes are female and the zygote with XY chromosomes are male.

**Kinds of gynandromorphy:**

According to presence of sex tissue type gynandromorphys are of following types.

(1) **Bilateral gynandromorph:** Half of body is male and other half is female.

(2) **Anterio-posterio gynandromorph:** Front portion of the body is one sex and back portion of the body is another sex.

(3) **Sex piebalds:** In the body of female there are patches of male.

**Importance of sex determination**

Horticulture, crop culture and poultry have lot of importance of sex determination. Several fruits and crops are monosexual and also bisexual. It is important to identify the female and male sexes. Similarly in poultry it is also essential to identify male and female at the time of birth.

Chapter 16

# Sex Linked Inheritance

It has been explained in the chapter on sex determination that sex chromosome play a great role in heredity. Experiment by Morgan and Bridges have proved that sex chromosomes have special genes which are not found in autosomes (somatic chromosomes). By observations of Wilson it has been clear that both the heterogamic sex chromosomes x and y differs in structure and types of genes. Various genes which are present in sex chromosomes X and Y are known as sex linked genes. The characters governed by the sex linked genes are called sex linked characters. Trasmission of such sex linked characters from one generation to another is known as sex linked inheritance.

**Structure of sex chromosomes:**

There is enough difference between structure of X and Y chromosomes. Y chromosome is smaller in size. X chromosome is rod like and straight. Y chromosome is slightly bent on one end. It has been also observed that x and Y chromosomes have unequal arms. The small arm of X chromosome matches with one arm of Y chromosome. Other two arms are dissimilar (Fig-34) and nonhomologous. It has been seen that in non homologous parts of X and Y chromosomes there are incomplimentary genes. On the other hand on homologous parts there are complimentary genes. During meiosis non homologous parts never do pairing and never have crossing over. In this way the genes on non homologous parts are completely linked. On the other hand homologous parts do have pairing at synopsis stage of meiosis and also form chiasma and crossing over. Therefore genes of homologous parts are incompletely linked.

**Kinds of sex linked inheritance:**

Inheritance of sex linked genes is different from inheritance of ordinary genes. Non homologous parts behave in different ways. Sex linked inheritance are of following three types.

(1) Digenic sex linked inheritance.
(2) Holandric sex linked inheritance.
(3) Hologenic sex linked inheritance.
(4) Diandric sex linked inheritance.

**These 3 sex liked inheritance are described below:**

**(1) Digenic sex linked inheritance:**

Digenic sex linked inheritance is those genes which are present in nonhomologous parts of completely linked genes and behave like ordinary genes. These genes are passed on in offsprings through the female. There never go directly from father to offsprings. Such inheritance is called digenic inheritance and such genes are known as digenic genes.

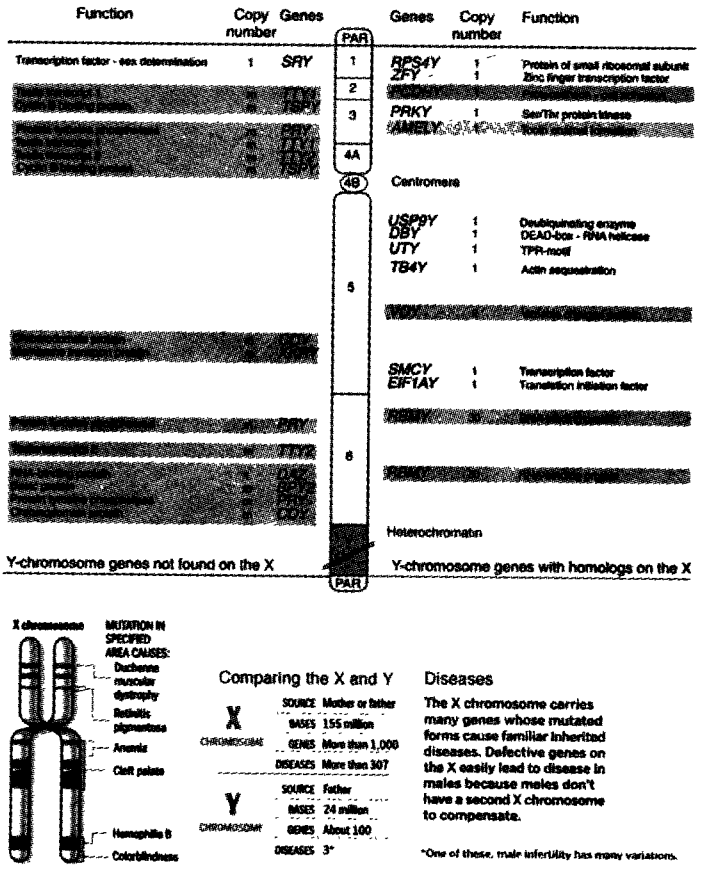



**Fig. 34: Structure of human sex chromosomes.**

**(2) Holandric sex linked inheritance:**

These are those genes which are present in non homologous parts of Y chromosome. These genes are completely linked to Y chromosome. Those genes are directly transmitted from father to offsprings. This type of inheritance is called holandric inheritance and such genes are known as holandric genes.

**(3) Hologenic sex linked:**

The two X chromosomes of female behave in such a close way that these chromosomes are completely homologous. One X chromosome of female passes on directly to daughters. Such inheritance is called hologenic sex linked inheritance and such genes are called hologenic genes.

**(4) Diandric sex linked inheritance:**

If the X chromosome from mother first passes on to sons and then it get transferred to daughters, then such a inheritance is called diandric sex linked inheritance and such genes are called diandric genes.

**Sex linked inheritance in Drosophila:**

Based on structure of sex chromosomes *Drosophila melanogaster* and human being both have XX in female and XY in male. In this fly Morgan for the first time studied sex linked inheritance. When Morgan was rearing cultures of red eyed *Drosophila* at that time he saw white eyed fly. First he thought it to be a mutant. But when Morgan hybridized this white eyed male *Drosophila* with red eyed female Drosophila he observed that all the offsprings (males as well as females) has red eye (designated by the letter W). Morgan concluded that W for red eye was dominant over w which was responsible for white eye. When male and female of F1 red eyed Drosophila were hybridized then in the F2 he found 3 red eyed and 1 white eyed Drosophila. But noteworthy matter was that all the white eyed *Drosophila* were male only. Red eyed *Drosophila* were also present in equal number.

The most astonishing matter was that all the females had red eyed only. From this observation Morgan concluded the w gene responsible for white eye was located in X chromosome of male Drosophila and w responsible for white eye expressed only in absence of W gene.

In the above mentioned example the gene w is first transmitted into daughters. Afterwards it get transferred in sons. To verify these results Morgan hybridized F1 heterozygous red eyed females with

hemizygous or homozygous white eyed male parent (test cross). He observed that in female Drosophila half number was with white eye and in male Drosophila half number was with red eye.

**Sex linked inheritance in human**

One good example of sex linked inheritance in human being is color blindness. Color blindness is of several types and in different grades. The most common of color blindness is the inability to differentiate between red and green. It is known as 'red-green' color blindness. If a person is unable to identify red color then technically it is called 'protonopia' If a person is unable to identify green color then it is technically called as 'deuteranopia'. Protonopia and deuteranopia both are hereditary. The responsible genes are recessive and sex linked. Following facts have been observed in this regard:

(1) Women having normal eye whose father were a color blind, when married with a normal eyed male produces children of who half have normal eyes and other half have color blind eyes.

(2) Color blind women produce color blind sons. If husband of such a women is color blind then in the offsprings only the girls will be color blind.

(3) Generally father of a color blind women is also a color blind.

(4) Color blindness disease is more common in gents as compaired to ladies.

In USA color blindness is found in 8% gents and 0.5% ladies. Why this disease is more common in gents? The answer of this question can be found if we imagine that the gene responsible for color blindness is present on X chromosome and the heterogametic sex XY is male.

If a normal eyed man marries a color blind women, in that case XX chromosome of that man will be transferred to his daughters only, it does not reach to sons at all. From that woman out of two X chromosomes one XX goes to daughter and the other goes to son. Because the gene for color blindness is recessive to the gene responsible for normal eye. In man (male) there is only one XX chromosome which comes from color blind mother. Therefore all their sons will be color blind. In daughters there are two XX chromosomes, out of which one come from father and the other come from mother. Because one XX chromosome from father there is gene for normal eye which is dominant therefore all daughters of F1 have normal vision. In these daughters the gene for color blindness is in

recessive form. Therefore if these girls are married with man having normal vision, then all the female offsprings will have normal eye but half of the male offsprings will have normal eyes and other half male offsprings will have color blind eyes. When color blind man marries women who have homozygous or heterozygous color blindness in that case they will produce color blind female offsprings. But such marriages generally do not take place. This inheritance is fully dependent on X chromosome. It can be also understood by Fig-35.

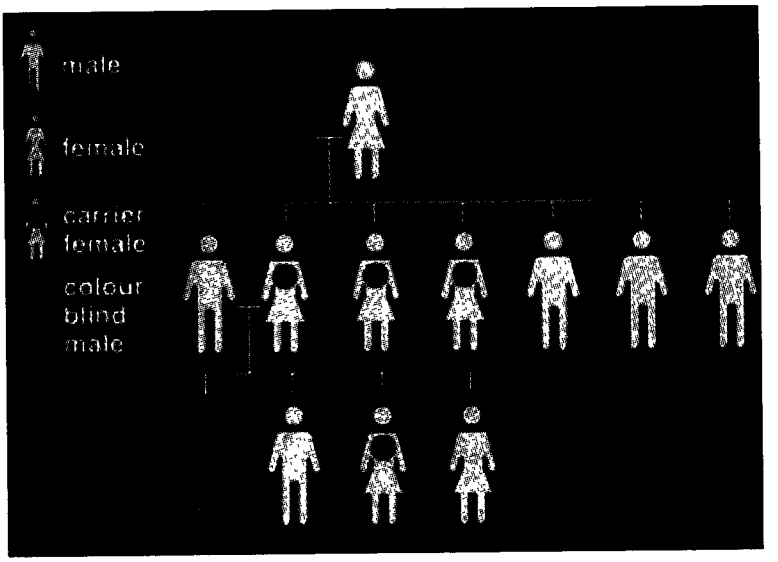



**Fig.35: Inheritance of color blindness.**

**Inheritance of sex linked characters through Y chromosome:**

We have understood before that the sex linked genes present on X chromosome, do not have any allele on Y chromosome. This is the reason that when the recessive gene is alone in male then only male express itself. Compared to X chromosome Y chromosome is rather inert. In other words the genes of X chromosome do not have allele on Y chromosome. But in *Drosophila melanogaster* there is bobbed gene. This gene has allele on X as well as on Y chromosome. Due to bobbed gene the Drosophila produces normal bristle. The mutant gene which is recessive produces slender bristle. When in male X chromosome has mutant of bobbed gene and Y chromosome has bobbed gene then the offspring produces normal bristles. If a male having such normal bristle, is crossed with a female having bobbed mutant genes on both

X chromosomes then all the male offspring will have normal bristles. But all the females will have slender bristle. In other words normal bristle is transmitted from father to male offsprings. Therefore we can say that inheritance of bobbed character is dependent on Y chromosome. Another example of auditory condition can be well understood by Fig-36.

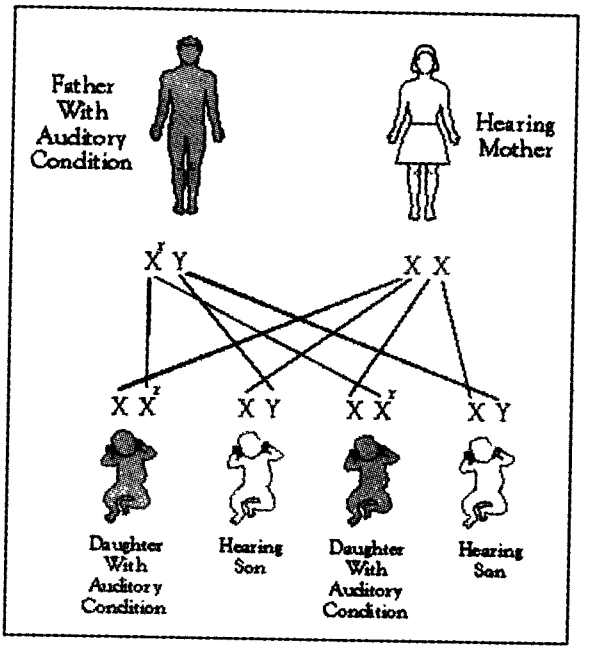



**Fig.36: Inheritance of sex linked character through Y chromosome.**

**Sex influenced characters:**

There are some genes whose two alleles express dominant effect in male and female sexes. In other words the allele which is dominant in male, the same allele expresses recessive in female. Similarly the allele which is dominant in female the same allele expresses recessive in male. Such genes are called sex influenced characters or genes.

**Sex limited character:**

There are certain characters which are expressed in one sex only. The governing genes are present on autosomes. Such characters are called secondary sexual characters or sex limited characters. Its inheritance is called sex limited inheritance. Appearance or no appearance of such characters depends on sex hormone which is produced in ovary or testis.

# Chapter 17

# The Gene

From Mendel time importance of gene increased tremendously. In chapters so for, on every step we were concerned with gene. In genetics every action is dependent on gene. Physical nature of gene and its chemical structure were not understood well till recently. In modern times genes have same place in science which was had earlier by hormones and vitamins. Now we very well know where the genes are located, how they express themselves and what is their physical and chemical nature. Geneticists, cytologists and biochemists all over the world are busy in analysis and trying for artificial production of genes. After artificial synthesis of genes many difficulties of human beings will disappear. Then man can manipulate so many important affairs such as mental capabilities, sex power, excellent health etc. He will be able to produce more desirable varieties ofplants and animals. Detail information about genes are given below:

**Definition of gene:**

Based on studies of Watson and Crick (1958) and Wilkins (1962) genes can be defined as mentioned below:

Gene is a molecule or a chemical radicle made of carbon, oxygen, hydrogen, phosphorus, nitrogen and deoxyribonucleic acid which are attached on a string chromonema made of protein. Genes are transmitted from one cell to another, from one generation to another without any change in their shape and structure.

**Physical nature of gene:**

Complete information on physical nature of gene is still not available. Whatever knowledge available on that basis it will be explained that where genes are located and what is their shape and size:

**(a) Position of genes:**

We all know that genes are located on chromosomes. But in chromosome where are the genes located? Answer to this question was first presented by Demerec (1949). He explained that most

important part of chromosome is chromonema, which is in form of thin string in full length of the chromosome. It is homogenous in the whole length and uniform in chemical and physical nature. Genes are located on this chromonema. Genes differ from each other chemically.

**(b) Shape of gene:**

Gene could not be seen so for. So regarding its shape nothing can be mentioned. However by electron microphotographic technique it could be now possible to through light on its shape. In this respect work done by Slizynski (1952) explained that on salivary gland chromosome and work done by Stanley (1952) is on tobacco mosaic virus. Slizynski (1952) explained that on salivary gland chromosome of *Drosophila melanogaster* there are numerous dark coloured bands which are probably genes. Stanley (1952) expressed that virus looks like a rod and behave similar to gene in all fundamentals. Based on conclusions drawn by these scientists genes are very minute and rod likes structures. This idea is supported by x ray induced mutation's target theory. In addition to it, this also get support from a observation that if gene has dot like structure then there will be much less surface for hitting with radiation. But it is a fact that with hit by a small dose of radiation there is a change in gene. Therefore it is clear that shape of gene is not like a dot but it is small rod like.

For determining the shape of gene the most important work was the work of Watson and Crick (1958) and Wilkins (1062). They have presented the virtual shape of gene. According to these scientists gene's shape is spiral where two rod like structures overlaps on each other spirally in a helix shape.

**Size of gene:**

To find out size of gene is a very difficult work. Electron microscope has helped to some extent in this task. But to see by electron microscope the genetic material has to be treated with several chemicals. Due to these treatments the gene undergoes changes. The result is that it becomes extremely difficult or almost impossible to find out exact size of gene. Therefore beside electron microscope ionizing radiation are used for this purpose. After hitting chromosome by ionizing radiation (particles) mutations are measured and size of gene is computed. But this technique is not fool proof. Therefore scientists have put forth another technique. In this technique first number of genes is found in a chromosome. Afterwards by calculation size of gene is found out. Though this technique also has shortcomings, even

then possible size of gene can be presented which can be compared by virus. According to geneticists gene is a macromolecule which measures between 15 mu x 100 mu and 20 m u x 150 mu .

**Chemistry of gene structure:**

Gene is made of 5 elements viz. carbon, hydrogen, oxygen, phosphorous and nitrogen. These elements unite and form nucleic acid. Nucleic acids are of two types viz. 1- DNA (Deoxy-ribonucleic acid) which is present in nucleus. 2- RNA (Ribo nucleic acid) which is mostly present in cytoplasm. DNA and RNA both are made of numerous nucleotides. These nucleotides are arranged well on each strand of helix. Each nucleotide is made of a phosphate group and a nucleoside. It is a complex molecule.

Phosphate group is a derivative of phosphoric acid (H3 PO4). A nucleotide is of primary phosphate type. If two or more nucleotide unites then it become secondary phosphate type. The difference between these two types of phosphate group is in number of hydrogen atoms

Nucleoside is a chemical unit like nucleotide which is made of sugar and a nitrogenous organic base. Sugar by which nucleoside are made of have pentose nature like glucose. In that there are 5 atoms of carbon

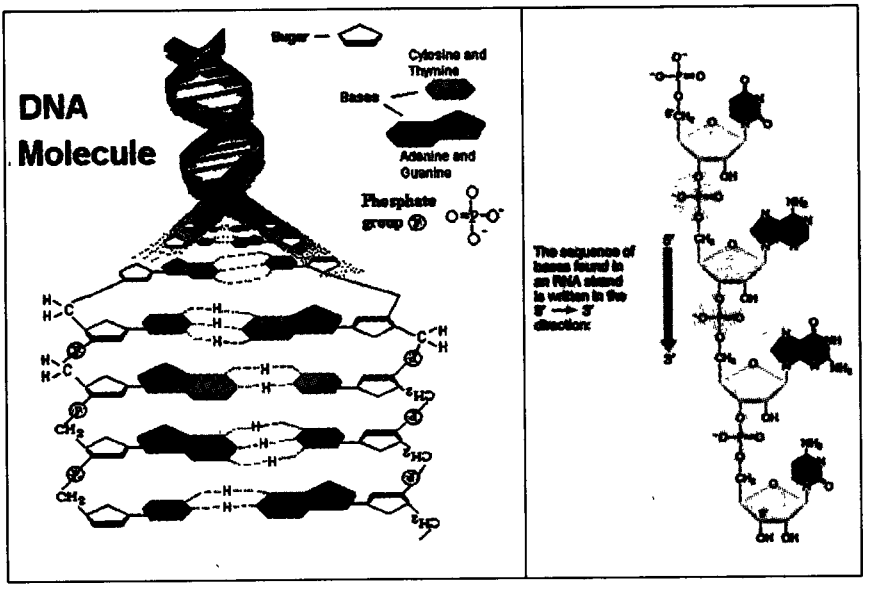



**Fig. 37: Double helical structure of DNA (gene)**

in a chain. These sugars differ from DNA and RNA nucleoside. In RNA it is ribose and in DNA it is deoxyribose. Because the nucleoside obtained from nucleus is deoxyribose therefore this nucleic acid is called deoxyribose nucleic acid or DNA. DNA is considered to be a gene (Fig-37).

Sugar may be ribose or deoxyribose made of 5 carbon atoms. On first and fourth carbons there are a independent and a solitary oxygen atoms. In the structure there are hydrogen atoms and hydroxyl groups (OH). In ribose sugar there are 6 hydrogen atoms. In deoxyribose sugar there are 7 hydrogen atoms. Another difference is that in ribose sugar there are 4 hydroxyl group, but in deoxyribose sugar there are 3 hydroxyl (OH) group (Fig-38 ).

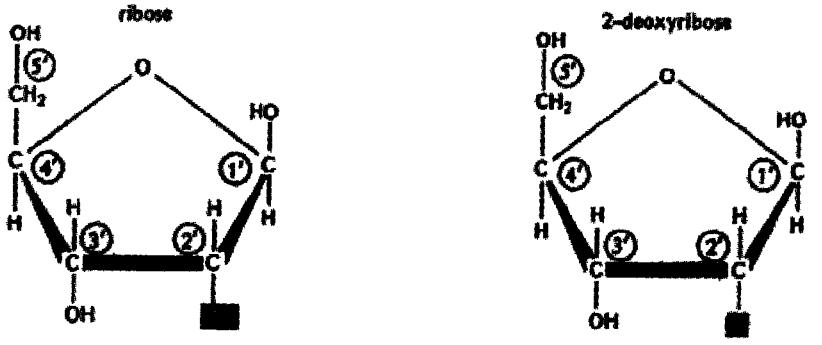



**Fig. 38: Chemical structure of ribose and deoxyribose.**

Nitrogenous base are ring like organic compound. These are of two kinds eg. 1) Single ringed pyrimidines and 2) double ringed purines.

Pyrimidine are of three types eg. Cytosine, Thymine and Uracil. Purines are of 2 types e.g. Adenine and Guanine.

In DNA there are adenine, guanine, cytosine and thiamine. But in RNA there are adenine, guanine, cytosine and uracil. In every nucleotide sugar is joined at one side with phosphate and at other side with purine or pyrimidine base ( Fig-39).

**Following are the main features for chemical nature of nucleic acid or genes** (Fig-40):

(1) Nucleosides of both the strands of helix are joined together with 2 or 3 unstable hydrogen bonds.

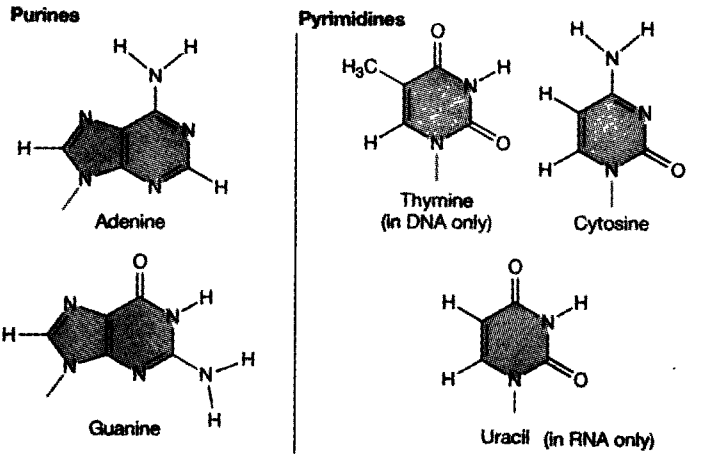



**Fig. 39: Structure of purine and pyrimidine base.**

(2) Pyrimidine nucleotides of one strand of helix are joined with purine nucleotides of other strand of helix.

(3) Adenine base (purine) of a nucleotide are joined with thymine base ( pyrimidine) of opposite nucleotide in DNA, but in RNA it is joined with uracil base.

(4) In DNA and RNA guanine base ( purine) of one strand are joined with cytosine base of other strand .

(5) Nucleotides are arranged one behind the other in straight line. Thus it confirms linear arrangement of genes.

(6) Nucleotides of strands of helix are joined to each other by sugars.

(7) On each strand of helix nucleotide are arranged in sets of 3 viz. adenine- cytosine - guanine. The set of 3 nucleotide is known as triplet or trinucleotide

In DNA molecule the exact number of nucleotides and actual sequence of triplets in gene are yet not clearly known. In addition to it exact length of helix is yet to be known because in the process of extraction helix gets broken. However based on available records number of nucleotide pairs is around 300000 and molecular weight of nucleotide is about 300. It has been calculated that difference between nucleotides is 3 to 4 Angstron units. Length of DNA molecule is about 60000000 or $6 \times 10^6$ Angstron units. Sequence of nucleotide in DNA is called genetic code.

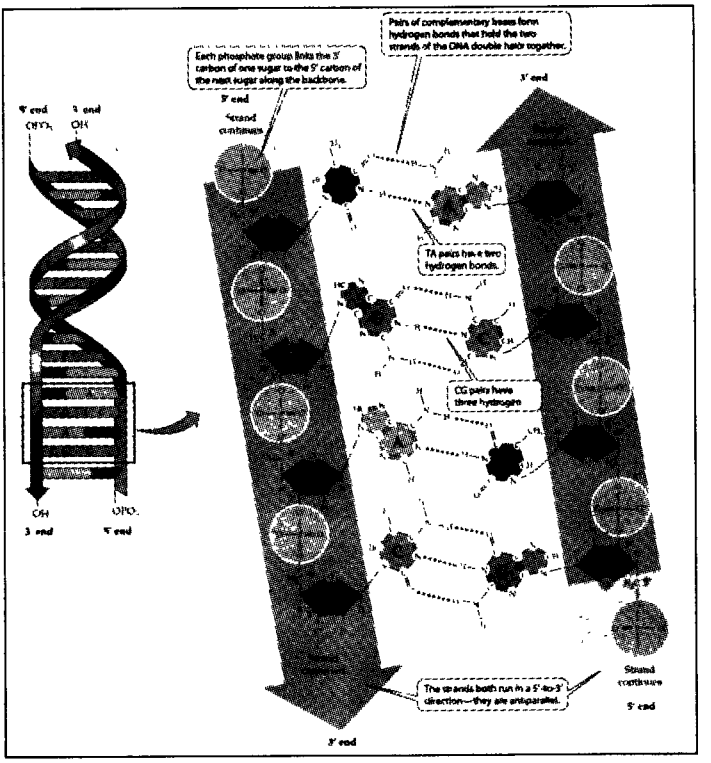



**Fig. 40: DNA double hehix.**

**Sub units of gene:**

Gene is made of following sub units:

**1. Cistron:**

Benzer (1962) named the largest segment of gene as Cistron. This name is based on that segment of DNA which has polypeptide chain. In Cistron there is a long chain of nucleotides. It is capable of mutation. Mutation and recombination takes place in Cistron.

**2. Muton:**

According to Benzer (1962) mutton is that smallest part or Cistron which is capable of mutation. Experiments on bacteria and several proteins have indicated that muton can be as small as a nucleotide.

**3. Recon:**

The third subdivision of gene is recon ( Benzer, 1962) Recon is the

smallest unit of DNA which is capable of crossing over and recombination. A recon may be as small as a nucleotide of DNA or RNA.

**Properties of gene:**

Following properties of genes have been found out by a lot of research work.

(1) Physical nature of gene is similar to virus.

(2) Except mutated genes all other genes are useful to the organisms. Some mutated genes are also useful.

(3) Gene has the capacity to replicate to its own type.

(4) Any gene can mutate.

(5) Every gene is made of molecules of sugar, phosphate, purine bases (adenine and guanine) and pyrimidine bases ( cytosine and thiamine).

(6) Gene is made of numerous nucleotides.

(7) Every gene has the capacity of crossing over and recombination.

(8) In chromosomes genes are arranged in linear fashion.

(9) In one cell division genes replicate only once. Every gene controls a type of enzyme production. The enzyme controls some physiological and metabolic functions.

(10) Every gene produces m RNA and sends it to cytoplasm. The m messenger control synthesis of protein.

(11) Process of gene replication occurs only in living cells.

(12) Every gene is made of 3 sub units viz. cistron, muton and recon.

(13) Very closely located genes exhibit linkage.

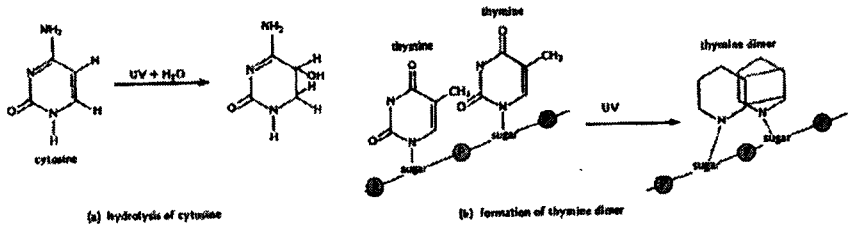
**Chemistry of gene mutation:**

Till chemical structure of gene was not known, the process of gene mutation was only an imagination. But now it is clear that due to mutation there is some change in the sequence of nucleotide- it is infact gene mutation. For example if in a gene the sequence of nucleotides is ATCTGA / TAGACT. If sequence of a nucleotide gets changed due to mutagen then the character and effect of the gene also get changed. Therefore on this basis it can be said that a change in sequence of nucleotide is mutation.

**Action of physical mutagen on gene:**

Among all physical mutagen the heat (through sun light) is an important mutant because due to increase in temperature in nucleotide pairs, the hydrogen bond of purine and pyrimidine bases become week. Because of weekness of hydrogen bonds in nucleotides ir. place of adenine the guanine comes and in place of cytosine the thymine comes. This is also a type of mutation. Ultraviolet (UV) rays may also cause mutation.

In case of mutation induced by radiant energy, in cells, free water molecules are produced which when collides with nucleotides, hydrolyses the bond between bases. In addition to it sequence of nucleotides get changed. In other words mutation occur (Fig-41).




(a) hydrolysis of cytosine (b) formation of thymine dimer

**Fig.41: Action of ultraviolet rays on gene in producing mutation.**

**Action of chemical mutagen on gene:**

For induction of mutation several chemicals have been discovered. Mustard gas is quite effective. In addition to it Methyl Ethane Sulfonate, Ethyl Methane Sulfonate, 5 Br-Uracil and HNO 2 are quite important.

**Action of HNO 2 on gene:**

Action of HNO 2 on gene has been explained by Fig-42. With effect of HNO 2 adenine gets changed to Guanine and Cytosine gets changed to Uracil or Thiamine. Due to these changes the sequence of nucleotides also get changed. So this becomes mutation.

**Action of 5 Br-Uracil:**

The process of gene mutation production by 5 Br-uracil can be well understood by (Fig-43). If a gene has nucleotide sequence of ABCT / TCGA. When 5 Br-uracil acts on this gene then this chemical mutagen come in contact with nucleotides ACGT. This DNA (gene) during replication breaks off into two strands AGCT and TCGA. Because 5

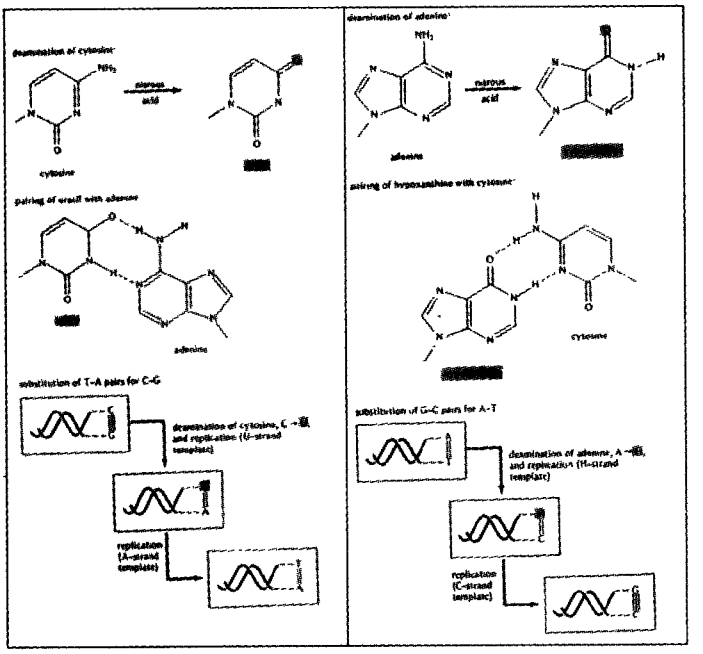


**Fig. 42: Action of HNO 2 on gene in producing mutation.**

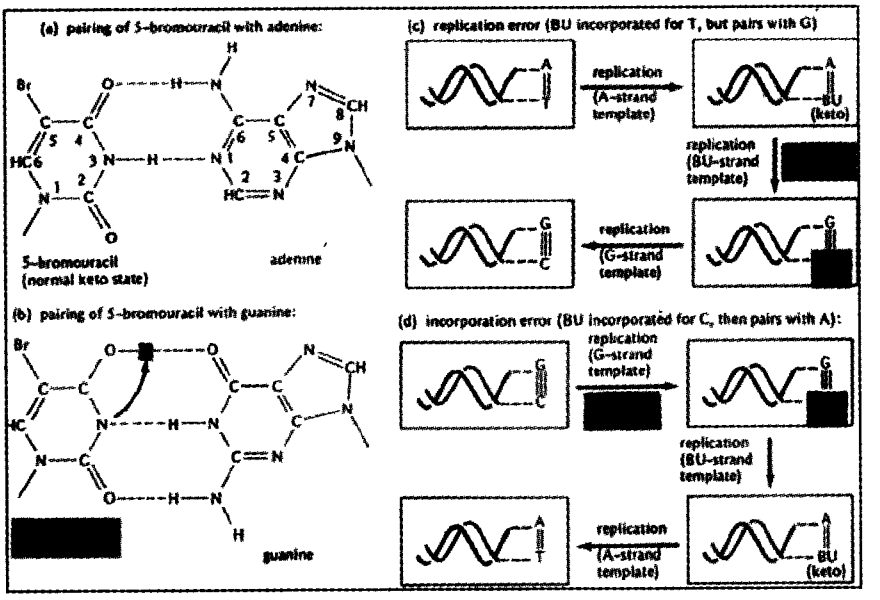



**Fig. 43: Action of 5 Br-uracil on gene in producing mutation.**

Br-uracil ( 5 BU) is just like thymine. Therefore 5 Br-uracil get replaced in place of T ( Thymine). Therefore after replication along with TCGA strand there is a modified strain AGC 5 Br-uracil. Similarly TCGA becomes 5 Br-uracil CGA. Therefore from old DNA the two new DNA will be AGCT / AGC 5 Br-uracil and TCGA / 5 Br-uracil CGA. This change in nucleotide is the mutation. During second replication 5 Br-uracil (5 BU) replace Guanine (G) instead of Thiamine (T).

# Chapter 18

# The Gene Complex

It is clear that every character of organism is affected by gene. Till Mendel's time it was believed that every character was controlled separately by individual gene. Afterwards the researches of several geneticists (e.g. Bateson, Punnet, Shull, Nelson, Ehle etc.) have proved that every trait (character) is controlled by combined effect of more than one gene. Scientists have also observed that different genes control characters differently. Sometimes one gene controls more than one character and sometimes 2 or 3 genes control only one character. These facts reveal that genes behaviour is quite complicated. Therefore studies of gene behavior is known as gene complex.

According to behavior, genes can be classified as mentioned below:

(1) Basic genes
(2) Lethal genes
(3) Multiple genes
(4) Cumulative genes
(5) Pleiotropic gene
(6) Modifying gene

These are described below:

**(1) Basic genes:**

Basic genes are most common. These are of following types:

**(A)** Normal gene: Single pair of gene which express one character is called normal gene. For example in rat gene CC for skin colour.

**(B)** Complimentary gene:Genes which independently have certain effect, but together the genes express different effect. Such genes are called complimentary genes.

**(2) Lethal gene:**

The gene which has effect of killing the organism is known as lethal gene. Lethal gene generally expresses their effect at embryonic stage.

Some times lethal gene expresses their effect later on.

Lethal genes are found in almost all types of plants and animals. However they express only in homozygous phase. When they are in heterozygous phase then development of individual is normal and presence of lethal genes is not noticed. But the organism having lethal gene in heterozygous condition when produce offsprings then those offsprings die which get lethal gene in homozygous condition.

**Example of lethal gene:**

Lethal gene can be easily understood by examples of rats. When hybridization is done between such rats which express yellow color in heterozygous condition. Then the offsprings do not show the ratio of 3:1, but the offsprings show a ratio of 2:1. The reason for this abnormality is presence of lethal gene.

Suppose yellow heterozygous rats have the genotype Yy, then at gamete formation Y and y genes will go to different gametes. Now if Y and y sperms of male rat join with Y and y egg cells of female rat then the offsprings will be of 4 types. But the offspring having YY in homozygous condition will die. This type of lethal effect has been observed in several organisms.

**(3) Multiple genes:**

Some times two or more pairs of genes interact and produce a phenotype. Such genes are called multiple gene. These are of following two types:

(A) Duplicate genes: If two pairs of genes control a phenotype then these are called duplicate genes.

(B) Triplicate genes: Triplicate genes also have effect like duplicate genes. The difference is that instead of two pairs there are three pairs of genes.

**(4) Cumulative genes:**

Due to cumulative action of dominant genes expression of characters is intensified. Such genes are called cumulative genes.

**(5) Pleiotropic gene:**

Gene which expresses more than one character at a time is called pleiotropic gene.

**Example of pleiotropic gene:**

In house rat the gene dw not only causes dwarfness but also causes other characters such as sterility, improper development of thyroid gland and absence of secondary sexual characters, The reason is that dw gene control harmone development by pituitary gland. Because pituitary gland controls almost every part of body. Therefore if there is some disturbance in pituitary gland that causes change in tissues, organs and systems. Infact dw gene stops the growth of eosinophilous cells which forms harmone in the gland. Disturbance in pituitary gland stops or effects the secretion by gonadotrotin, thyropin and stops the development of growth harmone. Stopage of these harmones results sterile condition of gonads, defective growth or dwarfness. In rats having dw gene which inhibits function of pituitary gland, if by surgery a functional pituitary gland is implanted then the growth, fertility etc become normal.

**(6) Modifying gene:**

Those genes which do not produce an effect themselves but has capacity to modify the character are called modifying genes. These genes are of two following types:

(A) **Supplimentary genes**: These genes do not produce any effect but by interaction with other genes intensify or dilute the expression of character.

(B) **Inhibiting genes:** Inhibiting genes do not produce any effect itself but interact with other genes to prevent or reduce the action of other genes. Inhibitary gene is also called epistatic gene. It has been presented in detail in the chapter laws of inheritance.

# Chapter 19

# Gene Action

Characters of organism express because of cumulative effect of some chemical action. It is now clear that genes responsible for one character do not act at one time. However they come in action one after the other. Each gene produces enzyme. Enzymes are produced by autocatalysis activity with the chemical substance present in cell. One enzyme performs only one chemical action which is in fact required for activation with second enzyme. On changes brought by second enzyme, the third enzyme exerts its effect. That is how this activity continues on. In this chain of reactions if there happens any mistake by any enzyme then the whole chain gets disturbed. For example if enzyme a, b, c act on substance A and changes it in to the substance D. If the enzyme b is not produced due to any reason then as explained below only the substance B will be formed.

Gene a gene b gene c

Enzyme a′ enzyme b′ enzyme c′

I A_____B_____C_____D

II A _a′____c′_____B

(no more changes afterwards because b′ enzyme is absent)

The above mentioned diagram clearly express that any enzyme acts only when there is proper background. For example the enzyme c′ will act only when the substance C is available. One more fact is clear from this diagram that indirect effect of gene also produce character.

Some important actions of genes are mentioned below:

(A) Genes and pigment: Before understanding of gene action it is important to know about pigment. In different organisms different pigments are produced. Knowledge has been available about chemistry of pigment production.

We will discuss production of pigment and color production in some plants and animals as mentioned below:

**Genes and flower color:**

In flowers pigments are produced generally by anthocyanins & anthoxanthins and their chemical derivatives. Both of these pigments are dissolved in cell sap. Anthocyanin produces various colours such as red and blue and also pink, purple, magenta & lavender. Anthoxanthins produces yellow and ivory colours in flowers. Anthocyanin and anthoxanthin are made of one type of ring structures which are derivative of sugar.

Anthocyanin pigment's one or two molecules are of sugar those are joined with phenyl ring - peragonidin. In phenyl ring of peragonidin on fourth carbon there is one OH group. It produces scarlet colour. By the increase of OH (hydroxyl group) in phenyl ring the colour changes from red to blue. In Delphindin there are 3 OH groups. Therefore it produces blue black or violet color. Researches have revealed that number of OH group is controlled by special genes.

In this connection one thing we should know that anthocyanin in acidic medium is red colour and in alkaline medium it become blue color. Therefore by growth in acidic and alkaline medium redness and blueness increases respectively.

Several geneticists such as Lawrence, Scottt Moucrieff, Sturgess and Wheldale on basis of several experiments have proved that in flowers several colours such as red, blue, yellow, ivory etc. are produced by action of special genes. These actions can be of several types for example number of hydroxyl group, oxidation & reduction action on third, fourth & fifth carbon atoms on phenyl ring and acidity & alkalinity of cell sap or in other words change in pH. Actions of genes can be well understood by following examples:

**Example-1**

In case of flowers of Streptocarpus due to actions of several genes seven different colours are produced. *Streptocarpus rexii* flowers are of blue colour. *Streptocarpus dunnii* flowers are of red colour. In these flowers anthocyanin pigment is produced by gene A. Flowers having aa gene do not produce anthocyanin so the flowers attain ivory (white) colour. In Pelargonidin the pigment anthocyanin is produced in absence of R and 0 genes. In Cyanidin anthocyanin is produced in presence of gene R. In Delphinidin it is produced in presence of gene 0. In presence of gene R there is increase of one OH or CH 2 group in phenyl ring. It makes the colour as rose or magneta. Due to presence of gene 0 there is increase of CH 2 and OH groups. With the result

the colour changes to blue or mauve. In addition to it gene action takes place through substitution of molecules also. For example on third or fifth position of carbon in anthocyanin molecule, a molecule of hexose get substituted. In a molecule of gene dd hexose and pentose are located on third carbon atom and at fifth carbon atom there is no substitution. This type of changes produces blue and salmon color.

**Example-2**

In *Dahalia variabilis* plant gene B produces anthocyanin and gene I produces opigen (a type of anthxanthin). It may happen that due to polyploidy number of genes B and I increases. Accordingly intensity of colour gets affected as mentioned below.

bbbbiiii = No pigment (white)

Bbbbiiii = Cyanin (chocklate)

bbbbIIii = Epigen (ivory)

BbbbIIii = Cyanin & Epigen (purple)

BbbbIIIi = Cyanin, Epigen (carmine) & Pelargonidin (magneta)

BBbbIIii = Cyanin (carmine) & Pelargonidin

**Genes and pigments in animals:**

Like in plants color (pigment) production in animals take place due to gene action. In animals black color occurs due to melanin pigment. Melanin pigment is produced by enzyme action on colorless chromogen tyrosine (amino acid).

In Negro and black animals amont of melanin is more. Therefore their colour is black. On the other hand in white animals and fair colour persons the amount of melanin is less. In the above mentioned figure oxidation of tyrosine to form dihydroxyphenylanine happens because of dominant gene C. The same gene C produces the enzyme tyrosinase. Whenever the gene c is in homozygous condition (cc) then production of tyrosinase stops. Therefore melanin production also stops. The result is albinism or white colour.

Wright on basis of experiment has stated that in animals colour development takes place due to action of two enzymes. In white as well as black both animals chromogen is found in equal amount. Both enzymes have capacity to oxidize the chomogen. But the process of oxidation takes place in two different types.Wright has also stated that if the enzyme I is present then chromogen is oxidized into yellow

pigment but in addition to I if there is double dose i.e. II then II enteract with I and produce brown or black pigment. But if II are alone then no pigment is formed. Appearance of different colors depends on quantity of both enzymes as mentioned below:

(1) Chromogen (equal number of Enzyme II and Enzyme I) >Black

(2) Chromogen (unequal amount of Enzyme I & II) >Black

(3) Chromogen (enzyme I half quantity) >Red

(4) Chromogen (enzyme II) >No colour (white)

**Gene and eye color:**

For appearance of different colours in eyes of insects gene action was explained by Kuhn, Beadle and Ephrussi based on their experiments. In *Drosophila melanogaster* and caterpillar of Ephastia, eye colour and body colour depends on the presence of Ommochrome pigment. This pigment is produced from amino acid tryptophan by action of enzymes produced by some genes. In these organisms there are two genes V and Cn which produce two types of enzymes V′ or *Tryptophane pyrholase* and enzyme Cn′. By the action of enzyme V′ on tryptophan one amino acid Kynurenine is produced. On this the enzyme Cn acts and changes it to 3-hydroxyl amine. This gets changed into amino acid ommochrome which in presence of protien carrier provide brown color to eyes and body (Fig-44).

In the above mentioned Fig-44, if gene V by spontaneous mutation is changed to v then the insect looses its capacity to produce Kynurenine. In such a situation if extract from V gene insect is injected in the body of v gene insect, then again that insect start production of normal pigment. Instead of injection the geneticist compare transplanted testes of VV or Vv insect in that mutated insect. The result was that the mutated insect again started production of normal pigment. Afterwords Kynurenine injection was found to be successful.

Similarly it has been observed that if Cn gene changes to cn by mutation then the insect becomes unable to produce 3-hydroxyl Kynurine from Kynurenine. Therefore the insect is unable to produce that wild type of pigment. But if testes of Cn Cn insect is transplanted in the cn cn (mutated) insect. Then this mutated insect starts production of wild eye color.

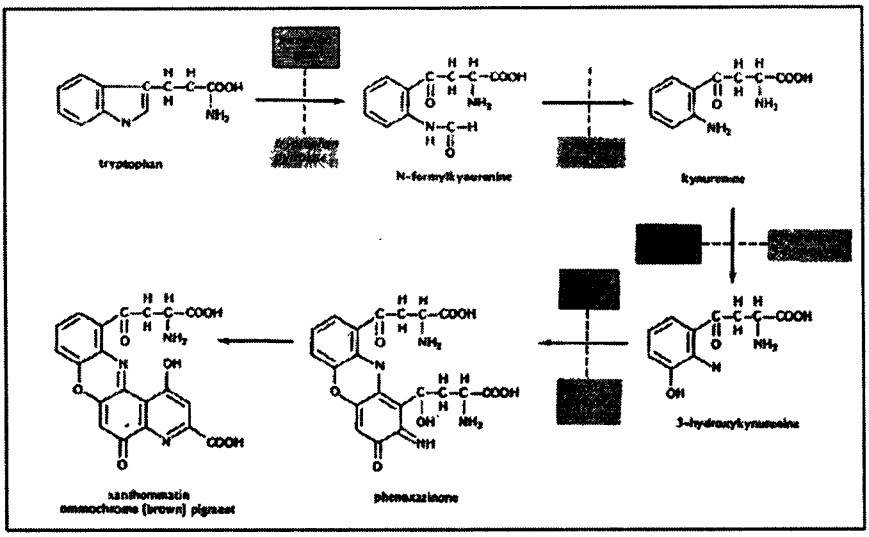


**Fig.44: Formation of pigment from Tryptophan.**

These observations suggest that in insects development of eye colour depends on production of special enzymes which are effected by special genes. But due to mutation these genes are unable to produce the enzyme. The result is that the insect becomes unable to produce the normal pigment. But if that desired enzyme is provided to the mutated insect by injection or transplantation of testes then the disturbed chemical reaction again starts and the insect start production of normal pigment (color). In this connection the researches of Efrussi and Beadle are worth mentioning. These geneticists have transplanted eye disk in developing embryo. Their observations are listed below:

(1) When eye disk of wild type fly was transplanted in Vermilion type or Cinnabar type of flies then these flies developed wild type eye colour. Body enzyme of Vermilion or Cinnabar fly had no effect.

(2) When Vermilion fly eye disk was transplanted in wild type fly then the fly developed wild type eye only.

(3) When Vermilion fly eye disk was transplanted in Cinnabar fly then the fly developed cinnabar eye only.

(4) When Cinnabar fly eye disk was transplanted in Vermilion fly then the fly developed Cinnabar eye.

From above mentioned observations Ephrussi and Beadle concluded that there is some deficiency in eyes of Vermilion fly and Cinnabar

fly which can be corrected by the cells of wild type fly. Beside these one more suggestion they made that because by the effect of Cinnabar eye disk the eye disk of Vermilion fly changes to Cinnabar eye disk but vermilion eye disk do not change the Cinnabar eye to vermilion eye. Therefore it suggests that perhaps vermilion eye has some type of deficiency.

The observations of Ephrussi and Beadle can be also understood by biochemical facts. In case of Vermilion eyes enzyme V and Cn both are absent and the amino acid tryptophan is present. In Cinnabar eyes the enzyme V is present but the enzyme Cn is absent. In Cinnabar eyes Kynurenine is present. Any of the both eye disk when transplanted in wild fly then because of presence of V and Cn enzymes in that fly wild eyes are produced. When Cinnabar eye disc is transplanted in Vermilion disc eyed fly then in that fly only cinnabar eye is produced because in vermilion disc eye Cn enzyme is absent. When vermilion eye disc is transplanted in cinnabar disc eyed fly, then in that fly only cinnabar disc eye develops because in vermilion the enzyme V is present.

Above discussions make it clear that vermilion colour is due to trytophan, cinnabar colour is due to Kynurenine, and wild type colour is due to sommochrome. These facts indicate that genes play a great role in production of different colours.

**Gene and protein:**

Proteins are made of amino acids, complex and chained molecules. The amino acids are joined together by peptide linkages. In various varieties of a species proteins are of different types. Nature and behavior of some special proteins such as enzymes and haemoglobin are controlled by special genes. It can be understood by following examples:

**Example:**

Sickle cell disease is caused by abnormal haemoglobin. Abnormal haemoglobin is produced by difference in electrical charge in normal haemoglobin. An intermediate stage has also been found when normal and abnormal both type of haemoglobin coexist. These two types of haemoglobin are produced because of difference in genes. Normally si /si genes, abnormally Si /si genes and at intermediate stage Si /si genes are found. During electrophoresis all the three types of haemoglobins have movement at different speed because they have

different electrical charge. Normal haemoglobin has negative charge (-), abnormal haemoglobin has positive charge (+) and intermediate haemoglobin has negative and positive charge.

The geneticist Ingran has discovered that in normal and abnormal haemoglobin one peptide linkage is different. From these facts it is clear that when there is difference in gene action (gene si mutates to Si) then one peptide linkage gets changed. This results such a defect in haemoglobin that the person succumbs to death.

**Genes and antigens:**

Presence of different kinds of antigen in blood is because of genes. It may be well understood that red blood cells (RBC) have antigen. By this antigen during blood transfusion RBC reacts with antibodies of donor blood serum. Every antibody and antigen has their own chemical configuration. Land Stiener (1900) demonstrated that if blood of incompatible blood group is transfused then RBC of the blood clumps the RBC of the person. The result is that blood vessels and capillaries of the person get blocked and therefore the person dies.

In human beings 4 types of antigen are found eg. A,B,AB, and O (none). Persons of antigen A have such antibodies which if the blood is in person of B antigen will agglutinate its red blood cells. Similarly persons of B antigen have such antibodies which if the blood is transfused in person of B antigen will agglutinate its red blood cells. Persons with AB antigen do not have antibody. Persons with O antigen have both the antibodies due to which A and B antigen red blood cells get agglutinated. Experiments have revealed that these antigens are produced by three genes viz. IA, IB and I as detailed below:

AB antigen — —> IA IB genes

B antigen — — >IB IB genes or IB I genes

A antigen — —> IA IA genes or IA I genes

O antigen— —> ii genes

Therefore,

Gene IA produces antigen A

Gene IB produces antigen B

Gene i produces antigen O

Genes and human disease

Due to sufficient progress in biochemical genetics now it is clearly known that several human diseases viz. albinism, alcaptonuria, tyrocinosis, colour blindness, phenylketonuria etc. are produced by action of genes. We will discuss some diseases below

**1. Genes and albinism:**

In human beings colour development is because of phenylalanines melanine which is produced by amino acid - in subcutaneous cells. Change of amino acid into melanin is controlled by genes K and C. If due to mutation these genes are changed to k and c or due to any reason the action of these genes are changed then formation of enzyme is stopped. Therefore the amino acid - phenylalanine can not change to melanin.

**2. Genes, Tyrosinosis and Alcaptonuria:**

In urine of some individuals p-hydroxy phenyl pyruvic acid is present. Due to this the disease tyrosinosis develops. In urin of some persons instead of p- hydroxyl phenyl pyruvic acid there is homogenistic acid. Due to this a disease alcaptonuria develops. In reality both of these diseases are because of incomplete oxidation of phenyl pyruvic acid.The reason for incomplete oxidation are mutated gene tt and aa.

**3. Genes and Phenylketonuria:**

Presence of phenylalanine causes a serious disease phenylketonuria. The reason is that due to gene KK phenylalanine after oxidation is unable to produce tyrosine. Actually due to mutation the gene KK is converted into kk so that it is unable to produce the enzyme to convert phenylalanine into tyrosine as shown in Fig-45.

Normal gene KK

Phenylalanine - - - - - - - - - - - - - - - - - - - → Tyrosine

Mutated gene kk

Phenylketonuria

**Fig.45: Action of genes KK and kk in producing disease phenylketonuria.**

**Gene and metabolism in neurospora:**

Neurospora or red fungus is an important material for study of gene action and biochemical studies. Beadle and Tayum and other scientists based on their experiments have reported that for one biochemical

reaction only one gene is responsible. They have proved that enzymes are produced by this fungus which controls several reaction activities. These enzymes are controlled by special genes.

For example a normal neurospora has the gene V+ which produces the enzyme trytophan synthetase which synthesize tryptophan . If the gene V+ is mutated then it looses its capacity to produce trytophan synthetase. So synthesis of tryptophan is not possible. But it has been observed that if from normal neurospora fungus the obtained enzyme trytophan synthetase extract is used for treatment of mutated fungus, then the fungus will produce tryptophan. Therefore this experiment shows the relationship between metabolism producing enzyme and gene.

**Gene and body size and body form:**

Ductless glands in animals produces several types of harmones which control characters such as body size and body form and many other characters. Development of these ductless glands & other glands and harmones are controlled by multiple gene complexes.

**Gene and growth of plants:**

In plants auxins or growth promoting hormones are found. Production of growth promoting harmone is controlled by genes. For example in maize the two varieties e.g. dwarf and normal are controlled by Na and na. The scientist Vanoverbeck has found out that the gene na produces an enzyme which disturbs the auxins, therefore growth of the maize plant stops and the plant becomes dwarf.

# *Part II*

# Applied Genetics

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Chapter 1

# Cancer Genetics

Cancer is a multistage carcinogenesis process during which normal dividing cells accumulate multiple somatic mutations (point mutations and mutations due to chromosomal damage), and then become cancer cells by several pathway events. Most human tumors are spontaneous but some are age and sex related, and a few are inherited or familial. There are many etiologic factors such as genetic history, diet, lifestyle, and environment may contribute to the development of these tumors. In human latency period ranges from a few years to 30 years or more. Cancer results from uncontrolled proliferation of monoclonal line with aneuploidy. Almost all differentiated cells can become neoplastic by the process called transformation. Malignant tumor (cancer) is a multistage carcinogenic process (progression) requires more than single genetic alteration (pathway events) to produce metastasis. Fig-1 shows the sequence of Adenocarcinoma.

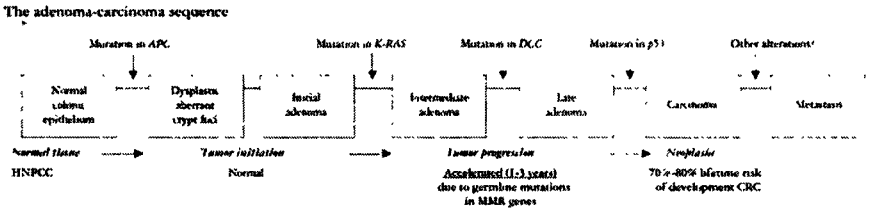


**Fig. 1: Sequence of Adenocarcinoma.**

Immortalization refers to the process in which cells lose the capacity to differentiate and then die, and thus continue to divide. Immortalized cells, however, do not produce tumors and are non-tumorigenic. Transformation refers to the process in which immortalized cells acquire the capacity to produce tumors. Thus, the transition from a normal cell to a cancer cell involves at least two distinct steps: immortalization and transformation.

Accordingly, the process of immortalization in normal cells can be called a "tumor initiation phase," whereas the process of transformation of immortalized cells can be referred to as a "tumor

promotion phase." Thus, genes that cause immortalization can be considered "tumor initiating genes," and genes that cause immortalized cells to become cancer cells can be considered "tumor promoting genes."

**Neoplastic cells differ from normal cell in following respects:**

They are immortal, loose contact inhibition, recognization and communication, decreased adhesiveness, shows invasiveness and low serum requirement, disorganized cytoskeletal structure, reduced number of gap junctions, increased nuclear cytoplasmic ratio etc. Uncontrolled growth of cells produce a cell mass called neoplasm or tumor

**The process of tumorgenesis:**

Normal cell ——→ neoplasm (tumor) ——→ Malignant tumor (cancer)

Immortalization — Transformation

Invasiveness

Metastasis

Following Fig- 2 describes the different stages of cancer

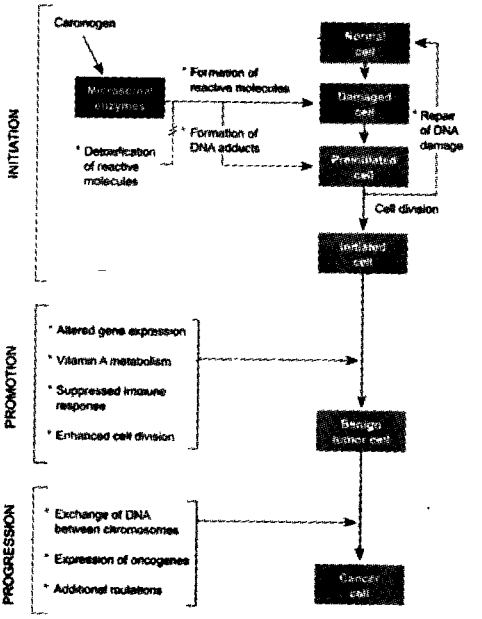


**Fig. 2: Different stages of cancer.**

**Cancer and cell cycle:**

The uncontrolled proliferation of cancer is due to the failure of cell cycle check point function at G/S or G2/M or M phase. The check points are the transitional periods where a decision is made to proceed or halt progression through cell cycle. The decision is controlled by physical interaction between CDKS & cyclins. G1/S check is controlled by CDKS/cyclins D & G2/M by CDKI/cyclin B. Any mutation in genes coding CDKS & cyclin may cause uncontrolled cell division leading to malignancy.

**Genetics of cancer:**

Control of cell division is regulated in following ways.

(i) By genes: Genes suppressing cell division. Example tumor suppressor genes.

(ii) Genes that normally function to promote cell division is **proto-oncogenes**. Proto-oncogenes by gain of function mutation becomes **oncogene**. There should be a balanced product of tumor suppressor gene and proto-oncogene to maintain the normal cell division.

**The uncontrolled growth can be caused by following reasons :**

(a) Loss of functional mutation of tumor suppressor gene.

(b) Permanent switch on of proto-oncogenes.

(c) Gain of functional mutation of proto-oncogene to oncogene.

These above three process lead to transformation, immortality. The clonal expression of a malignant cancer may be due to following reasons.

(a) Loss of function of differentiation genes.

(b) Epigenetic gene expression.

(c) Loss of heterozygosity of tumor suppressor genes.

(d) Non functional DNA repair mechanism.

Several hypotheses are there to explain the biology of carcinogenesis and cancer those includes - two-stage model, chromosomal aberrations, somatic mutation, activation of cellular oncogenes due to point mutations and chromosomal re-arrangements, inactivation of tumor suppressor genes due to point mutations or deletion of chromosome segments and downregulation of "differentiation genes".

**Two-Stage Model Hypothesis:**

It says that at least two distinct stages of carcinogenesis, initiation and promotion. Initiating events occurred at the nuclear DNA level, whereas promoting events occurred at the epigenetic levels (membranes, organelles, and post-translation protein modification). This model is most valuable cancer model for public health to identify many tumor- and antitumor-initiating agents, and treatment trials.

**Chromosomal Damage Hypothesis:**

This hypothesis is proposed by Boveri in 1914. According to this hypothesis, chromosomal anomalies and damage by carcinogens (carcinogens (ionizing radiation and chemicals) are the key initial events in carcinogenesis. But chromosomal damage by itself is not always sufficient to induce cancer in normal cells. However, this type of damage can make the genome more sensitive to mutagens, and thus can decrease the latency period during which downregulation of a differentiation gene can occur as a random event.

**Somatic Mutation Hypothesis:**

This hypothesis states that normal dividing cells become cancer cells and exhibit immortalization or transformation after accumulation of multiple, selective and specific somatic mutations due to genome instability. Mutations that downregulates differentiation genes cause immortalization of normal cells.

**Viral Infection Hypothesis:**

In rodents, some retroviruses (RNA viruses) like Rous sarcoma virus (RSV), avian lymphomatosis viruses, murine leukemic viruses, feline leukemic viruses and murine mammary tumor viruses causes cancer. RNA viruses such as human-T-leukemia virus and hepatitis C virus increases risk of T-cell leukemia and hepatic carcinoma respectively in human. Genes from certain DNA viruses like large T-antigen (Lta) gene from the SV40, E6, and E7 from HPV and E1A from the adenovirus are responsible for immortalization of human cell. Expression of the Lta protein of SV40 virus is for maintaining the cell proliferation of immortalized rat fetal dopamine neurons.

**Bacterial and Parasitic Infection Hypothesis:**

*Helicobacter pylori* increase the risk of gastric cancer, *Opistorchis viverrini* and *Schistosoma haematobium* are considered risk factors for cholangiocarcinoma and urinary bladder cancer. A latency period of

at least 14 years of chronic infection is needed for the development of these tumors. Generation of free radicals and release of cytokines during chronic infection may be one of the common intermediary risk factors that increases the risk of cancer in infected organs.

**Telomerase Hypothesis:**

Telomerase enzyme has potential role in proliferation, aging, and carcinogenesis. An increased activity of telomerase occurs subsequent to immortalization and/or transformation, and that it pays no direct role in immortalization. Shortening of telomere length causes aging and cell death.

**Proto-Oncogene Hypothesis:**

This hypothesis says that, proto-oncogenes are normal cellular genes those when get mutated become oncogenous and cause cancer.

**Classifications of Proto-Oncogenes:**

According to the function of the particular proto-oncogene product and function, Proto-Oncogenes can be classified in several groups. Those are

**1. Growth Factors:**

The c-Sis gene (the v-sis gene is the oncogene in simian sarcoma virus) encodes the PDGF B chain. The v-sis gene was the first oncogene to be identified as having homology to a known cellular gene. The int-2 gene encodes an FGF-related growth factor. The KGF (also called Hst) gene also encodes an FGF-related growth factor and was identified in gastric carcinoma and Kaposi's sarcoma cells.

**2. Receptor Tyrosine Kinases:**

The c-Fms ("fims") gene encodes the colony stimulating factor-1 (CSF-1) receptor and was first identified as a retroviral oncogene. The Flg ("flag") gene (named because it has homology to the Fms gene, hence fms-like gene) encodes a form of the FGF receptor. The Neu ("new") gene was identified as an EGF receptor-related gene in an ethylnitrosourea-induced neuroblastoma. The conversion of proto-oncogenic to oncogenic Neu requires only a single amino acid change in the transmembrane domain. The Trk ("track") genes encode the NGF receptor-like proteins. The first Trk gene was found in a pancreatic cancer. Subsequently, two additional Trk-related genes were identified. These three are now identified as TrkA, TrkB and

TrkC. The Met gene encodes the hepatocyte growth factor(HGF)/ scatter factor (SF) receptor. The c-Kit gene encodes the mast cell growth factor receptor.

**3. Membrane Associated Non-Receptor Tyrosine Kinases:**

The v-src gene was the first identified oncogene. The c-Src gene is the archetypal protein tyrosine kinase. The Lck gene was isolated from a T cell tumor line (LYSTRA cell kinase) and has been shown to be associated with the CD4 and CD8 antigens of T cells.

**4. G-Protein Coupled Receptors:**

The Mas gene was identified in a mammary carcinoma and has been shown to be the angiotensin receptor.

**5. Membrane Associated G-Proteins:**

There are three different homologs of the c-Ras gene, each of which was identified in a different type of tumor cell. The Ras gene is one of the most frequently disrupted genes in colorectal carcinomas.

**6. Serine/Threonine Kinases:**

The Raf gene is involved in the signaling pathway of most RTKs. It is likely responsible for threonine phosphorylation of MAP kinase following receptor activation.

**7. Nuclear DNA-Binding/Transcription Factors:**

The Myc gene was originally identified in the avian myelocytomatosis virus. A disrupted human c-Myc gene has been found to be involved in numerous hematopoietic neoplasias. Disruption of c-Myc has been shown to be the result of retroviral integration and transduction as well as chromosomal rearrangements. The Fos gene was identified in the feline osteosarcoma virus. The protein interacts with a second proto-oncogenic protein, Jun to form a transcriptional regulatory complex. The p53 gene was originally identified as a major nuclear antigen in transformed cells. The p53 gene is the single most identified mutant protein in human tumors. Mutant forms of the p53 protein interfere with cell growth suppressor effects of wild-type p53 indicating that the p53 gene product is actually a tumor suppressor.

**Oncogene Hypothesis:**

Gain of function mutation or overexpression of cellular proto-oncogenes is the primary events that initiate neoplastic changes. Viral

oncogenes such as v-src, v-ras, and v-myc were isolated from retroviruses and were found to be tumorigenic and are counterparts (cellular proto-oncogenes) from the mammalian genome. But mutation in cellular proto-oncogenes cannot be considered an initial event in human carcinogenesis but are the secondary events in human carcinogenesis. Over expression of multiple oncogenes such as ras, myc, c-erbB2, and Bcl2 occur in human lung cancer and 30% of adenocarcinomas show mutation in K-ras, and overexpression of c-erbB2 is found in 25% of non-small-cell lung cancer (NSCLC).

**List of oncogene and their related cancers**

| Oncogene | Function | Cancer |
|---|---|---|
| Abl | Tyrosine kinase activity | Chronic myelogenous leukemia |
| abl/bcr | New protein created by fusion | Chronic myelogenous and acute lymphotic leukemia |
| akt-2 | Encodes a protein-serine/threonine kinase | Ovarian cancer |
| Alk | Encodes a receptor tyrosine kinase | Lymphomas |
| Aml1/mtg8 | New protein created by fusion | Acute leukemias |
| Axl | Encodes a receptor tyrosine kinase | Hematopoietic cancers |
| bcl-2, 3, 6 | Block apoptosis (programmed cell death) | B-cell lymphomas and leukemias |
| c-myc | Cell proliferation and DNA synthesis | Leukemia; breast, stomach, lung, cervical, and colon carcinomas; neuroblastomas and glioblastomas |
| Egfr | Tyrosine kinase | Squamous cell carcinoma |
| erg/c16 | New protein created by fusion | Myeloid leukemia |
| erbB | Tyrosine kinase | Glioblastomas, and squamous cell carcinomas |
| erbB-2 (originally neu) | Tyrosine kinase | Breast, salivary gland and ovarian carcinomas |
| Fos | Transcription factor for API | Osteosarcoma |
| Fps | Tyrosine kinase | Sarcoma |
| Gli | Transcription factor | Glioblastomas |
| Gsp | Membrane associated G protein | Thyroid carcinoma |
| HER2/neu | New protein created by gene fusion | Breast and cervical carcinomas |
| Hox11 | Over-expression of DNA binding protein | Acute T-cell leukemia |

| | | |
|---|---|---|
| Hst | Encodes fibroblast growth factor | Breast and squamous cell carcinomas |
| IL-3 | Over expression of protein | Acute pre B-cell leukemia |
| int-2 | Encodes a fibroblast growth factor | Breast and squamous cell carcinomas |
| Jun | Transcription factor for API | Sarcoma |
| K-sam | Encodes growth factor receptors | Stomach carcinomas |
| L-myc | Cell proliferation and DNA synthesis | Lung carcinomas |
| Mdm-2 | Encodes a p53 inhibitor | Sarcomas |
| MLM | Encodes p16 a negative growth regulator that arrests the cell cycle | Melanomas |
| MSH2 | Mismatch repair in DNA | Hereditary nonpolyposis colorectal cancer |
| Mtg8/aml 1 | New protein created by fusion | Acute leukemias |
| Myb | Encodes a transcription factor with DNA binding domain | Colon carcinoma and leukemias |
| Neu (now erb-2) | Tyrosine kinase | Glioblastomas and squamous cell carcinomas |
| N-myc | Cell proliferation and DNA synthesis | Neuroblastomas, retinoblastomas and lung carcinomas |
| NPM/ALK | New protein created by fusion | Large cell lymphomas |
| nrg/rel | New protein created by fusion | B-cell lymphoma |
| Ost | Guanine nucleotide exchange factor | Osteosarcomas |
| pax-5 | Relocation of transcription factor to the IgH gene | Lympho-plasmacytoid B-cell lymphoma |
| PRAD-1 | Encodes cyclin D1 that is important in G1 of the cell cycle | Breast and squamous cell carcinomas |
| Raf | Serine/threonine kinase | Many cancer types |
| rasK | Involved in signal transduction of the cell | Lung, ovarian, and bladder carcinoma |
| rasN | Involved in signal transduction of the cell | Breast carcinoma |
| Ros | Tyrosine kinase | Sarcoma |
| Src | Tyrosine kinase | Sarcomas |
| tal-1, 2 | Over-expression of transcription factor | Acute T-cell leukemia |
| tan-1 | Over-expression of protein | Acute T-cell leukemia |
| Tiam-1 | Guanine nucleotide exchange factor | T-lymphoma |
| TSC2 | GTPase activator | Renal and brain tumors |
| Trk | Recombinant fusion protein | Colon and thyroid carcinomas |

**Tumor Suppressor Gene (Anti-Oncogene) Hypothesis:**

This concept was derived from the cytological study of retinal cells and retinoblastoma cells in which one of the alleles of chromosome 13 was missing from the retinal cells and both were missing from the retinoblastoma cells. This suggested that chromosome 13 must carry a tumor suppressor gene, the loss of which was essential for tumor formation. Wild-type Rb gene also inhibited the growth of normal cells.

Another well-known tumor suppressor gene, p53 and mutations in p53 are present in up to 50% of NSCLC and in 80% of SCLC.

**List of tumor suppressor genes and their related cancers**

| Syndrome/ Cancer | Cloned Gene | Function | Chromosomal Location | Tumor Types |
|---|---|---|---|---|
| Li-Fraumeni Syndrome | P53 tumor suppressor | cell cycle regulation, apoptosis | 17p13 | brain tumors, sarcomas, leukemia, breast cancer |
| Familial Retinoblastoma | RB1 tumor suppressor | cell cycle regulation | 13q14 | retinoblastoma, osteogenic sarcoma |
| Wilms Tumor | WT1 tumor suppressor | transcriptional regulation | 11p13 | pediatric kidney cancer |
| Neurofibromatosis Type 1 | NF1 protein=neurofibromin 1 tumor suppressor | catalysis of RAS inactivation | 17q11.2 | neurofibromas, sarcomas, gliomas |
| Neurofibromatosis Type 2 | NF2 protein = merlin or neurofibromin 2 tumor suppressor | linkage of cell membrane to cytoskeleton | 22q12.2 | Schwann cell tumors, astrocytomas, meningiomas, ependynomas |
| Familial Adenomatous Polyposis | APC tumor suppressor | signaling through adhesion molecules to nucleus | 5q21 | colon cancer |
| Tuberous sclerosis 1 | TSC1 protein = hamartin tumor suppressor | | 9q34 | facial angiofibromas |

| | | | | |
|---|---|---|---|---|
| Tuberous sclerosis 2 | TSC2 protein = tuberin tumor suppressor | GTPase activation | 16 | benign growths (hamartomas) in many tissues, astrocytomas, rhabdomyosarcomas |
| Deleted in Pancreatic Carcinoma 4 | DPC4 also known as Smad4 tumor suppressor | regulation of TGF-BMP signal transduction | 18q21.1 | pancreatic carcinoma, colon cancer |
| Deleted in Colorectal Carcinoma | DCC tumor suppressor | transmembrane receptor involved in axonal guidance via netrins | 18q21.3 | colorectal cancer |
| Familial Breast Cancer | BRCA1 tumor suppressor | repair of double strand breaks by association with Rad51 protein | 17q21 | breast and ovarian cancer |
| Familial Breast Cancer | BRCA2 tumor suppressor | Similar to BRCA1? | 13q12.3 | breast and ovarian cancer |
| Peutz-Jeghers Syndrome | STK11 tumor suppressor protein = serine-threonine kinase 11 | potential regulation of vascular endothelial growth factor (VEGF) pathway | 19p13.3 | hyperpigmentation, multiple hamartomatous polyps, colorectal, breast and ovarian cancers |
| Hereditary Nonpolyposis Colorectal Cancer type 1 HNPCC1 | MSH2 tumor suppressor | DNA mismatch repair | 2p22-p21 | colorectal cancer |

| | | | | |
|---|---|---|---|---|
| Hereditary Nonpolyposis Colorectal Cancer type 2 HNPCC2 | MLH1 tumor suppressor | DNA mismatch repair | 3p21.3 | colorectal cancer |
| von Hippel-Lindau Syndrome | VHL tumor suppressor | regulation of transcription elongation | 3p26-p25 | renal cancers, hemangioblastomas, pheochromocytoma |
| Familial Melanoma | CDKN2A protein = cyclin-dependent kinase inhibitor 2A tumor suppressor | inhibits cell-cycle kinases CDK4 and CDK6 | 9p21 | melanoma, pancreatic cancer, others |
| Gorlin Syndrome: Nevoid basal cell carcinoma syndrome (NBCCS) | PTCH protein = patched tumor suppressor | transmembrane receptor for hedgehog signaling protein | 9q22.3 | basal cell skin cancer |
| Multiple Endocrine Neoplasia Type 1 | MEN1 tumor suppressor | Unknown | 11q13 | parathyroid and pituitary adenomas, islet cell tumors, carcinoid |
| Multiple Endocrine Neoplasia Type 2 | RET, MEN2 | transmembrane receptor tyrosine kinase for glial-derived neurotrophic factor (GDNF) | 10q11.2 | medullary thyroid cancer, type 2A pheochromocytoma, mucosal hartoma |
| Beckwith-Wiedmann Syndrome | p57, KIP2 | cell cycle regulator | 11p15.5 | Wilms tumor, adrenocortical cancer, hepatoblastoma |

| | | | | |
|---|---|---|---|---|
| Hereditary papillary renal cancer (HPRC) | MET | transmembrane receptor for hepatocyte growth factor (HGF) | 7q31 | renal papillary cancer |
| Hereditary prostate cancer numerous loci: HPC1(PRCA1), HPCX, MXI1, KAI1, PCAP | HPC1 and PRCA1 are same designation ribonuclease L (RNaseL) maps to this locus | RNaseL involved in mRNA degradation | 1q24-q25 | prostate cancer |
| Ataxia telangiectasia (AT) | ATM 4 complementation groups: ATA, ATC, ATD, ATE, are associated with mutations in the ATM gene | gene product likely halts cell cycle after DNA damage | 11q22.3 | lymphoma, cerebellar ataxia, immunodeficiency |
| Bloom syndrome | BLM | DNA helicase RecQ protein-like-3 | 15q26.1 | solid tumors, immunodeficiency |
| Xeroderma pigmentosum (XP) 7 complentation groups<br><br>XPA XPC XPD | XPA - XPG | DNA repair helicases, nucleotide excision repair | XPA = 9q22.3<br>XPC = 3p25<br>XPD=19q13.2-q13.3<br>XPE=11p12-p11<br>XPF=16p13.3-p13.13 | skin cancer |

**Function of p53:**

TP53 level in G1 of a healthy cell is very low. But damage in DNA rapidly increases the level. Rising of TP53 activates p21 gene product and cell cycle is arrested at G1/S point providing the cell DNA repair time before initiation of DNA replication in S phase.

When both the copies of p53 are mutated, it no longer can produce p21 inhibitor to prevent the entry to S phase. It results mutated DNA

replication that leads production of transformed cells. Following Fig-3 describes different pathway events involved in carcinogenesis

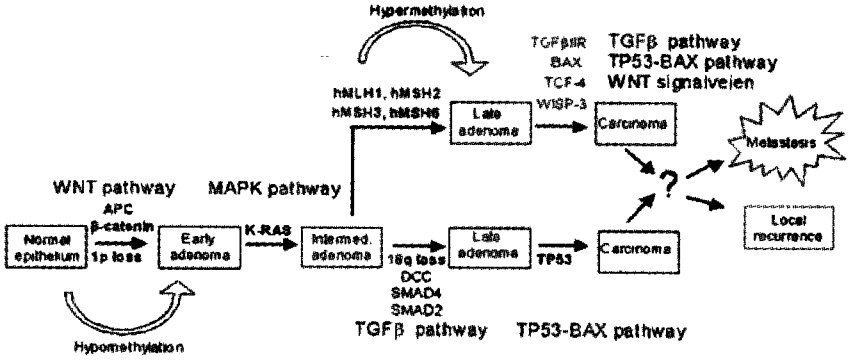


**Fig. 3: Pathway events in cancer showing involvement of different types of oncogenes and P53.**

## Differentiation Gene Hypothesis:

A gene, which induces terminal differentiation in normal cells referred to as a differentiation gene and its mutation may be an initiating event that causes cell to become immortalized and that mutations (point mutation or overexpression) in cellular oncogenes, growth regulatory genes, and tumor suppressor genes may represent secondary events that convert immortalized cells into transformed cells. Mutation in differentiation gene prevents the cell from undergoing terminal differentiation and subsequent cell death and consequently, the cells continue to proliferate (hyperplasia), leading to the formation of pre-neoplastic lesions such as adenomas. Differentiation gene is regulated by at least two sets of genes, "differentiation suppressor genes" and tumor suppressor genes, in an opposite manner. For example, inactivation of a "differentiation suppressor gene" or activation of a tumor suppressor gene downregulates the expression of a differentiation gene. Mutation in a differentiation gene is sufficient to downregulate this gene. The evidence for the existence of a differentiation suppressor gene is indirect. A differentiation gene may be downregulated by one of the following mechanisms: mutation within the differentiation gene; overexpression of "differentiation suppressor genes;" and inactivation of tumor suppressor genes (Fig-4). Some candidate differentiation genes (c-fos, c-fes, and RAG-1 gene activator) that are upregulated during cAMP-induced terminal differentiation of NB cells.

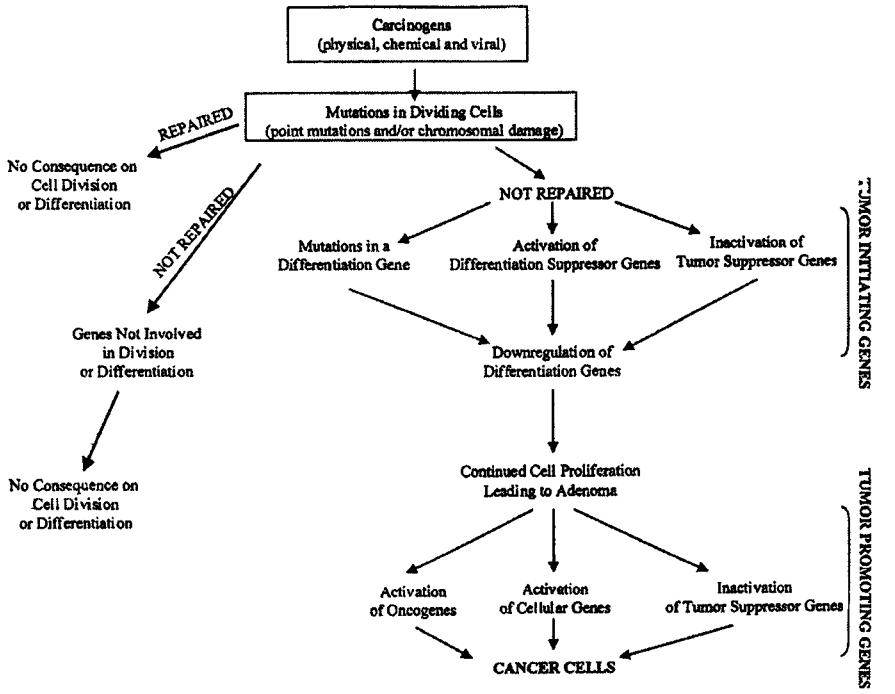


**Fig. 4: Differentiation Gene hypothesis.**

**Cancer: Types, Diagnosis and treatment:**

Cancer is an abnormal, continuous and uncontrolled multiplication of cell that may grow into adjacent tissue or spread to distant parts of the body (metastasis). The mass of cancer cells eventually become large enough to produce lumps, masses or tumors that can be detected, which can be benign or malignant:

**Benign tumors:** These are not cancerous and can usually be removed and do not spread to other parts of the body, and the cells do not invade other tissues

**Malignant or metastatic tumors (cancer):** These are cancerous and can invade and damage nearby tissues and organs and metastasize. This type of cancer can spread from its original location to other parts of the body by direct extension or invasion into adjacent tissues or by Systemic spread throughout the body by blood and lymphatic system or by cerebrospinal fluid (Fig-5)

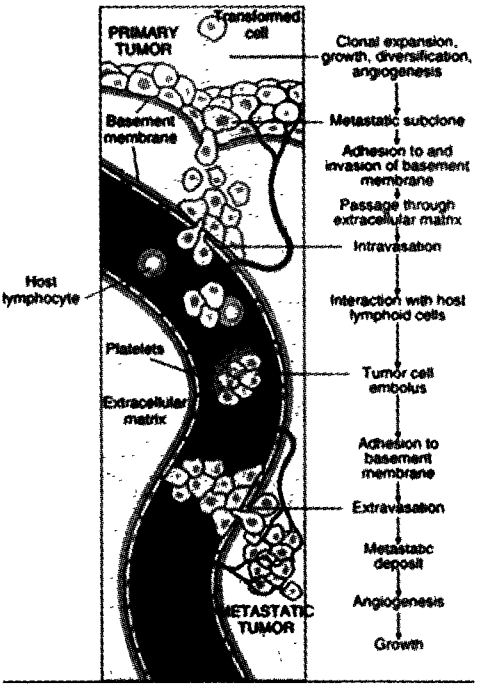


**Fig. 5: Process of metastasis.**

**General categories of cancers:**

There are four general categories of cancer and adenocarcinomas are the most common type.

I. **Carcinomas** are cancers that occur in epithelial surfaces - the cells that form the outer surface of the body to line or cover the body's cavities, tubes and passageways.

II. **Adenocarcinomas** are cancers that form on a glandular surface, such as the lung, breast, prostate, ovary, or kidney.

III. **Sarcomas** are cancers that occur in supporting structures, such as bone, muscle, cartilage, fat or fibrous tissue.

IV. **Leukemias and lymphomas** are cancers that occur in blood cell elements.

Carcinoma and sarcoma fall under solid tissue tumor. Lymphoma and leukemia are soft tissue cancer.

Tissue and organ specific cancers such as liver cancers are those that originated from a liver cell are similarly Brain cancers, nerve cancers, melanomas, and certain testicular and ovarian cancers etc.

**Diagnostic procedures for cancer:**

When the symptoms of cancer appears, the diagnosis can be done by several methods. First a detailed medical history (family and personal) is to be considered to know the hereditary relation, then physical examination can be done. Other diagnostic procedures that may be requested include

**Imaging tests:**

1) X-ray, computed tomography (CT or CAT scan) for brain or other internal organs, lymph nodes cancers.
2) Radionuclide scan - to detect any abnormal areas or tumors.
3) Ultrasound - for abdominal organ tumors, such as the uterus, liver, and kidneys.
4) Magnetic resonance imaging (MRI) for internal organ or structure, especially the brain and spinal cord, nodules in bones or lymph nodes.
5) Endoscopy - allow to see inside the hollow organs, such as the bladder or uterus. Biopsy samples are taken through this.

**Laboratory tests:**

To examine blood, urine, other fluids or tumor tissue by microscopy and by immunohistochemistry.

**Biopsy:**

It is an invasive method to remove a sample of the suspicious tissue for examination in a laboratory.

After diagnosis stage of progression can be evaluated to determine the extent (spread) of the cancer which can be done by staging. Staging is important for treatment, prognosis and treatment results. Stage refers to the extent, or the size, of the cancer. Each cancer, by organ, has its own staging system.

**Stages of cancer:**

There are five stages in cancer and are non reversible

1. **Stage 0** or carcinoma in situ: It is very early cancer. The abnormal cells are found only in the first layer of cells of the primary site and do not invade the deeper tissues.
2. **Stage I**: Cancer involves the primary site, but has not spread to nearby tissues.

   a. Stage IA: a very small amount of cancer - visible under a microscope - is found deeper in the tissues.
   b. Stage IB: a larger amount of cancer is found in the tissues.

3. **Stage II**: Cancer has spread to nearby areas but is still inside the primary site.
   a. Stage IIA: cancer has spread beyond the primary site.
   b. Stage IIB: cancer has spread to other tissue around the primary site.
4. **Stage III**: Cancer has spread throughout the nearby area.
5. **Stage IV**: Cancer has spread to other parts of the body.
   a. stage IVA: cancer has spread to organs close to the pelvic area.
   b. stage IVB: cancer has spread to distant organs, such as the lungs.

Recurrent cancers again come back (recurred) after it has been treated but not cured properly.

**Treatment of cancer:**

The goal of cancer treatment is successful treatment, prevention, prolonged life and palliation (pain relief).

Specific treatment for a cancer is determined by several factors like- overall health and medical history, extent of the disease - type, grade, stage and location , tolerance for specific medications, procedures, or therapies, expectations for the course of the disease etc.

The conventional and primary methods of cancer treatment are:

1. Surgery
2. Radiation therapy
3. Chemotherapy
4. Hormone therapy
5. Immunotherapy / biological therapy
6. Adjuvant therapy - combining two or more treatments
7. Prophylactic or preventive treatment.
8. Gene Therapy

**Surgery:**

It is a surgical procedures used to diagnose or destroy cancerous tissue include by several surgical techniques like biopsy, endoscopy, laparoscopy, laparotomy, laser surgery , cryosurgery, electrosurgery, excisional etc.

**Radiation Therapy:**

It uses special kinds of energy waves or particles to fight cancer (destroy cancer cells or prevent cells from growing or reproducing). Depending on the type and location of the cancer, the therapy varies. Radiation Therapy provides a cure for cancer, control the disease, or help relieve its symptoms. Radiation treatments are painless and usually last a few minutes.

**Procedures in Radiation Therapy:**

**Simulation process**: It is "mapping" out the position for treatment and the exact location of body (referred to as treatment field or port) where the radiation will be given (the simulation process). Imaging studies may are performed duringthe simulation process to plan how to direct the radiation during the treatments.

**Treatment plan:** After simulation process treatment plan is determined, including the type of machine to use, the amount of radiation that is needed and the number of treatments that will be given.

**Types of radiation therapies:**

Depending on the type of cancer, method of Radiation therapy varies. The main considerations are location of the cancer and the patient's health. Radiation therapy can be used in combination with other treatments. The most common types of radiation therapy are -

**External radiation (external beam therapy):** Here radiation is administered by a large machine that points the energy waves directly at the tumor to kill cancer cells. Special shields are made to protect the tissue surrounding the treatment area.

**Internal radiation (brachytherapy, implant radiation):** Here a high dose of radiation is given inside the body as close to the cancer as possible by administering a higher dose of radiation in a shorter time span when compared with external radiation. The radiation treatment may be swallowed, injected or implanted directly into the tumor. Some of the radioactive implants are called "seeds" or "capsules".

## Chemotherapy:

Chemotherapy is the use of anticancer drugs to treat cancerous cells. It is one of the most common treatments for cancer. Different groups of drugs work in different ways to fight cancer cells by interfering with the cancer cell's ability to grow or reproduce. But there are many side effects of chemotherapy.

Chemotherapy can be given as a pill, as an intramuscular injection, intravenously (directly to the bloodstream; also called IV), topically (applied to the skin) or directly into a body cavity

**Some of the chemotherapy drugs and their side effects**

| **Chemotherapy Drug** | **Possible Side Effects (Not all side effects are listed. Some of those listed may be short-term side effects; others are long-term side effects.)** |
|---|---|
| **Carboplatin** (Paraplatin): Usually given intravenously (IV) and used for cancers of the ovary, head and neck, and lung. | Decrease in blood cell counts, hair loss (reversible)<br>Confusion, nausea, vomiting and/or diarrhea (usually a short-term side effect occurring the first 24 to 72 hours following treatment) |
| **Cisplatin** (Platinol, Platinol-AQ) Usually given intravenously (IV) and used for cancers of the bladder, ovary and testicles. | Decrease in blood cell counts, allergic reaction, including a rash and/or labored breathing, nausea and vomiting that usually occurs for 24 hours or longer ringing in ears and hearing loss, fluctuations in blood electrolytes, kidney damage. |
| **Cyclophosphamide** (Cytoxan, Neosar):<br>Can be given intravenously (IV) or orally and used for lymphoma, breast cancer, and ovarian carcinoma | Decrease in blood cell counts, nausea, vomiting, abdominal pain, decreased appetite, hair loss (reversible), bladder damage, fertility impairment, lung or heart damage (with high doses), secondary malignancies (rare). |
| **Docetaxel** (Taxotere): Usually given intravenously (IV) and used for breast cancer, lung and prostate | Decrease in blood cell counts, nausea, vomiting, abdominal pain, diarrhea, decreased appetite, hair thinning, rash, numbness and tingling in hands and feet. |
| **Doxorubicin** (Adriamycin): Usually given intravenously (IV) and used for breast cancer, lymphoma, and multiple myeloma | Decrease in blood cell counts, mouth ulcers, hair loss (reversible), nausea and vomiting, heart damage |
| **Erlotinib** (Tarceva) Usually given orally and<br>used for non small cell lung cancer | Rash and other skin changes and diarrhea |

| | |
|---|---|
| **Etoposide** (VePesid): Can be given intravenously (IV) or orally and used for cancers of the lung, testicles, leukemia and lymphoma | Decrease in blood cell counts, hair loss (reversible)<br>nausea and vomiting, allergic reaction<br>mouth ulcers<br>low blood pressure (during administration), decreased appetite, diarrhea and abdominal pain, bronchospasm and flu-like symptoms |
| **Fluorouracil** (5-FU): Usually given intravenously (IV) and used for cancers of the colon, breast, stomach, and head and neck | Decrease in blood cell counts, diarrhea, mouth ulcers<br>Photosensitivity, dry skin |
| **Gemcitabine** (Gemzar): Usually given intravenously (IV) and used for cancers of the pancreas, breast, ovary and lung | Decrease in blood cell counts, nausea and vomiting, fever and flu-like symptoms and rash |
| **Imatinib mesylate** (Gleevec): Usually given orally and used for chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor (GIST) | Nausea and vomiting, fluid retention (swelling around ankles, eyes), muscle cramps, diarrhea , gastrointestinal bleeding and rash |
| **Irinotecan** (Camptosar): Usually given intravenously (IV) and used for cancers of the colon and rectum | Decrease in blood cell counts, diarrhea and hair loss (reversible) |
| **Methotrexate** (Folex, Mexate, Amethopterin): May be given intravenously (IV), intrathecally (into the spinal column), or orally and used for cancers of the breast, lung, blood, bone, and lymph system | Decrease in blood cell counts, nausea and vomiting<br>mouth ulcers, skin rashes and photosensitivity<br>dizziness, headache or drowsiness, kidney damage (with a high-dose therapy), liver damage, hair loss (reversible) and seizures |
| **Paclitaxel** (Taxol, Abraxane): Usually given intravenously (IV) and used with cancers of the breast, ovary, and lung | Decrease in blood cell counts, allergic reaction, nausea and vomiting, loss of appetite, change in taste, thin or brittle hair joint pain (short term), numbness or tingling in the fingers or toes |
| **Sorafinib** (Nexavar): Usually given orally<br>for advanced kidney cancer | high blood pressure (during first few weeks of treatment), rash, other skin changes, diarrhea, fatigue, hair loss, nausea and vomiting |
| **Sunitinib** (Sutent): usually given orally<br>for gastrointestinal stromal tumor (GIST) and kidney cancer | Diarrhea, nausea and vomiting, mouth ulcers, upset stomach skin changes, including skin discoloration and rash, fatigue, high blood pressure, bleeding, swelling and taste disturbance |

| | |
|---|---|
| **Topotecan** (Hycamtin): Usually given intravenously (IV) for cancers of the ovary and lung | Decrease in blood cell counts, diarrhea,hair loss (reversible), nausea and vomiting |
| **Vincristine** (Oncovin, Vincasar PFS): Usually given intravenously (IV) for leukemia and lymphoma | Numbness or tingling in the fingers or toes, weakness, loss of reflexes, jaw pain, hair loss (reversible), constipation or abdominal cramping |
| **Vinblastine** (Velban): Usually given intravenously (IV) for lymphoma and cancers of the testis and head and neck | Decrease in blood cell counts, hair loss (reversible), constipation or abdominal cramping, jaw pain, numbness or tingling in the fingers or toes |

**Hormone Therapy:**

Hormones help some types of cancer cells to grow, such as breast cancer and prostate cancer and in other cases; hormones can kill cancer cells, slower cancer cells growth or stop their growth. Hormone therapy for cancer treatment involves surgically removal of a hormone producing gland that helps growth of cancer cells. Hormone therapy depends upon many factors such as the type and size of the tumor, the age of the person, the presence of hormone receptors on the tumor etc.

If the hormone receptor test indicates that the hormones are affecting cancer, the cancer may be treated in one of following ways:

1. treating cancer cells to keep them from receiving the hormones they need to grow
2. treating the glands that produce hormones to keep them from making hormones
3. surgery to remove glands that produce the hormones, such as the ovaries that produce estrogen, or the testicles that produce testosterone

If hormone therapy is given before the primary treatment, it is called neoadjuvant treatment. Neoadjuvant treatments help to kill cancer cells and contribute to the effectiveness of the primary therapy. If hormone therapy is given after the primary cancer treatment, it is called adjuvant treatment. Adjuvant therapy is given to improve the chance of a cure.

**Biological Therapy:**

Also called immunotherapy, biological response modifier therapy, or biotherapy. It uses the body's immune system to fight cancer. Biological therapies are designed to boost the immune system, either

directly or indirectly, by assisting in the following:

1. Making cancer cells more recognizable by the immune system, and therefore more susceptible to destruction by the immune system.
2. Boosting the killing power of immune system cells.
3. Changing the way cancer cells grow, so that they act more like healthy cells.
4. Stopping the process that changes a normal cell into a cancerous cell.
5. Enhancing the body's ability to repair or replace normal cells damaged or destroyed by other forms of cancer treatment, such as chemotherapy or radiation.
6. Preventing cancer cells from spreading to other parts of the body

There are many different types of biological therapies used in cancer treatment.

Biological response modifiers (BRMs) include nonspecific immunomodulating agents, interferons, interleukins, colony-stimulating factors, monoclonal antibodies, cytokine therapy, and vaccines those can boost the body's ability to fight the disease, direct the immune system's disease fighting powers to disease cells and strengthen a weakened immune system.

**Nonspecific immunomodulating agents:**

Stimulate the immune system to produce more cytokines and antibodies to fight cancer.

**Interferons (IFN)**: Naturally occurs in the body and also can be produced in the laboratory for use in cancer therapy. IFN improves immune system and acts against cancer cells by slow down growth or by changing cancer cell to normal behaviour. Some interferons may also stimulate natural killer cells (NK) cells, T cells, and macrophages that help to fight cancer cells.

**Interleukins (IL):** Interleukins are proteins (cytokines) and can stimulate the growth and activity of immune cells, such as lymphocytes, which work to destroy cancer cells.

**Colony-stimulating factors (CSFs):** Can encourage stem cells within the bone marrow to produce more blood cells. CSFs are given, along with chemotherapy, to boost the immune system.

**Monoclonal antibodies:** Are produced in the laboratory. They can

bind to cancer cells and when cancer-destroying agents are introduced into the body, they seek out the antibodies and kill the cancer cells. For example Trastuzumab (Herceptin®) is used for breast cancer and Rituximab (Rituxan®) for lymphoma.

**Cytokine therapy:** Cytokine helps immune system to recognize and destroy cancerous cells. Cytokine therapy kills cancer cells and prevents tumors from growing.

There are few side effects of biological therapy, which often mimic flu-like symptoms and varies according to the type of therapy. These are fever, chills, nausea, vomiting, loss of appetite, fatigue etc. Specifically, cytokine therapy often causes fever, chills, aches and fatigue. Other side effects include a rash or swelling at the injection site. Therapy can cause fatigue and bone pain and may affect blood pressure and the heart.

Chapter 2

# Apoptosis

Apoptosis is the term coined by Andrew Wyllie in 1972 to describe morphological description of a dying cell that contrast with necrosis.

Programmed cell death (PCD) is a term used to describe cells that die at predictable time and place during development. Programmed cell death is a gene directed cellular suicide mechanism that eliminates unwanted, superfluous cells during development as well as tissue homeostasis, control proliferation and differentiation and defends against viral infection. All Programmed cell deaths are apoptotic and so these terms are used interchangeably.

Programmed cell death is associated with ALPS- Autoimmunity, Myoicordial infraction, Stroke, Diabetes, Neurodegenerative diseases, Alzheimer's disease, Infertility, Sepsis, Viral infection etc.

**When Apoptosis does occur:**

| | |
|---|---|
| **In embryonic and fetal development** | • Tissue developmental programs, which control sculpturing of embryonic form.<br>• Limb and organ differentiation and development.<br>• Developmental organization of nervous system.<br>• Elimination of self-reactive components of immune system. |
| **In adult** | • On stimulation by T-Lymphosites.<br>• In response to DNA damage, viral infection or transformation, radiation etc.<br>• In certain organ or tissue on withdrawal of supporting hormone. |

**Characteristics of Apoptosis:**

1. Cytoplasm shrinks with out membrane rupture.
2. Blabbing of plasma membrane and nuclear membrane.
3. Chromatin condenses and migrates to nuclear membrane.

4. Inter-chromosomal cleavage in nucleosomal size by Mg+2 dependent endonuclease leads to laddering of DNA at nucleosomal repeat length of 200bp.
5. Fragmentation of cell with retention of cell membrane.
6. Flipping up phosphatidal serine from outer plasma membrane.
7. Cell contents are packed in membrane bounded bodies (apoptotic bodies).
8. Internal organneles still remain functional, mitocondria swells and engulfed by neighbors.
9. Epitopes appear on plasma membrane, making cell as phagocytotic target.
10. No spillage and inflammation.

**Differences between apoptosis and necrosis**

| Apoptosis | Necrosis |
|---|---|
| 1. A normal physiological response to specific suicide signals or lack of survival signals. | 1. Pathological response to cellular injury. |
| 2. Chromatin condenses. Cell shrinks. | 2. Nuclear swelling and chromatin clumps. |
| 3. Preservation of cell organelle. | 3. Cell swells |
| 4. Blabbing of plasma membrane and nuclear membrane. | 4. Mitochondria swell and rupture. |
| 5. No lysis takes place. | 5. Plasma membrane lyses |
| 6. Rapid engulfment of dying cells by neighboring cells. | 6. Slow phagocytosis |
| 7. Prevent inflammation and pus formation. | 7. Triggers inflammation and pus formation |
| 8. DNA breaks early in internucleosomal pattern. | 8. Cell ruptures and out of cell content. |
| 9. DNA breakage by gene activated, endonucleases | 9. DNA breaks later and randomly, DNA breakage by random injury and ATP depleted |
| 10. Moderate Calcium influx. | 10. Massive Calcium influx. |

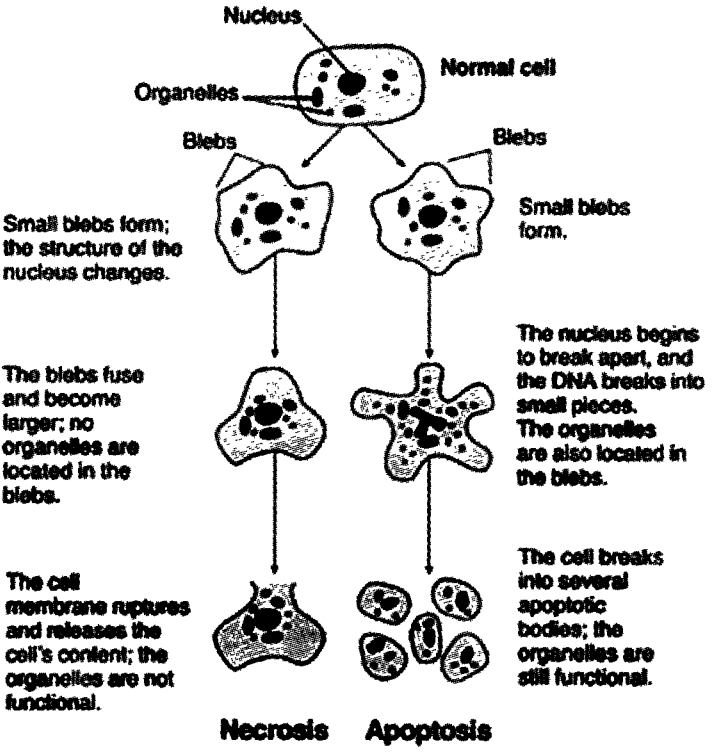


**Fig. 6: Differences between apoptosis and necrosis.**

## Genes involve in apoptosis:

| Gene Family | Mammals | C. elegans | Drosophila |
|---|---|---|---|
| Caspase (1-14) | Casp-1 to Casp-14 | Ced-3 (casp-9) | Dcp-1, drICE |
| Bcl-2 (19)<br>Survival factors<br><br>Death promoter | Bcl-2, Bcl-Xl<br><br>Bax, Bad, Bak, Bid, Bim, Bik | Ced-9 | <br><br>Drob-1, buffy |
| Death domain proteins ( these are apoptosis inducer and contain long prodomain) | Fas, CD-95, APO-1, TNFR? | Ced-3, Csp-1, Csp-2 | dredd (Casp-8), dronc, dream. |
| IAP (Inhibitor Apoptotic Proteins) | N-AIP, CIAP-1, X-IAP, CIAP-2, DIAP-1, SURVIVIN | Ces, Mes | dIAP-1, dIAP-2, Deterin. |

| | | | |
|---|---|---|---|
| AIF ( 'nuclear Apoptotic Inducing Factors, released from Mitochondria) | AIF | ? | dAIF |
| Ced-4 (Apoptosis activator) | APAF-1 (apoptotic protease activating factor-1) | Ced-4 | Ark (apaf-1 related killer) |
| Acinus | Acinus | ? | Acinus-d |

**Mechanism of apoptosis:**

There are 3 different mechanisms of apoptosis (Fig-7)

A. Death signals generated within the cell (Intrinsic/ mitochondrial pathway).
B. Activation of death activators by extrinsic death signals (Extrinsic or death receptor signaling pathway).
C. By reactive oxygen species and DNA damage.

**Intrinsic or mitochondrial pathway:**

Mitochondrial outer membrane displays Bcl-2 on their surface in healthy cells due to internal damage to the cell (e.g., from reactive oxygen species, DNA damage, UV radiation) causes Bcl-2 to activate related protein, Bax, that causes leackage in the outer mitochondrial membrane to release cytochrome-c from mitochondria. Upon released cytochrome-c binds to Apaf-1 with the help of ATP and form aggregate called apoptosomes. Apoptosome then binds to and activates caspase-9. Caspase-9 cleaves and do activates other down stream caspases or executioner caspases (caspase-3 and -7) leading to digestion of structural proteins in the cytoplasm, chromosomal DNA degradation and phagocytosis of the cells those are the phenomena of apoptosis.

**Extrinsic or death receptor signaling pathway:**

Fas and the TNF receptor are integral membrane proteins. Their receptor domains remain exposed at the cell surface. When complementary death activator (FasL and TNF respectively) binds to these receptors, it transmits a signal to the cytoplasm leading to caspase 8 activation. Activated Caspase 8 initiates a cascade of Caspase activation (caspase-3, 6, and 7) that causes apoptosis.

**Apoptosis-Inducing Factor (AIF):**

Neuronsdoes not use Caspases for self-destructuction. It uses Apoptosis-inducing factor (AIF) which is normally located in the mitochondrial intermembrane space. Upon receiving death stimulus, AIF is released out from the mitochondria and migrates into the nucleus, where it binds to DNA and cleaves it causing cell death.

**Free Radical signaling pathway:**

Oxidative stress can trigger apoptosis. Activated neutrophils produce reactive oxygen species like superoxide free radicals those can induce endothelial cell apoptosis. Endothelial cells also produce reactive oxygen species inside the cell during reperfusion injury following ischemia. Superoxide dismutase (SOD) converts highly reactive and damaging superoxide free radicals to peroxides. Peroxides and hydroxyl radicals activate NF-kB and activate expression of inflammatory genes including adhesion molecules, TNF and IL-8.

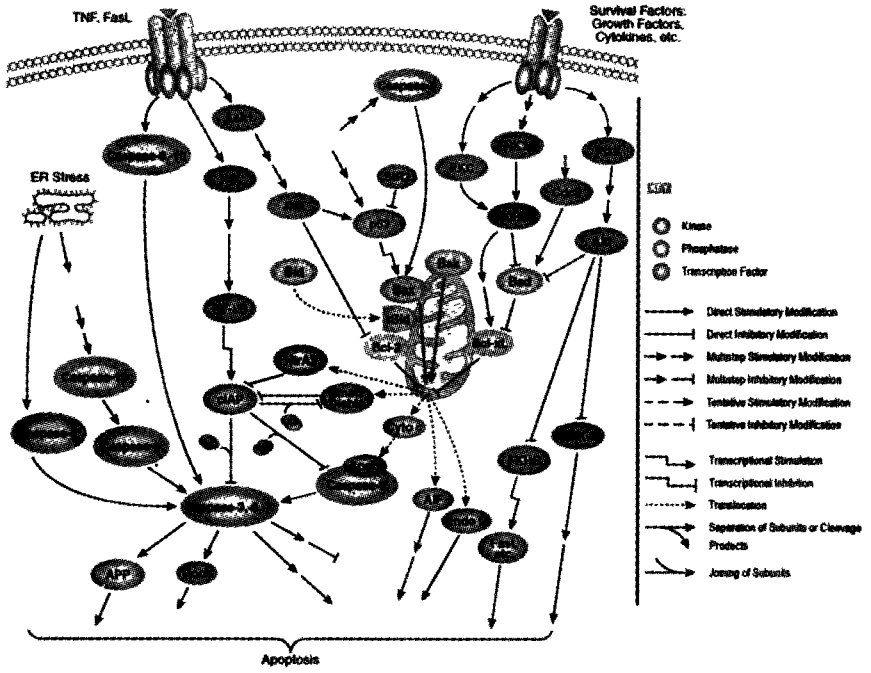


**Fig. 7: General Apoptotic pathways.**

**Other pathways are:**

- Apoptosis Pathway mediated by Granzyme A.
- HIV Induced T Cell Apoptosis.
- Induction of apoptosis through DR3 and DR4/5 Death Receptors.
- Multiple antiapoptotic pathways from IGF-1R signaling lead to BAD phosphorylation.
- TSP-1 Induced Apoptosis in Microvascular Endothelial Cell.

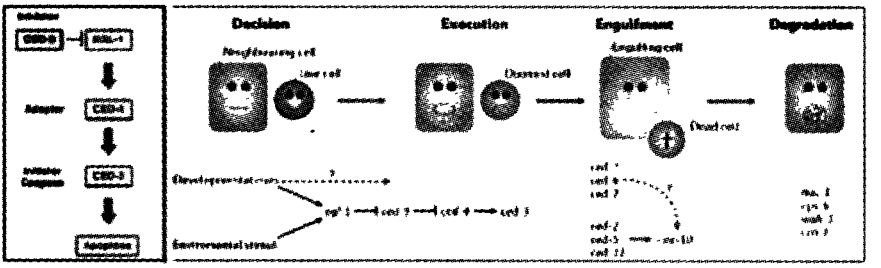


**Fig-8: Apoptosis in *C. elegans*.**

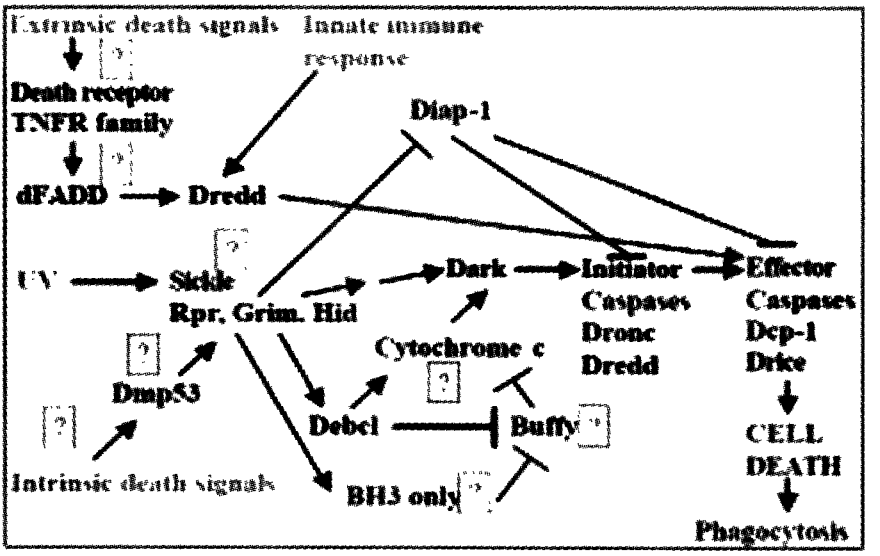


**Fig.9: Apoptosis in *Drosophila*: Intrinsic and extrinsic pathways.**

**Cancer and apoptosis**

Cancer is a multistage carcinogenic process where there is a net accumulation of atypical cells arising from excess proliferation, an insufficient apoptosis or a combination of the two. There are also

various side effects of chemotherapy. Thus from therapeutic point of view, the best strategy is " induced apoptosis in the neoplastic cell line without affecting the normal cells of the body. Several diatary phytochemicals are now a days are used for chemoprevention those do not cause side effects. The list provides few of those phytochemicals used for cancer prevention.

A list of antioxidant, anticancer and dietary phyto-chemicals those can induce apoptosis to prevent various types of cancers:

| Phytochemical | Source | Action |
|---|---|---|
| Geraniol | Bergamot, carrot, coriander, lavender, lemon, lime, nutmeg, orange, blueberry and blackberry | Inhibits Colon and Pancreatic cancer Growth |
| Cyanidin | present in skin of most red coloured berries such as bilberry, blackberry, cherry, cranberry, elderberry, and also in apples, pears, peaches and plums | Exhibits an inhibitory effect on the migration and invasion of human lung cancer cell lines. |
| Allicin | Predominant thiosulfinate in garlic (Allium sativum) and responsible for the typical and offensive odor of garlic. | Lowers the risk of stomach cancer. |
| Indole-3-Carbinol | Found in cruciferous vegetables such as cabbage, cauliflower, broccoli, kale and brussels sprouts. | Reduces the risk of breast, prostate and cervical cancer |
| Isothiocyanates | Found in cruciferous vegetables such as broccoli, cauliflower, kale, turnips, Brussels sprouts, cabbage, radish and turnip. | Helps to prevent lung cancer and esophageal cancer. Lowers the risk of gastrointestinal cancer. |
| Sulforaphane | Found in cruciferous vegetables such as broccoli, cauliflower, cabbage and kale. The richest source of sulforaphane is broccoli sprouts. | Reduces the risk of breast cancer and prostate cancer. |
| Proanthocyanidins | skin and seeds of grapes | Acts against highly metastatic breast carcinoma. |
| Quercetin | Found in apple, tea, onion, nuts, berries, cauliflower and cabbage. | Reduces the cancer risk of prostate, ovary, breast, gastric and colon cells. |

| | | |
|---|---|---|
| Resveratrol | A flavonol of red grapes, blueberries, mulberries and peanuts | Reduces the cancer risk of prostate, ovary and breast |
| Lycopene | Pigment of ripe tomatoes. | Shows an inverse relation ship between blood lycopene levels (or tomato consumption) and cancer risk of prostate, lung and stomach. |
| Saponins | Best known sources are peas and soybeans. | Helps to prevent colon cancer |
| Daidzein | Soy beans, kudzu, red clover | Reduces incidences of prostate cancers and prevents mammary tumours. |

**Detection of apoptosis:**

There are numerous techniques available for the detection of apoptosis. Morphological changes that occur during the process of apoptosis can be detected by light and electron microscopy.Cell shrinkage and increase ingranularity are detected by using density-gradient centrifugation or flow cytometry. Apoptosis can be measured by utilizing factors such as changes in calcium ion flux, annexin V binding and transglutaminase (tTG) activity. Some of the common techniques used to detect apoptosis include PCR analysis, comet assays, agarose gel analysis, in vitro and in situ DNA end-labeling and ELISA systems. There is no gold standard for the specific detection of apoptosis is available.

**TUNEL:**

Apoptosis Detections in TdT-mediated dUTP nick end labeling (TUNEL): TUNEL technique is highly influenced by the extent of proteolytic digestion. It is used to identify the nuclei in the areas of necrosis. This method is also used in biparametric analysis and retrospective studies of cell death. Apoptotic cells of origin can be identified by biparametric stain. This method labels the free 3′-ends of DNA by terminal transferase (TNT), and the label is then visualized by immunohistochemical techniques. TUNEL reaction seems to be prone to false positive or negative findings and several improvements of the methods have been proposed. The staining is very dependent on 1) fixation time of the tissue samples, 2) proteolytic pretreatment of the section and 3) the concentration of the nucleotides and terminal transferase used for labeling

**Comet Assay:**

Comet assay is a technique used for measuring DNA strand breaks in each cell. This technique was first developed by Swedish researchers Ostlingand Johansson. It is also used to detect DNA damage, in nutritional research, and genetic toxicology testing.

**DNA laddering:**

DNA is cleaved at sites located between nucleosomal units, thereby generating. DNA mono- and oligonucleosomal fragments (180bp multimers), which may be visualized on agarose gels, but is difficult to quantify.

**ELISA:**

Can detects and quantifies cytosolic mononucleosomes and oligonucleosomes Poly ADP-Ribose Polymerase

**Annexin:**

Annexin V is an anticoagulant protein that preferentially binds negatively charged phospholipids. An early step in the apoptotic process is disruption of membrane phospholipid asymmetry, exposuring phosphatidylserine (PS) on the outer leaflet of the cytoplasmic membrane. Fluorescently conjugated Annexin V can be used to detect this externalization of phosphatidylserine on intact living cells. Propidium iodide is often combined as a second flurochrome to detect necrotic cells.

**Caspase 3:**

Induction of apoptosis leads to procaspase-3 proteolytic cleavage to generate an active 18 kDa caspase-3 fragment which then targets key modulators of the apoptotic pathway including poly-ADP-ribose polymerase and other caspases, for cleavage.

# Chapter 3

# Stem Cell

**Stem cell unique properties:**

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have three general properties:

1. They are capable of dividing and renewing themselves for long periods.
2. They are unspecialized.
3. Regulated by intrinsic signals and the external microenvironment and
4. Can give rise to specialized cell types.
5. Are very few in population.

Self-regeneration is the ability of stem cells to divide and produce more stem cells. During early development, the cell division is symmetrical i.e. each cell divides to gives rise to daughter cells each with the same potential. Later in development, the cell divides asymmetrically with one of the daughter cells produced also a stem cell and the other a more differentiated cell.

Depending on differentiation potentiality,stem cells are of three types

1. **Pluripotent**- (can give rise to all the cells of three germ layers) i.e. Embryonic Stem Cell (ESC)
2. **Multipotent**- (can transdifferentiate into few types of specialized cells) i.e. Adult Stem Cells (HPSC, BMSC etc.)
3. **Unipotent** -(can only differentiate into one types of specialized cell) i.e Cardiac Muscle Stem Cell.

**Different developmental potentialities of cells:**

| Differentiation Potential | Number of cell types | Example of stem cell | Cell types resulting from differentiation |
|---|---|---|---|
| Totipotential | All | Zygote (fertilized egg), blastomere | All cell types |
| Pleuripotential | All except cells of the embryonic membranes | Cultured human ES cells | Cells from all three germ layers |
| Multipotential | Many | Hematopoietic cells | skeletal muscle,cardiac muscle, liver cells, all blood cells |
| Oligopotential | Few | Myeloid precursor | 5 types of blood cells (Monocytes, macrophages, eosinophils, neutrophils, erythrocytes) |
| Quadripotential | 4 | Mesenchymal progenitor cell | Cartilage cells, fat cells, stromal cells, bone-forming cells |
| Tripotential | 3 | Glial-restricted precursor | 2 types of astrocytes, oligodendrocytes |
| Bipotential | 2 | Bipotential precursor from murine fetal liver | B cells, macrophages |
| Unipotential | 1 | Mast cell precursor | Mast cells |
| Nullipotential | None | Terminally differentiated cell e.g. Red blood cell | No cell division |

**Stem cells are involved in three processes** - development, repair of adult tissue and cancer.

Totipotent zygote is the ultimate if stem cell which has ability to produce trophoblast and the embryonic membranes. During development the 32- to 64-cell stage of zygote is called a blastomere which sticks together to form a tight ball of cells called a morula.

Each of these cells is totipotential. The next stage is blastocyst which consists of a hollow ball of cells called as trophoblast cells. In addition to cell division the development is also characterized by cell migration. The gastrula is composed of three germ layers i.e. the ectoderm, mesoderm and endoderm. The outer layer or ectoderm, middle layer is mesoderm and the inner layer is called endoderm.

The lineage differentiation of these three germ layers

| Embryonic Germ Layer | Differentiated Tissue |
|---|---|
| Endoderm | Thymus, Thyroid, parathyroid glands, Larynx, trachea, lung, Urinary bladder, vagina, urethra, Gastrointestinal (GI) organs (liver, pancreas), Lining of the GI tract Lining of the respiratory tract |
| Mesoderm | Bone marrow (blood), Adrenal cortex, Lymphatic tissue Skeletal, smooth, and cardiac muscle, Connective tissues (including bone, cartilage), Urogenital system Heart and blood vessels (vascular system) |
| Ectoderm | Skin, Neural tissue (neuroectoderm), Adrenal medulla, Pituitary gland, Connective tissue of the head and face, Eyes, ears |

As the developmental precedes, stem cells loose there differentiation potentiality and gain specific fate by determination process. Cells of the germ layers are more specialized than the fertilized egg or the blastomere. Germ layer stem cells give rise to progenitor cells or precursor cells Zygotic Stem Cell by symmetrical cell division produces two stem cells. But by asymmetrical cell division produces one stem cell and one progenitor cell. The progenitor cell produces two differentiated cell by an asymmetrical cell division (Fig-10)

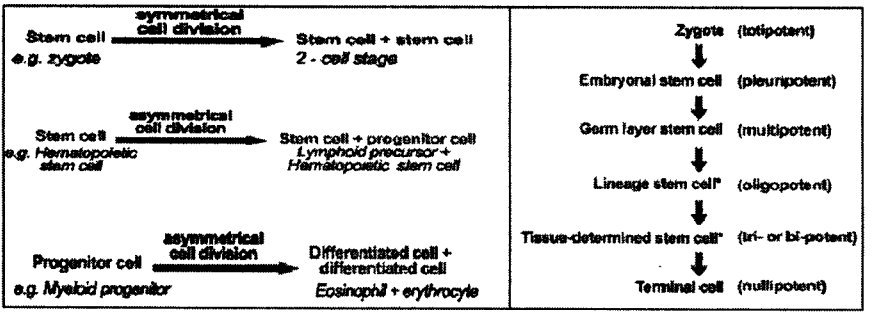


**Fig.10: Types of stem cell division.**

## Embryonic Stem Cell (ESC)

**Three types of mammalian pluripotent stem cell lines (Fig-11)**

A) Embryonal carcinoma (EC) cells which is derived from testicular tumours (teratocarcinomas)

B) Embryonic stem (ES) cells which is derived from blastocysts (pre-implantation embryos)

C) Embryonic germ (EG) cells which is derived from primordial germ cells of the post-implantation embryo

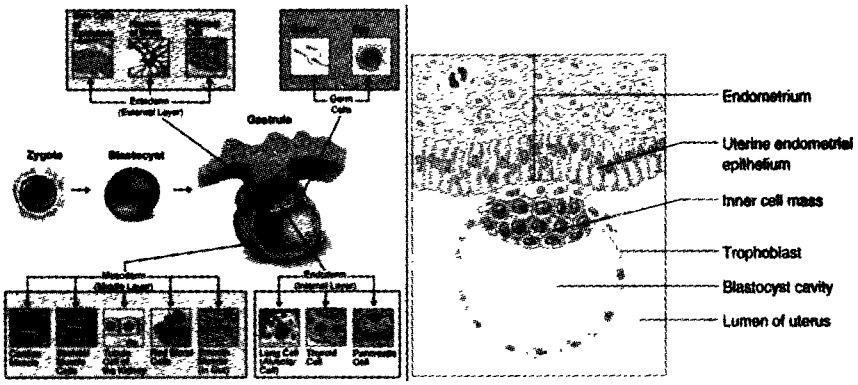


**Fig.11: Potentiality of ESC.** **ECS**

**Pluripotency can be tested in three independent assays:**

1. Ability to differentiate in vitro in a Petri dish
2. Differentiate into teratomas or teratocarcinomas when placed in adult histocompatible or immunosuppressed mice
3. In vivo differentiation when introduced into the blastocoel cavity of a pre-implantation embryo
   - ES and EG cells contribute to every cell type, including the germline
   - EC cells colonise most embryonic lineages, but generally do not colonise the germline

**Factors required for maintaining pluripotency:**

A very few of the factors regulating self-renewal of stem cell are known. Feeder layers *in vitro* provide some critical factors for maintenance of undifferentiated state, pluripotency and ESCs expansion. A feeder layer provide the factor(s) that suppress

differentiation or promotes self-renewal. Leukaemia inhibitory factor (LIF) is one of the several factors that has differentiation-inhibiting activity.

**LIF :** It is a Neuropoeitic and haematopoietic cytokine that activates a heterodimeric receptors. IL6 family of cytokine receptors activate the STAT family of transcription factors to inhibit cell differentiation.

**Stem Cell Markers:**

Markers are unique in their expression pattern and timing provides a useful tool for scientists to initially identify as well as isolate stem cells. Various combinations of markers are required for identifying and isolating cells for stem cell research.

**Embryonic Stem Cell Markers:**

**Oct-4:** Oct-4 (also termed Oct-3 or Oct3/4). First and most recognized marker used for the identification of totipotent ES cells. It isa transcription factor, that activates gene transcription via a *cis*-element containing octamer motif.It is expressed in totipotent embryonic stem and germ cells. Expression is required for stem cell self-renewal and pluripotency. Down- regulation of Oct-4 causes differentiation of embryonic stem (ES) cells. Oct-4 is one of the master regulator for pluripotency and lineage commitment.

**SSEAs (Stage Specific Embryonic Antigens):** There are three SSEAs (SSEA-1, -3 and - 4. SSEA-1) expressed on the surface of preimplantation eight cell stage murine embryos and also found on teratocarcinoma stem cell surface. SSEA-1 is also expressed in oviduct epithelium, endometrium and epididymis, some areas of the brain and kidney tubules in adult mice. SSEA-3 and -4 are synthesized during oogenesis and are present in the membranes of oocytes, zygotes and early cleavage-stage embryos. These SSEAs controlls cell surface interactions during development. Undifferentiated primate ES cells, human EC and ES cells express SSEA-3 and SSEA-4, but not SSEA-1. Undifferentiated mouse ES cells express SSEA-1, but not SSEA-3 or SSEA-4.

**Hematopoietic Stem Cell Markers:**

**CD34:** The cell surface sialomucin CD34. it is expressed on human bone marrow cells and the most critical marker for hematopoietic stem cells (HSCs). It plays a significant role in early hematopoiesis. But resent research showed that HSCs may be CD34+ or CD34-

depending on the primitiveness of the stem cells. But still $CD34^+$ cells are used as HSCs stem cell source for different therapeutic aspects.

**ABCG2:** ABCG2 is a member of the family of ABC transporters and expressed mainly on CD34- cells and is down-regulated in committed hematopoietic progenitors. It is a determinant of the Hoechst-negative phenotype of side population (SP) and expressed exclusively in monkey bone marrow, mouse skeletal muscle, ES cells and on SP cells. Used for positive selection of pluripotent stem cells from various adult sources.

**Sca-1:** Sca-1 (stem cell antigen 1, Ly-6A/E), an 18 kDa phosphatidylinositol-anchored protein, and the most recognized HSC marker in mice. Sca-1 can also been used to isolate non-hematopoietic stem cell population of adult bone marrow, fetal liver and spleen.

**CD133:** it is a 120 kDa, glycosylated protein and may provide an alternative to CD34 for HSC selection and *ex vivo* expansion. CD133 expression is not limited to primitive blood cells, but defines unique cell populations in non-hematopoietic tissues as well. Human neural stem cells can be directly isolated by using an anti-CD133 Ab.

**Mesenchymal/Stromal Stem Cell Markers:**

**STRO-1**: It is expressed by stromal elements in human bone marrow. STRO-1+ marrow cells is capable of differentiating into multiple mesenchymal lineages those include hematopoiesis-supportive stromal cells with a vascular smooth muscle-like phenotype, adipocytes, osteoblasts and chondrocytes. STRO-1 Ab is in common practice for the identification, isolation and functional characterization of human bone marrow stromal cell precursors.

**Neural Stem Cell Markers:**

**Nestin:** It is a class VI intermediate filament protein, that is predominantly expressed in stem cells of the central nervous system (CNS). Nestin is used xtensively to identify CNS stem cells form various nervous system and from in vitro culture. Pancreatic islet progenitors and hematopoietic progenitors also express Nestin in certain extent.

**p75 Neurotrophin R (NTR):** also known as low affinity nerve growth factor (NGF) receptor. $p75NTR^+$ cells are able to differentiate into neurons, smooth muscle and Schwann cells in culture. It is used to identify hepatic stellate cells and mesenchymal precursors.

**PSA-NCAM (Polysialic acid-neural cell adhesion molecule):** It is a critical factor for many neural developmental processes. PSA-NCAM, is the embryonic form of NCAM, and is mainly expressed in the developing nervous system that retain plasticity

**Differences between mouse and human ES cells:**

**NOTES:** Human ES cells form relatively flat, compact colonies. They can easily be dissociated into single cells. But mouse ES cells form tighter, more spherical colonies that are more refractory to standard dissociation methods. Human ES cells grow more slowly and population doubling time ~36 hours as compared to less than 22 hours in case of mouse.

**Properties of hESC and mESC cells:**

Human and nonhuman primate ES cells share a similar morphology that is distinct from human EG cells and mouse ES cells. Human ES cells form relatively flat, compact colonies that easily dissociate into single cells in trypsin or in $Ca^{+2}$- and $Mg^{+2}$-free medium, whereas human EG cells form tight, more spherical colonies that are refractory to standard dissociation methods, but which more closely resemble the morphology of mouse ES cell colonies. Moreover, human ES cells grow more slowly than mouse ES cells; the population-doubling time of mouse ES cells is ~12 hours, whereas the population-doubling time of human ES cells is about 36 hours.

Paralleling these differences in cellular morphology, human ES cells differ from their murine counterparts with regard to cell-surface antigen phenotype. Like undifferentiated primate ES cells and human EC cells, human ES cells express stage-specific embryonic antigens 3 and 4 (SSEA-3 and SSEA-4), high molecular weight glycoproteins TRA-1-60 and TRA-1-81, and alkaline phosphatase. Undifferentiated mouse ES cells do not express SSEA-3 or SSEA-4, but do express the lactoseries glycolipid SSEA-1, which is not expressed in human ES cells, rhesus ES cells, or human EC cells. The functional significance of these antigens is unknown.

Human ES cells also differ from mouse ES cells in their in vitro culture requirements for undifferentiated growth. Mouse ES cells require leukemia inhibitory factor (LIF) for undifferentiated proliferation. In contrast, LIF alone is not sufficient to prevent differentiation of human ES cells in vitro. Instead, continued undifferentiated propagation of human ES cells currently require feeder layers and either the presence of serum or, if cultured in serum-free medium, bFGF. Under

conditions of low cell density, human ES cell lines are more difficult to propagate in serum, with a cloning efficiency of approximately 0.25%. In contrast, culture in both serum replacement medium and supplemental bFGF significantly increases the cloning efficiency over culture in serum alone. Fibroblast feeder layers are currently required to prevent differentiation of human ES cells. How undifferentiated proliferation can be sustained in the absence of feeder cells is an area of active investigation. The critical factors produced by fibroblast feeder layers, which prevent differentiation of human ES cells, are entirely unknown. Further work is clearly needed to clarify the mechanisms involved in sustaining human ES cell proliferation, including specific receptor-ligand interactions, downstream signaling events, and cellular target molecules. Ultimately, it would be essential to establish feeder-independent culture conditions, which permit large-scale propagation of human ES cells in culture.

Human ES cells have demonstrated remarkably stable karyotypes. Human ES cell lines demonstrate normal XX and XY karyotypes, similar to ES cell lines from other species, but distinct from human EC lines derived from teratocarcinomas. This characteristic makes human ES cells more relevant as a model for the study of developmental biology mechanisms and for derivation of differentiated cells for transplantation therapy.

Human ES cells express high levels of telomerase. The expression of telomerase, a ribonucleoprotein that adds telomere repeats to chromosome ends, thereby maintaining their length, is highly correlated with immortality in human cell lines. Most diploid somatic cells do not express high levels of telomerase and enter replicative senescence after a finite proliferative life span in tissue culture, usually after 50-80 population doublings. Unique among normal somatic cells, some populations of adult stem cells (i.e., hematopoietic stem cells) in vivo also constituitively express telomerase; however, telomerase activity is not sustained when cells are placed in culture. In contrast, cells of the early embryo have high telomerase activity levels. Likewise, human ES cell lines exhibit high telomerase activity levels even after more than 300 population doublings and passage for more than 1 year in culture. In summary, properties of cells of the early embryo, such as normal karyotype and high telomerase activity, are sustained for an extended period of time by human ES cell lines in culture. This unique property among human cell lines has important implications as a tool to study cellular senescence and mechanisms of stem cell renewal.

**Multi lineage differentiation of ESC *in vivo***

Although ES cells can differentiate to multiple embryonic and adult cell types in vitro, pattern formation or organogenesis does not occur to a significant degree. Differentiation in the context of an in vivo environment, such as following injection into a host blastocyst or implantation into mice, unveils the full developmental potential of undifferentiated ES cell lines. In this context, many of the normal features of tissue architecture are reproduced. For example, epithelia exhibit polarity, are enveloped by a basement membrane, and are surrounded by mesenchyme; complex tissue structures such as hair follicles, teeth and gut are also formed. Human ES cells injected into severe combined immunodeficient mice form benign teratomas, with advanced differentiated tissue types representing all three EG layers.

Easily identifiable differentiated cells in human ES cell teratomas include smooth muscle, striated muscle, bone, cartilage, fetal glomeruli, gut, respiratory epithelium, keratinizing squamous epithelium, hair, neural epithelium, and ganglia. Compared with human EC cell lines, human ES cell lines exhibit both more advanced and more consistent developmental potential. For example, the human EC cell line NTERA2 c1.D1 injected into immunocompromised mice forms teratocarcinomas containing simple tubular structures resembling primitive gut, neural rosettes, and tissue resembling neuropile

Embryonic inductive events and complex epithelial-mesenchymal interactions control the formation of organized tissue structures during normal embryogenesis. These events and interactions begin to occur in teratomas but are less pronounced during in vitro differentiation. Unfortunately, the precise inductive events regulating embryonic pattern formation are still being elucidated and cannot yet be reliably reproduced in vitro.

Because in vivo differentiation of human ES cells is more complete than in vitro differentiation, it would be useful to explore means to extract the cells or tissue of interest from the heterogeneous mix of tissues comprising teratomas or to direct differentiation in vivo to a particular lineage.

**Multi lineage differentiation of ESC *in vivo*:**

When removed from feeder layers and transferred to suspension culture, ES cells begin to differentiate into multicellular aggregates of differentiated and undifferentiated cells, termed embryoid bodies

(EBs), which resemble early post-implantation embryos. Human EBs frequently progress through a series of stages beginning as simple, morula-like EBs eventually forming cavitated and cystic EBs between 7 and 14 days of post-differentiation development. As for mouse and nonhuman primate ES cells, differentiation in vitro is consistently disorganized and frequently variable from one EB to another within the same culture. A more comprehensive understanding of the morphology of human EBs and the relationships among different cell types comprising these complex embryo-like structures may yield important new information on early inductive events in human development (Fig-12)

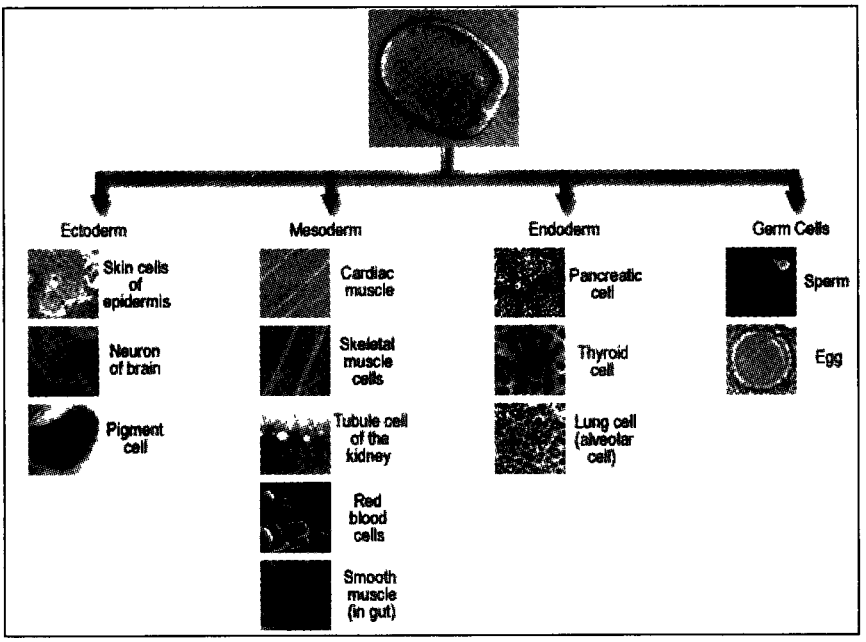


**Fig.12: *In vivo* differentiation of ESC.**

Human ES cells, like nonhuman primate ES cells, are able to differentiate into trophoblast in culture. Nonhuman primate ES cell lines spontaneously differentiate in vitro into extraembryonic endoderm lineages, including yolk sac, and into trophoblast, as evidenced by -fetoprotein and chorionic gonadotropin (CG) mRNA synthesis, and bioactive CG production. Similarly, human EBs synthesize -fetoprotein transcripts and secrete -fetoprotein and hCG into the culture medium. Human ES cells, therefore, represent a useful model in which to study human placental development and function

***In vitro* differentiation:**

Mouse ES cell lines are able to differentiate *in vitro* into a variety of embryonic and adult cell types from all three EG layers. These include cardiomyocytes, hematopoietic progenitors, yolk sac, skeletal myocytes, smooth muscle cells, adipocytes, chondrocytes, endothelial cells, melanocytes, neurons, glia, pancreatic islet cells, and primitive endoderm. From these experiments it is clear that ES cells induced to differentiate in culture follow many of the critical developmental stages found in the normal embryo and are ultimately able to generate post-mitotic terminally differentiated cell types depending on the particular growth factor conditions.

As a result of their ability to differentiate into many different cell types, ES cells have been recognized as a valuable model system for studying the mechanisms underlying lineage specification during the early stages of mammalian development. For example, by comparing downstream gene expression profiles between null mutant and wild-type ES cells, one can dissect the complex network of transcription factor genes regulating tissue-specific gene expression. Also, in vitro culture provides a unique setting enabling control of the extrinsic

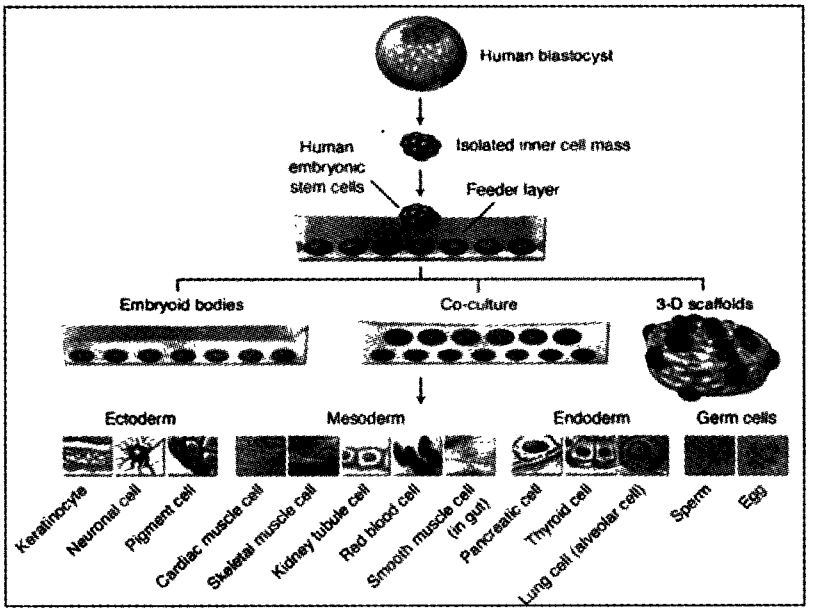


**Fig.13: *In vitro* differentiation of ESC.**

cytokine or growth factor environment to study how these factors influence cellular differentiation. Furthermore, ***in vitro*** differentiation of ES cells transduced with gene trap vectors can be used to discover novel developmentally regulated genes that are important in tissue-specific differentiation programs. Thus, developmental pathways of cell lineages, which can be derived from ES cells, can be studied using this in vitro model system (Fig-13)

Recent studies demonstrate that human ES cells differentiating in culture are able to activate the expression of genes restricted to each of the three EG layers. Human EBs derived from the human ES cell line, H9, transcribed genes for -fetoprotein, neurofilament 68kDa subunit, -globin, and -cardiac actin marking primitive endoderm, neuroectoderm, and mesoderm derivatives· Differentiating cells acquired morphologies characteristic of neurons and cardiomyocytes.

**Possible methods of achieving *in vitro* differentiation of human ES cells**

- adding specific combinations of chemical morphogens or growth factors;
- co-culturing or co-transplanting ES cells with inducer tissues or cells;
- implanting ES cells into specific organs or regions of animals;
- overexpressing tissue-specific homeobox transcription factor genes;
- selecting cells that activate a particular lineage-specific gene expression program
- Isolating cells of interest based on fluorescence-activated cell sorting

**Adult stem cells:**

An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Some scientists now use the term somatic stem cell instead of adult stem cell. Unlike embryonic stem cells cells, which are defined by their origin (the inner cell mass of the blastocyst blastocyst), the origin of adult stem cells in mature tissues is unknown.

**Trans differentiation:** it is the capacity of SC to be differentiated or to take in different fate as described in the following figure-14

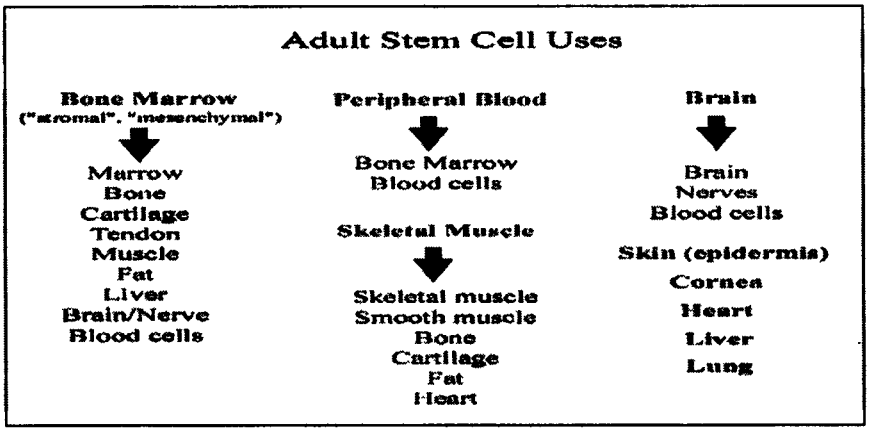


**Fig.14: Trans-differentiation of adult stem cell.**

**Differentiation pathways of adult stem cells:**

Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages, and platelets. Bone marrow stromal cells (mesenchymal stem cells cells) give rise to a variety of cell types:bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells such as those in tendons. neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non non-neuronal cells—astrocytes and oligodendrocytes. Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, Paneth aneth cells, and enteroendocrine cells. Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

**Developing Transplantation Therapeutics:**

Diseases that result from the destruction and/or dysfunction of a limited number of cell types, such as diabetes mellitus, in which pancreatic islet cells have been selectively destroyed, or Parkinson's

disease, which results from the destruction of dopaminergic neurons within a particular region of the brain, could be treated by the transplantation of differentiated derivatives of ES cells (Fig-15). Studies in animal models show that transplantation of either pluripotent stem cell derivatives, or fetal cells, can successfully treat a variety of chronic diseases, such as, diabetes, Parkinson's disease, traumatic spinal cord injury, Purkinje cell degeneration, liver failure, heart failure, Duchenne's muscular dystrophy, and osteogenesis imperfecta

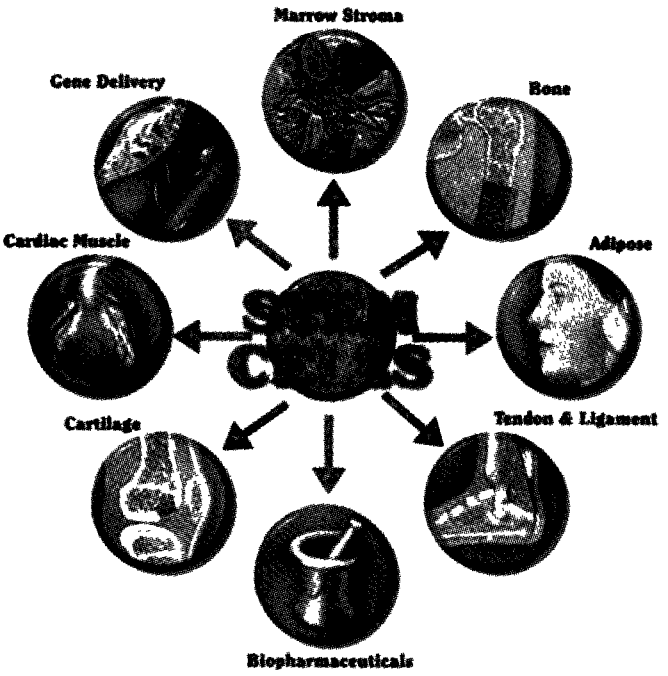


**Fig.15: Cell based therapeutics.**

Although considerable progress in human transplantation medicine, the chief obstacles in widespread application of cellular transplantation that face this field are -

- The need for massive doses of immunosuppressive drugs to prevent rejection of the transplanted tissue and the scarcity of organs from human cadaver donors. In light of these obstacles, a human ES cell-based strategy could permit the generation of an unlimited supply of cells or tissue from an abundant, renewable, and readily accessible source. Moreover, by virtue

of their permissiveness for stable genetic modification, ES cells could be engineered to escape or inhibit host immune responses.

The first step toward successful development of a stem cell-based therapy for human diseases is to establish that human ES cells are capable of differentiating to a particular cell type of interest and to purify this lineage from the mixed population. Unfortunately, the heterogeneous nature of development in culture has hampered the use of ES cell derivatives in transplantation studies.

**To overcome this**

- Use of a tissue-specific promoter to drive a selectable marker such as an antibiotic resistance gene.
- Transduction of a gene construct containing a tissue-specific promoter/enhancer controlling expression of a green fluorescence protein gene.

In this way, cells activating a lineage differentiation program of interest could be selected by fluorescence-activated cell sorting in much the same way that $CD34^+$ hematopoietic stem cells are selected and sorted for stem cell transplantation.

- A third major milestone on the road to clinical trials will be to demonstrate efficacy in rodent and large animal models of disease.

Rhesus ES cells and the rhesus monkey provide an excellent preclinical model for developing ES cell-based transplantation therapies and for testing strategies to prevent immune rejection.Indeed, for Parkinson's disease and diabetes mellitus, good models are already available in the rhesus monkey. Replacment of infarcted heart muscle or scar tissue with ES cell-derived cardiomyocytes will require that new muscle cells integrate with the existing muscle, contract in a coordinated and mechanically useful manner, and develop a new blood supply. Although complex structural integration would be essential for some cell transplants (e.g., neurons and cardiomyocytes), normal functioning of other ES cell-derived transplants will be more independent of such complex tissue interactions (e.g., islet cells and hematopoietic cells).

- Fourth, the possibility arises that transplantation of differentiated human ES cell derivatives into human recipients may result in the formation of ES cell-derived tumors.

**Preventing Immunologic Rejection of Transplanted Cells**

A fifth consideration is the prevention of immune-mediated rejection of the human ES cell-derived cellular graft. Currently used

immunosuppressive drugs are associated with numerous complications including wound healing, opportunistic infections, drug-related toxicities, skin malignancies, and low-grade lymphomas called post-transplant lymphoproliferative disorders. Instead, human ES cells could be genetically manipulated to reduce or eliminate immune-mediated rejection so that lifelong pharmacologic immunosuppression would not be required.

One potential method for limiting the immune response is to decrease the immunogenicity of transplanted cells. Homologous recombination has been used to "knock-out" major histocompatibility complex (MHC) class I and class II molecules in mouse ES cells. But, MHC class I- and class II-deficient skin grafts are still rejected, possibly on the basis of indirect allo-recognition-mediated rejection and/or natural killer cell-mediated destruction. Thus, in addition to deleting foreign MHC genes, it might be necessary to "knock-in" the desired MHC genes, so that ES cell-derivative transplants are seen as "self" by the prospective recipient.

Alternatively, genes for immunosuppressive molecules such as Fas-ligand could be inserted into ES cells, or important immune-stimulating proteins, such as B7 antigens or CD40-ligand, could be deleted from ES cells. Irrespective of the method used, the ability to stably integrate genetic modifications into ES cells provides an advantage over using adult somatic cells, which are less reliably genetically altered.The MHC expression profile of human ES cell derivatives will depend on the degree of differentiation and/or the specific cell type derived. For example, adult somatic cells normally only express MHC class I antigens, whereas B cells, macrophages, and dendritic cells typically express both class I and class II antigens. Furthermore, whereas most adult organs and tissues harbor immunostimulatory dendritic cells and vascular endothelial cells, these normal tissue components would be expected to be absent from ES cell-derived cellular or tissue transplants. Following figure -16 shows the strategy for stem cell therapy.

The specific removal of antigen-presenting cells from solid organ transplants generally increases graft survival. Consequently, some ES cell-derived tissues may be rather inert immunologically, while others, like hematopoietic stem cells, may be as immunogenic as normal adult tissues. Therefore, human ES cell-derived transplants may in some cases provide an inherent immunologic advantage compared to human cadaver tissue transplants.

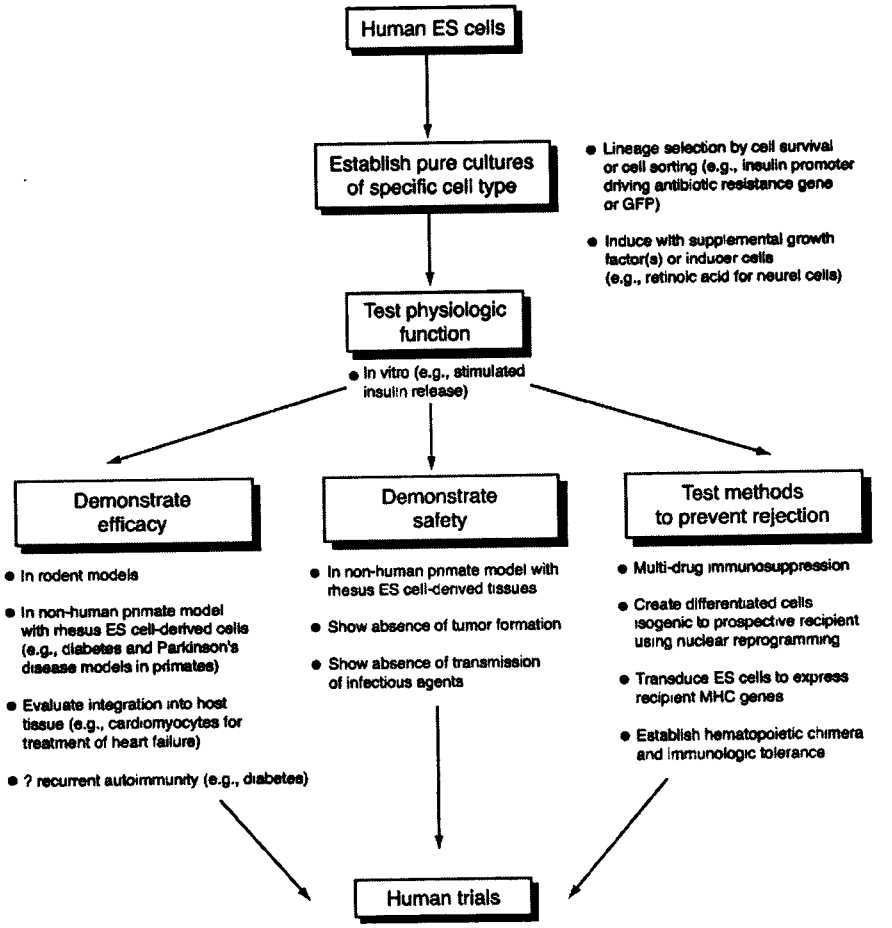


**Fig. 16: Strategy for stem cell therapy.**

Nuclear transfer technology may provide a more precise means to prevent rejection of transplanted cells. Since all nuclear genes here, including major and minor histocompatibility loci, would be seen as "self.".Establishing hematopoietic chimerism is another potential means of preventing rejection of transplanted cells. This relatively mild treatment can permit long-term engraftment and could potentially allow successful solid organ transplantation in humans without prolonged immunosuppressive therapy. By using the same ES cell lines to derive both hematopoietic stem cells and other lineages, it may be possible to initially achieve hematopoietic chimerism followed by engraftment of a second cell type.

**Bone marrow-derived stem cells:**

Bone marrow (BM) contains hematopoietic stem cells (HSCs), which differentiate into every type of mature blood cell; endothelial cell progenitors; and marrow stromal cells, also called mesenchymal stem cells (MSCs), which can differentiate into mature cells of multiple mesenchymal tissues including fat, bone, and cartilage. Recent findings indicate that adult BM also contains cells that can differentiate into additional mature, nonhematopoietic cells of multiple tissues including epithelial cells of the liver, kidney, lung, skin, gastrointestinal (GI) tract, and myocytes of heart and skeletal muscle (Fig-17).

**Types of marrow-derived stem cells:**

**A) Hematopoietic stem cells:**

The only true assay for the presence of hematopoietic stem cells (HSCs) is their ability to reconstitute the hematopoietic system of a myeloablated host as the hematopoietic reconstitution requires extensive self-renewal of the transplanted HSCs and their differentiation into every mature blood cell type.

In humans, the $CD34^{+}CD38^{-}$ cell population is enriched for HSCs in humans. Side population (SP) cells are also enriched for HSCs. They are called SP cells because they have a unique ability to extrude Hoechst dye and because, when examined by fluorescence-activated cell sorter (FACS) analysis, they fall within a separate population to the side of the rest of the cells on a dot plot of emission data in the blue rather than the red spectrum. SP cells express the ABCG2 transporter, a transmembrane protein, which allows them to actively exclude Hoechst dye and fluoresce in this specific manner. SP cells are also present in other tissues, including skeletal muscle. Data are conflicting; some suggest that SP cells can be tissue-specific stem cells within these organs and others suggest that they are actually bone marrow (BM)-derived SP cells lodged within these tissues. A single cell provides long-term hematopoietic reconstitution 20% of the time when injected into lethally irradiated recipients.

**B) Marrow stromal cells:**

Marrow stromal cells (MSCs) derived in different laboratories using different techniques share 2 features: growth in culture as adherent cells with a finite life span and ability to differentiate into osteoblasts, chondroblasts, and adipocytes in response to appropriate stimuli. One of the main hindrances to our understanding of the full potential of

MSCs has been confusion in the literature regarding what specifically defines an MSC and how it should be isolated and grown in vitro. A wide array of cytokines (eg, fibroblast growth factor 2 [FGF2], FGF4, platelet-derived growth factor-BB [PDGF-BB], leukemia inhibitory factor) and isolation techniques (eg, immunomagnetic and physical) have been used to identify and expand MSCs. Furthermore, no specific constellation of surface markers has been agreed on for these cells. Some of the surface antigens reported to be on these cells are Stro 1,CD13, -integrins (CD49a and CD49b), 1-integrins (CD29), CD44 (hyaluronate), CD71 (transferrin), CD90 (thy-1), CD106 (vascular cell adhesion molecule-1 [VCAM-1]), and CD124 (interleukin-4 [IL-4] receptor). MSCs uniformly lack antigens such as CD45 that typically identify hematopoietic cells.

Depending various isolation techniques and culture media used to, MSCs can differentiate *in vitro* into neuronal-type cells,

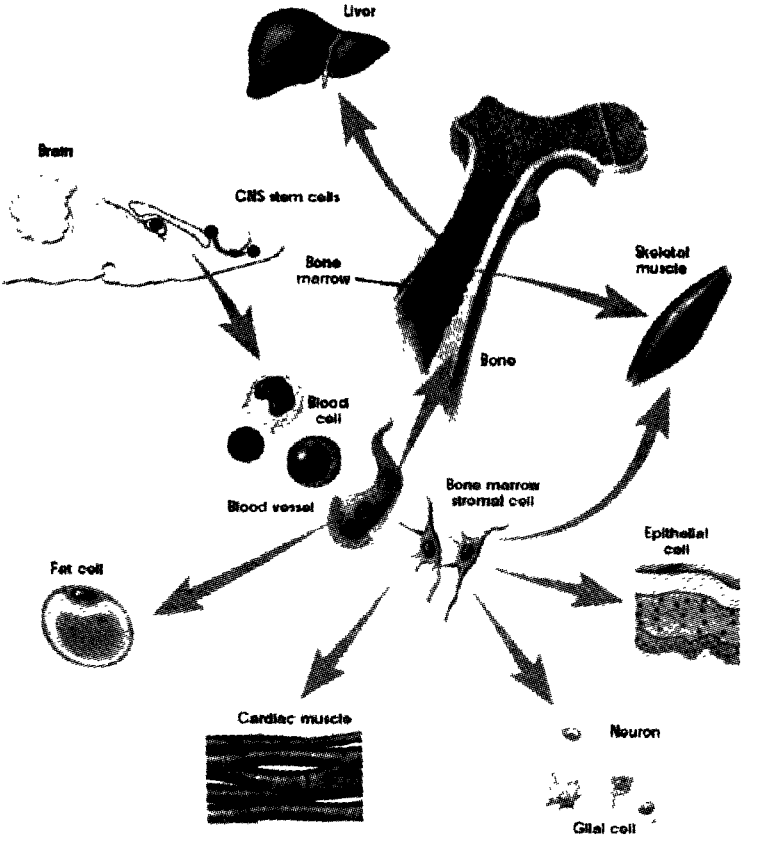


**Fig.17: *in vitro* differentiation of BMSC.**

**C) Multipotent adult progenitor cells:**

A population of highly plastic, adult-derived BM cells, referred to as multipotent adult progenitor cells (MAPCs), can be grown in vitro from postnatal marrow (and other organs) of mice, rats, and humans.They copurify initially with MSCs and grow as adherent cells in vitro. However, unlike MSCs, MAPCs can be cultured indefinitely in a relatively nutrient-poor medium. Specific changes in growth factors induce differentiation of MAPCs into cells bearing endodermal, mesodermal or ectodermal markers. MAPCs also display their broad differentiation potential in vivo. For these assays, MAPCs were derived from ROSA 26 mice, which express -galactosidase under a ubiquitous promoter so that cells from these animals can be tracked. ROSA-26-derived MAPCs injected into murine blastocysts resulted in chimeric mice with ROSA-26 cells contributing to nearly all somatic tissues, including brain, lung, myocardium, liver, intestine, and kidney. After intravenous administration into a sublethally irradiated immunodeficient mouse, MAPCs differentiate to varying degrees into hematopoietic cells in the marrow, blood and spleen and into epithelial cells in liver, lung, and intestine. Consistent with the ability to grow indefinitely in vitro and to self-renew in vivo, MAPCs express telomerase; telomere length is maintained after many cell doublings. It is not yet clear whether MAPCs are a distinct, rare subpopulation of MSCs normally present in humans or whether their in vivo potential is a phenomenon developed under unique in vitro cell culture conditions. Efforts to isolate naive, uncultured MAPCs are under way. In either case, the discovery of these highly plastic cells opens many promising new avenues of research.

**Bone marrow plasticity:**

BM can be transdifferenciated into various cell and type of tissues

| Source tissue | Multilineage differentiation potential |
|---|---|
| Bone marrow (BM) | Astrocyte, Neuron, Cardiomyocyte, Chondrocyte, Hepatocyte, Mesangial cell, Muscle, Neuron, Osteoblast, Stromal cell, Brain (neurons and macroglia), Liver, Cardiac myocytes, Kidney, gastrointestinal tract, Skin, lung, pancreas |
| BM (MAPC) | lung, Blood, Skin, gastrointestinal tract, Liver |
| BM (KTLS ) | Purkinje neuron, Liver |
| MSC | lung, Neuronal cells |
| Circulating cells | Cardiac myocytes |

| | |
|---|---|
| Muscle | Adipocyte, myotubes, osteocyte, Endothelial cell, neuron, Chondrocyte, Osteocyte |
| Trabecular bone | Adipocyte, chondrocyte, osteoblast |
| Dermis | Adipocyte, chondrocyte, muscle, osteoblast |
| Adipose tissue | Chondrocyte, muscle, osteoblast, Stromal cell |
| Periosteum | Chondrocyte, osteoblast |
| Pericyte | Chondrocyte, Osteoblast |
| Blood | Adipocyte, fibroblast, osteoblast, osteoclast |
| Synovial membrane | Adipocyte, chondrocyte, muscle, osteoblast |

Arthritis Res Ther. 2003; 5(1): 32–45.

**Adult mesenchymal stem cells and cell-based tissue engineering**

**Sources of mesenchymal stem cells:**

Examples of these tissues include adipose tissue, periosteum, synovial membrane, muscle, dermis, pericytes, blood, bone marrow and most recently trabecular bone. Currently, bone marrow aspirate is considered to be the most accessible and enriched source of MSCs, although trabecular bone may also be considered an alternative source,

**Trans-differentiation of MSC:**

Adult mesenchymal stem cell (MSC) can differentiate into chondrocytes, osteoblasts, adipocytes, fibroblasts, marrow stroma, and other tissues of mesenchymal origin. Interestingly, these MSCs reside in a diverse host of tissues throughout the adult organism and possess the ability to 'regenerate' cell types specific for these tissues (Fig-18)

**Bone marrow contains three main cell types**

1. Endothelial cells,
2. Hematopoietic stem cells, and
3. Stromal cells.

**Characteristics of mesenchymal stem cells**

MSCs are multipotent for their ability, to trans differenciate into a variety of different cells/tissue lineages

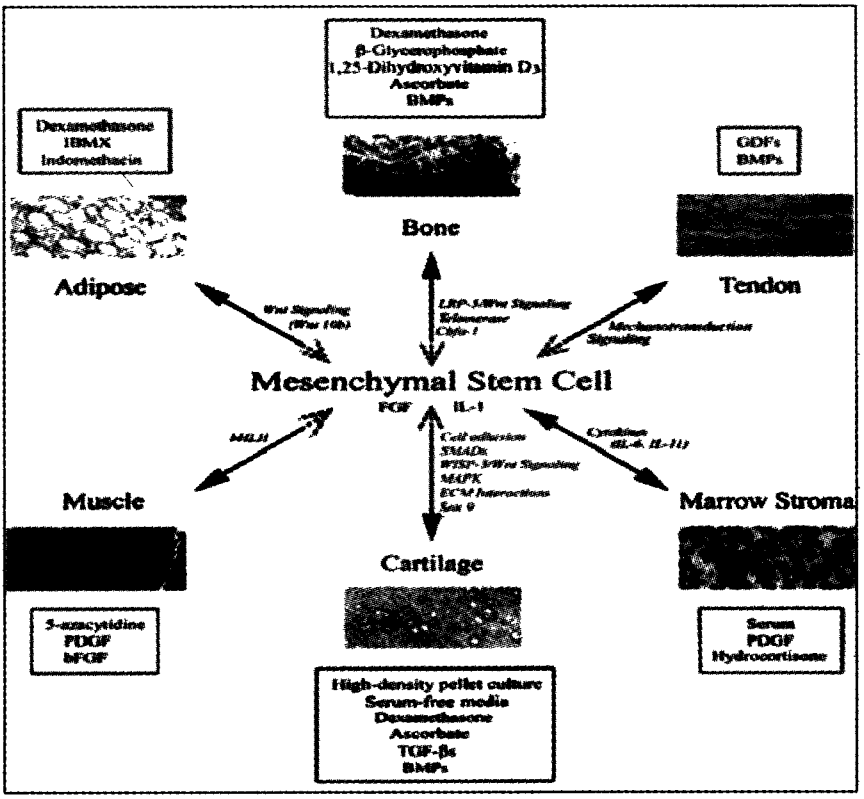


**Fig.18: Trans-differentiation of MSC.**

**Advantages of MSC**

1. long-term culture expansion,
2. MSCs display finite life spans, uncharacteristic of immortalized 'stem' cells.
3. full multilineage differentiation potential

**Characterization of human MSCs**

MSCs isolated directly from bone marrow are positive for CD34. Isolation and enrichment of the MSC population has been greatly facilitated by the Stro-1 monoclonal antibody. Marrow-derived MSCs express SH2, SH3, and SH4 antigens those are absent from osteocytes and osteoblasts MSCs from human bone marrow constitutively expresses cytokines, such as granulocyte-colony stimulating factor, stem cell factor, leukemia inhibitory factor, macrophage-colony stimulating factor, and IL-6 and IL-11 is consistent with the ability of

MSCs to support hematopoiesis and provide factors that regulate the marrow milieu itself.

Applications of mesenchymal stem cells in tissue engineering and regenerative medicine

**Bone:** Osteogenic differentiation is used for bone regeneration.

**Regulation of osteogenic differentiation:** Treatment with the synthetic glucocorticoid dexamethasone stimulates MSC proliferation and supports osteogenic lineage differentiation. Organic phosphates, such as â-glycerophosphate, also support osteogenesis by playing a role in the mineralization and modulation of osteoblast activities. ascorbic acid phosphate and 1,25-dihydroxyvitamin D3, are commonly used for osteogenic induction, with the latter involved in increasing alkaline phosphatase activity in osteogenic cultures and promoting the production of osteocalcin. Application of BMP-2 and basic fibroblast growth factor increases MSC osteogenesis both *in vivo* and *in vitro*

**Bone tissue engineering:** The use of natural and synthetic biomaterials as carriers for MSC delivery has shown increasing promise for orthopaedic therapeutic applications, especially bone formation. Porous ceramics of hydroxyapatite and â-tricalcium phosphate loaded with autologous MSCs for this purpose. These constructs are capable of healing critical-sized segmental bone defects not capable of being healed by resident cells or by the addition of the osteoconductive device alone.

**Cartilage:** Joint pain is a major cause of disability, results from damage to the articular cartilage by trauma or degenerative joint diseases such as primary osteoarthritis. Regeneration of cartilage tissue using autologous MSCs, thereby obviating any donor-site morbidity as is seen with current repair methods.

**Regulation of chondrogenic differentiation:** High-density pelleting and growth in serum-free medium containing specific growth factors and supplements. The TGF-â superfamily of proteins and their members, such as the bone morphogenetic proteins (BMPs), are well-established regulatory factors in chondrogenesis. Another TGF-â family member, BMP-6, appears to increase the size and weight of pellet cultures and to increase the amount of matrix proteoglycan produced. BMP-2 and BMP-9 have also been used in three-dimensional MSC culture systems

**Cartilage tissue engineering :** MSC-based repair of full-thickness articular cartilage defects has been attempted in animal models, using various carrier matrices. Natural polymers such as collagen have shown promise in early applications. Autologous MSCs dispersed in a collagen-type-I gel, can repair full-thickness defects on the weight-bearing surface of medial femoral condyles.

**Soft tissues:**

**Tendon:** MSC can differentiated into other connective tissues, such as muscle, tendons, and ligaments is also being investigated. For tenogenesis, key factors include culture conditions, growth factors, and physical stimulation, such as mechanical loading.

**Adipose tissue:** *In vitro* adipogenic induction requires specific medium supplementations, including dexamethasone and 3-isobutyl-1-methylxanthine. Indomethacin, a nonsteroidal anti-inflammatory drug, binds to and activates the transcription factor peroxisome proliferator-activated receptor gamma (PPAR-ã), which is crucial for adipogenesis . Stromal elements of the marrow, perhaps containing MSCs, can differentiate into either the osteogenic or the adipogenic lineage, depending upon micro environmental cues.

MSCs may also be considered for gene therapy applications for the delivery of genes or gene products. Another intriguing prospect for the future is the use of MSCs to create 'off-the-shelf' tissue banks.

**Cord blood stem cells:**

Umbilical cord is a non conventional and reach source of MSC and Blood stem cell.

**Advantages and Disadvantages of Cord Blood:**

| Advantages | Disadvantages |
|---|---|
| • Limitless supply<br>• Available on short notice for transplant<br>• No donor attritution compared with bone marrow registry<br>• Ethnic diversity easier to achieve<br>• Painless collection of stem cells<br>• Higher proliferative capacity<br>• Lower rate of acute graft-vs-host disease | • Unable to obtain additional "donor" cells for<br>• leukocyte infusion or second transplant<br>• Fewer total HPCs due to small volumes<br>• Slower engraftment (return of circulating<br>• neutrophil and platelet numbers)<br>• Large inventory product (high up-front costs;<br>• units may become "outdated" due to changes<br>• in banking standards |

## Cord blood over Bone marrow:

### Advantages

| Umbilical cord blood | Bone marrow |
|---|---|
| o Speed of availability<br>o Greater likelihood of suitable match<br>o Decreased transmission of viral diseases<br>o Reduced graft versus host disease<br>o Ease of scheduling transplant | o Faster engraftment<br>o More experience |

### Disadvantages:

| Umbilical cord blood | Bone marrow |
|---|---|
| ▪ Less experience<br>▪ Limited cell dose<br>▪ Slower engraftment | ▪ Lack of donors<br>▪ Longer search times<br>▪ More graft versus host disease |

### Cord blood can be transplanted for the therapeutic purpose & their Current Applications

| | |
|---|---|
| **Acute Leukemia's**<br>Acute Lymphoblast Leukemia (ALL)<br>Acute Myelogenous Leukemia (AML)<br>Acute Biphenotypic Leukemia<br>Acute Undifferentiated Leukemia | **Stem Cell Disorders**<br>Aplastic Anemia (Severe)<br>Fanconi Anemia<br>Paroxysmal Nocturnal Hemoglobinuria (PNH)<br>Pure Red Cell Aplasia |
| **Chronic Leukemia's**<br>Chronic Myelogenous Leukemia (CML)<br>Chronic Lymphocytic Leukemia (CLL)<br>Juvenile Chronic Myelogenous Leukemia (JCML)<br>Juvenile Myelomonocytic Leukemia (JMML) | **Other Inherited Disorders**<br>Lesch-Nyhan Syndrome<br>Cartilage-Hair Hypoplasia<br>Glanzmann Thrombasthenia<br>Osteopetrosis<br>Adrenoleukodystrophy |
| **Myelodysplastic Syndromes**<br>Refractory Anemia (RA)<br>Refractory Anemia with Ringed Sideroblasts (RARS)<br>Refractory Anemia with Excess Blasts (RAEB)<br>Refractory Anemia with Excess Blasts in Transformation (RAEB-T)<br>Chronic Myelomonocytic Leukemia (CMML) | Plasma Cell Disorders<br>Multiple Myeloma<br>Plasma Cell Leukemia<br>Waldenstrom's Macroglobulinemia<br>Amyloidosis |

| **Inherited Immune System Disorders** | **Inherited Metabolic Disorders** |
|---|---|
| Ataxia-Telangiectasia | Mucopolysaccharidoses (MPS) |
| Kostmann Syndrome | Hurler's Syndrome (MPS-IH) |
| Leukocyte Adhesion Deficiency | Scheie Syndrome (MPS-IS) |
| DiGeorge Syndrome | Hunter's Syndrome (MPS-II) |
| Bare Lymphocyte Syndrome | Sanfilippo Syndrome (MPS-III) |
| Omenn's Syndrome | Morquio Syndrome (MPS-IV) |
| Severe Combined Immunodeficiency (SCID) | Maroteaux-Lamy Syndrome (MPS-VI) |
| SCID with Adenosine Deaminase Deficiency | Sly Syndrome, Beta-Glucuronidase Deficiency (MPS-VII) |
| Absence of T & B Cells SCID | Adrenoleukodystrophy |
| Absence of T Cells, Normal B Cell SCID | Mucolipidosis II (I-cell Disease) |
| Common Variable Immunodeficiency | Krabbe Disease |
| Wiskott-Aldrich Syndrome | Gaucher's Disease |
| X-Linked Lymphoproliferative Disorder | Niemann-Pick Disease |
| | Wolman Disease |
| | Metachromatic Leukodystrophy |
| **Myelodysplastic Syndromes** | **Histiocytic Disorders** |
| Refractory Anemia (RA) | Familial Erythrophagocytic |
| Refractory Anemia with Ringed Sideroblasts (RARS) | Lymphohistiocytosis |
| Refractory Anemia with Excess Blasts (RAEB) | Histiocytosis-X |
| Refractory Anemia with Excess Blasts in Transformation (RAEB-T) | Hemophagocytosis |
| Chronic Myelomonocytic Leukemia (CMML) | |
| **Myeloproliferative Disorders** | **Inherited Erythrocyte Abnormalities** |
| Acute Myelofibrosis | Beta Thalassemia Major |
| Agnogenic Myeloid Metaplasia (myelofibrosis) | Sickle Cell Disease |
| Polycythemia Vera | |
| Essential Thrombocythemia | |
| **Lymphoproliferative Disorders** | **Inherited Platelet Abnormalities** |
| Non-Hodgkin's Lymphoma | Amegakaryocytosis / Congenital Thrombocytopenia |
| Hodgkin's disease | |
| **Phagocyte Disorders** | **Other Malignancies** |
| Chediak-Higashi Syndrome | Ewing Sarcoma |
| Chronic Granulomatous Disease | Neuroblastoma |
| Neutrophil Actin Deficiency | Renal Cell Carcinoma |
| Reticular Dysgenesis | Retinoblastoma |

## Diseases and Maladies Treated Using Adult Stem Cells

| Cancers: | Auto-Immune Disease: | Spinal Cord Injury |
|---|---|---|
| Brain Cancer | Multiple Sclerosis | Stroke Damage |
| Retinoblastoma | Crohn's Disease | Anemias/Blood Conditions |
| Ovarian Cancer | Scleromyxedema | Sickle Cell Anemia |
| Skin Cancer: Merkel Cell Carcinoma | Rheumatoid Arthritis | Sideroblastic Anemia |
| Testicular Cancer | Juvenile Arthritis | Aplastic Anemia |
| Tumors Abdominal Organs Lymphoma | Systemic Lupus | Amegakaryocytic Thrombocytopenia |
| Non-Hodgkin's Lymphoma | Polychondritis | Chronic Epstein-Barr Infection |
| Hodgkin's Lymphoma | Sjogren's Syndrome | Fanconi's Anemia |
| Acute Lymphoblastic Leukemia | Behcet's Disease | Diamond Blackfan Anemia |
| Acute Myelogenous Leukemia | Myasthenia | Thalassemia Major |
| Chronic Myelogenous Leukemia | Autoimmune Cytopenia | Red Cell Aplasia |
| Juvenile Myelomonocytic Leukemia | Systemic Vasculitis | Primary Amyloidosis |
| Cancer of the Lymph Nodes: Angioimmunoblastic Lymphadenopathy | Alopecia Universalis | **Wounds/Injuries:** |
| Multiple Myeloma | Cardiovascular | Limb Gangrene |
| Myelodysplasia | Heart Damage | Surface Wound Healing |
| Breast Cancer | **Ocular:** | Jawbone Replacement |
| Neuroblastoma | Corneal Regeneration | Skull Bone Repair |
| Renal Cell Carcinoma | **Immunodeficiencies:** | **Other Metabolic Disorders:** |
| Various Solid Tumors | X-Linked Hyper Immunoglobuline-M Syndrome | Osteogenesis Imperfecta |
| Soft Tissue Sarcoma | Severe Combined Immunodeficiency Syndrome | Sandhoff Disease |
| Waldenstrom's Macroglobulinemia | X-linked Lymphoproliferative Syndrome | Hurler's Syndrome |
| Hemophagocytic Lymphohistiocytosis | Neural Degenerative diseases/injuries | Krabbe Leukodystrophy |
| POEMS Syndrome | Parkinson's Disease | Osteopetrosis |
| | | Cerebral X-Linked Adrenoleukodystrophy |

**Potential Future Stem Cell Applications:**

Alzheimer's Disease, Cardiac Disease, Diabetes, Lupus, Multiple Sclerosis, Muscular Dystrophy, Parkinson's Disease, Rheumatoid Arthritis, Spinal Cord Injury and Stroke.

**Possible Approaches to Stem Cell Therapy for Cerebral Infarction:**

Organ-specific stem cells are harvested from the brain, expanded in vitro, and reimplanted into the patient. New neurons may be derived from the neural stem cells to replace those lost during infarction, enabling the patient to regain lost neurologic function. B, Human embryonic stem cells from allogeneic donors are reprogrammed in vitro into neural precursor cells and then reimplanted into the patient. C, Somatic cells (eg, skin cells) are obtained from the patient, and somatic nuclei are harvested and transferred to enucleated human oocytes. A blastocyst is formed from the resulting cell. Cells from the inner mass of the blastocyst are cultured and reprogrammed in vitro to create neural precursor cells, which are then used to repopulate the damaged tissue without risk of immunologic rejection. D, Bone marrow stem cells are harvested from the patient, reprogrammed in vitro to become neural precursor cells, and reimplanted into the patient to repopulate the damaged area. E, Combination therapy in which bone marrow stem cells are harvested, genetically altered through gene transduction, reprogrammed in vitro to become neural precursor cells, and reimplanted into the patient (Fig-19).

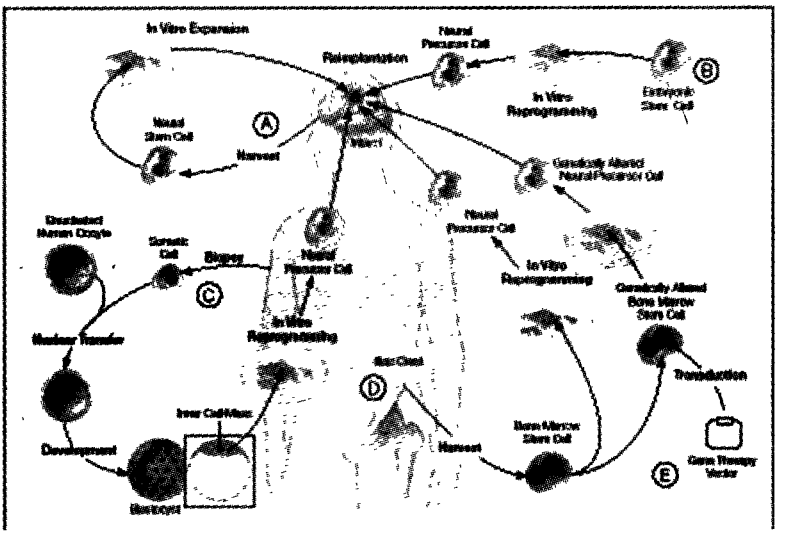


**Fig.19: Approaches to Stem Cell Therapy for Cerebral Infarction.**

Chapter 4

# Eugenics

Every mental and physical characters of human being are controlled by genes. Some characters are useful to human and some characters are harmful to society. One question may arise. Whether some change is occurring in genetic composition? If it is, in which direction? What should be done for this problem? Sir Francis Galton was the first scientist who paid attention to it. Galton agreed with Darwin's theory of evolution. He stated that like in other animals, human beings also have process of evolution. Sir Francis Galton in the year 1883 evolved the word eugenics. He defined it as eugenics is the study of those factors by which development of mental and physical characters takes place in human beings. In other words we can say that the branch of genetics in which the laws of inheritance are used to improve human beings is called eugenics. Eugenics is a Greek word meaning well born.

**Aims and ideals of eugenics**

It is a matter of common agreement that human being is not complete. In other words there is some mental or physical problems which needs improvement. Following characters of human being may need improvement:

1. **Health:** Mental and physical healths of human being are very important. From genetics point of view health means that man should be free from hereditory defects, disease and weakness which may reduce the work efficiency, happiness or life. Beauty is a part of good health because good health and beauty have positive correlation.
2. **Intelligence:** Meaning of intelligence is the capacity to think and understand. High level of intelligence is useful to human being and society.
3. **Moral character:** It means that human beings should have cordial and good relationship with the neighbourers and society. He/she should have high character and excellent behaviour.

4. **Variability:** To attain specialization in knowledge is very useful to the person and society. Generally the capacity for specialization is hereditary.

**Methods of improving mankind**

To improve human being two methods may be used:

First method is **euthenics**. It means by improving the environment and surroundings, improvement is brought in human beings. By the word environment we mean education, nutrition, medical facilities, dress, house etc.

The second method is **eugenics**. Under it improvement in hereditary constitution are studied. This method is of following two types.

1. **Positive eugenics:** Under positive eugenics studies are made for production of superior hereditary characters.
2. **Negative eugenics:** Under this studies are made to get rid of undesirable germplasms

**Positive eugenics:**

Positive eugenics is used to develop person with superior hereditary characters. In addition to it efforts are made to prevent good germplasm from deterioration. Therefore it is important that persons of exellent heredity and inheritance should produce more children. Hence it is important that these persons should be provided sound financial conditions by the government.

Generally it is seen that persons of excellent hereditary characters which have specialized knowledge and excellent human beings produce less children. One of the reason is late marriage. This reason is being discussed below.

**Late marriage:**

There are several reasons for late marriage. Details and their possible reasons are mentioned below.

1. **Higher education:** Higher education is one of the main reason for late marriage. Boys and girls attaining higher education usually marry at the age of 30 - 35 years. Reproductive age in man is upto age of 45 years and in women is upto age of 40 years normaly. Therefore highly educated men and women get reproductive stage only for 10 - 15 years.

On the other hand lower grade persons marry at the age of 17 - 22

years. Therefore they get quite a long reproductive phase.

**Solution to the problem:**

(a) To stop late marriage in higher educated men and women. One step is to encourage such persons while studying. They should be provided all financial help for reasonable good living. Beside this following restrictions & imposition may help further:

(b) There should be restriction for late marriage in highly educated persons by imposing tax.

(c) Boys and girls attaining higher education and willing for early marriage must be given scholarship, freeship, free books, stationary etc. from the government.

**2. Reduction in sexual instinct:** Persons who pass a long period of their life for higher education sometimes suffer from temporary suppression of other psychological feelings. This temporary suppression some times develop physiological disturbances due to which their gonads do not function properly. That is the reason that why high intellectuals sometimes becomes sexually incapable. Therefore in them the feeling develop for not to marry.

**Solution to the problem:**

For solution, entertainment must be made a part of higher education and presence of those high intellectuals must be made compulsory. By doing so their sexual instinct will be alive and they will marry and produce children.

**3. Poor economic position in early years of employment:** Poor economic situation is also a cause for late marriage. Now a days there is competition everywhere including employment. It has been seen that employment is not given as per the qualification. Therefore a person who has passed a long part of his life to get higher education does not get employment for long time, or such person gets underemployment which does not suit to their qualification. In that case the person gets disappointed and think that it would be better to marry only after getting proper job so that it would be possible to maintain the family properly.

**Solution to the problem-**Solution can be done by following methods:

a. Persons with high education must get such a suitable & dignified employment which has high salary as well as respect.

b. Such persons after marriage must be given enough marriage allowance.

c. After birth of every child the person must get sufficient increase in pay.

**4. High cost of living:** All of us know that now a day's price of every item is increasing. After marriage when the lady is in carrying stage and maternity stage, that time she requires several items. Due to high prices it becomes a problem how to procure them. Due to this reason they tries to postpone the conception till they have sound financial condition.

In addition to it **housing is another problem.** Reasonable good house is the requirement of newly married couple. But the price of land and price of construction is so much high that it becomes extremely difficult or sometimes impossible.

The problems mentioned above have effect on number of children. They prefer to have less children so that their expenses can be born.

In addition to the above mentioned problems **education of the child is another problem** for high intellectuals. Now a days standard of education in government school is poor. Therefore they try to admit their children in convent school. But admission fee and monthly fee are exorbitant in convent school. Due to these reasons high intellectual persons produce fewer children so that they can get them educated properly.

**Solution to the problem:**

(a) For delivery government must provide financial help to such persons.

(b) High intellectuals must get special grant to build a house. They must get land at reasonably cheap price.

(c) High intellectuals must get maternity allowance.

(d) Such persons must be given additional money for education of children, otherwise low cost convent school should be provided.

**5. Lack of freedom of marriage with desired mate:** In Indian society sometimes does not allow marriage between girls and boys who love each other. During long period of education sometime boys and girls of other cast starts love affair. Mostly parents does not allow such marriages. Due to which those boys and girls of high caliber decide not to marry.

**Solution of the problem:**

Government should make a rule that boys and girls of high caliber

even may be of different cast can marry against their parent's wishes.

Thus it is clear that if above mentioned points are executed properly then there will be definitely improvement in human race.

**Negative eugenics:**

For welfare of human beings negative eugenics can be of great help. Following methods may be used:

1. **Sterilization:** Undesired persons not fit for the nation should be sterilized so that they may not produce children.
2. **Stopage of child birth** in parents of defective inheritance and avoid marriage between blood relation.
3. **Avoid marriage between** defective persons.
4. **Check on immigration.**

These are detailed below:

**1. Sterilization:**

Sterilization is a good technique for welfare of the country. This should be applied on undesirable persons to prevent them producing children. Sterilization is dependent on surgery. Under this in men vasectomy and in women salpingectomy operation is done. These surgical operations are explained below:

**Vesectomy:**

It is a minor surgery. Doctor open the scrotum and cut the spermduct upto some length and rest is tied properly. Afterwards scrotum is stitched. By this surgery the man looses the power of reproduction. But production of male harmone in testes continues. Though seminal vesicles and prostrate glands ejaculatory fluid is produced as before but sperm production stops and thus the man can not produce child.

**Salpingectomy:**

Salpingectomy is a complicated surgery. It is done in ladies. In this stomach is cut . Then the oviduct is cut. The cut ends are tied. Afterwards the stomach is stiched. After this surgery production of endocrine harmone continues. So the lady carries on the married life as before. However because ovum is not produced so she is unable to produce child.

In the above mentioned operations male and female have permanent sterility. However if in males spermduct (vasadiference) and in female

oviduct (fallopian tube) are tied with surgical thread which can be joined when desired, then in both cases sterility will be temporary.

Now a days sterilization techniques are being widely practiced. United States of America has also legalised this surgery. Sterilization surgery is specially recommended in case of undesirable persons such as idiotic, insane, tuberculosis, cancer, leprocy,etc. If these surgeries are done in right sense then these hereditary diseases can be eliminated from the world one day.

**2. Stopage of child birth in parents of defective inheritance and avoid marriage between blood relation:**

Different type of sex linked characters for example night blindness etc. are definitely inheritable whichare controlled by dominant genes, heterozygous recessive condition, sex linked recessive gene and autosomal recessive gene.

If disease caused by recessive genes occurs in a family, then it may pass from generation to generation without expression, because generally husband or wife are normal, but if by chance marriage takes place between first cousin in that case the children gets this gene in homozygous recessive stage. So they develop the disease of colour blindness.

Looking the above mentioned facts it is very important for welfare of the nation we should avoid marriage between close relatives. Some advanced countries such as United States of America has abandoned marriage between close relatives by rule.

**3. Avoid marriage between defective persons:**

To avoid proliferation of bad germplasm another method is to avoid marriage between defective & undesirable persons. By doing so the society will be benefited in two ways viz.(i) persons who are habitual criminals, beggars, drinkers, mads will not be able to produce children because their children may become like the parents. (ii) such bad persons will not be spoiling good persons of the society.

**4. Check on immigration of foreigners:**

Under negative eugenics the last important suggestion is check on immigration of foreigners. The reason is that if foreigners settle permanently then there are chances of transfer of undesirable genes in Indians. This fact can be understood by an example. Suppose a foreigner having epilepsy or/ and tuberculosis in recessive form if

settle in India, he may marry with a women who has recessive genes for these diseases. In that case in their children both recessive genes will appear in homozygous stage. The result will be that their children will express those bad diseases.

Therefore before allowing any foreigner to settle in India it is very important to find out properly that whether that person has any disease in heterozygous or homozygous phase.

The above mentioned methods of positive eugenics, negative eugenics and euthenics if used in good combinations then there will be good success in improvement of human race.

Chapter 5

# Plant Breeding

India is an agricultural country. Seventy percent of its population are farmers. Despite this, food problem is an important problem of our country. Beside India other countries of the world are facing the same problem. How to solve this problem? It appears that answer to this question is quite difficult because population of the world is increasing at the rate of over 2.2 crores every year. To solve this problem plant breeding may help. Therefore it is essential that plant breeders should develop such improvement in crops so that farmers may reap more and more yield of better quality.

Plant breeding is a subject which is related to genetics, cytology and cytogenetics and has its own special features. Plant breeding is based on principles of genetics and studies in details the technologies of crop improvement. A good plant breeder has good understanding of genetics and cytogenetics. In addition to these he/she should know agronomy, horticulture, plant pathology, plant physiology, statistics, agricultural chemistry and entomology.

Plant breeding has been defined by G.M. Poehlman and D.N. Borthakur (1959) as a science to improve heredity of plants. When by hybridization between close relatives offspring are produced, it is known as inbreeding. But if the parents are least related or distantly related then the breeding is known as out breeding.

Under plant breeding stigma of other plant is artificially pollinated and offspring are produced for various types of selections. Therefore plant breeder has to know merits and demerits of the plant and requirement of the country.

**Research stations of plant breeding in India:**

In India plant breeding researches are done by state government, central government and non government agencies. Indian Council of Agricultural Research (ICAR), Council of Scientific and Industrial Research (CSIR) and the important central agencies, state agricultural universities in various states of India are the important state agencies.

Commercial private seed breeding stations are non government agencies. Breeding research centers in important crops are mentioned below:

1. **Wheat:** Main breeding centre on wheat is at Indian Agricultural Research Institute, New Delhi. Breeding work on this crop is also being done in various state agricultural universities. Name of some famous wheat breeders are B.P.Pal, Ram Dhan, B.S. Kadam, Pathak, Bhatia etc.
2. **Rice:** There are several breeding centers for rice in various states. The Central Rice Research Institute is located in Cuttack of Orissa. In Uttar Pradesh rice breeding centers are in Nagina, Faizabad, Gorakhpur, Panchperva, Tisuhi etc. Several rice breeding stations are located in various state agricultural universities of north and south India. Name of some famous rice breeders are Ramiah, Kadam, Nagavo, Parthasarthy, etc.
3. **Pulse:** Breeding researches on pulses are being done in various states of India. Central Pulse Research Station is located in Kanpur, Uttar Pradesh. Central Research Station for Soyabean is located in Madhya Pradesh.
4. **Potato:** Breeding work on potato is being done at Potato Breeding Station, Shimla, Himachal Pradesh. Some breeding work is also being done at Ootachmund (Tamil Nadu), Poona and Shilong. Name of some famous potato breeders are B.P.Pal, Pushkar Nath, M.S. Swaminathan, Mundkar etc.
5. **Cotton:** For cotton breeding work in the country certain committee have been formed eg. Indian Central Cotton Committee (ICCC). This committee manage cotton breeding researches done by various states. Central Cotton Research Institute is located in Nagpur. Name of famous cotton breeders are Ansari, Avtar Singh, Dastur, Ghose, Ayangar, Jagannath Rao, Joshi, Kalyan, Raman, Majumdar, Pandey,Lather, etc.
6. **Sugarcane:** Central Sugarcane Research Institute, Coimbatore is doing excellent breeding work on this crop. It has a branch at Lucknow, Uttar Pradesh.
7. **Horticultural crops:** Indian Institute of Horticultural Research (IIHR), Bangalore is doing excellent breeding work on **fruit crops** such as mango, banana, papaya, guava, citrus, grape, pineapple, **floricultural crops** eg. rose, marigold, chrysanthemum, bougainvillea, **vegetables** eg. tomato, brinjal, okra, chilies, pumpkin, beans, spinach, watermelon, muskmelon, etc.Vegetable

breeding is also being done by the Central Vegetable Research Institute, Varanasi. IIHR, Bangalore is also doing breeding research on **medicinal & aromatic crops** such as vinca, khasikateri, aloe, kevanch, memory plant, jasmines, tuberose, scented geranium, patchouli, etc. Breeding researches on these crops is being done by Central Institute of Medicinal and Aromatic Plants, Lucknow & its regional research stations, National Research Centre on Medicinal & Aromatic Plants, Anand and various state agricultural universities.

In addition to above mentioned research centers India has also other research centers on various other crops such as Indian Coffee Board, Indian Tea Association, Jute Research Institute, Barrakpur & Neelgang, West Bengal, Indian Coconut Committee, Kerala, Central Arecanut Committee, Kerala, Central Tobacco committee, Chennai, and other research institutes.

**Objectives of plant breeding:**

Objective of plant breeding is to combine various useful characters into one variety of plant. The useful characters which a plant breeder must understand are mentioned below:

1. **High yield:** It is the most important objective of plant breeding.
2. **Early maturity:** In addition to high yield, early maturity of the crop is also an objective.
3. **High quality:**In addition to above mentioned qualities the crop should be of high quality. It should have sufficient nutrient, attractive colour, should be tasty and easily digestible. As per the crop requirement the produce should be of excellent quality.
4. **Non shedding of grain:** Due to shedding of grain there is reduction in yield. This problem is more in case of paddy grown in Kashmir. Therefore plant breeding has the objective to prevent the shedding . Non shedding varieties of paddy developed are S22 and mtu 7.
5. **Resistance to drought and frost:** If these objective are met then the crop can be grown at a place where it was earlier not possible to grow. For example ground nut (*Arachis hypogea*) was not grown in Punjab. However because of development of new varieties F15 and Samrala now ground nut can be grown in Punjab.
6. **Disease and insect resistance:** Plants of vegetables, cereals, pulses, oil seeds, fruits, wooden plants, medicinal and aromatic

crops are attacked by several insects and diseases caused by fungus, bacteria and virus. Every year this leads to loss of several crores rupees in the country. Therefore one objective of plant breeding is to develop varieties which are resistant to diseases and insects. For example in case of wheat varieties resistant to rust are NP 809 and NP 770.

7. **More medicinal property:** In case of breeding of medicinal crops the objective is to increase medicinal properties, yield and resistance to biotic & abiotic stress.
8. **More yield of superior quality essential oil:** For breeding of aromatic crops the objective is to increase yield, quality and resistance to diseases & pests.

In addition to above mentioned objectives a plant breeder makes efforts to develop varieties which by application of more fertilizers and manure can produce more yield, the fruits should have uniformly good size and colour. The crop should have resistance to lodging and flood.

**Methods of plant breeding**

Main methods of plant breeding are the followings:

1. Plant introduction
2. Selection
3. Hybridisation
4. Mutation
5. Polyploidy

Before adopting the method of plant breeding the breeder should know how the plant breeds and propagate. According to method of reproduction plant can be of two types.

**(a) Asexual group:** In some crops and fruits the propagation is by vegetative means such as cutting, layering, budding, grafting, inarching, tissue culture etc. for example potato, sweet potato, sugarcane, mango, rose, jasmine, scented geranium, patchouli etc.

**(b) Sexual group:** Most of the cereals, pulses, vegetables, some fruits have sexual reproduction. Under sexual group some crops have self pollination viz. barley, oat, wheat, paddy, gram, pea etc. where as some crops have cross pollination system such as maize, rye, cucurbits, jute etc.

Now brief description is being presented for methods of plant breeding below:

**1. Plant introduction:**

For improvement of plants, amongst all the methods plant introduction is the cheapest and also most simple method. Plant introduction means to procure new varieties and to grow and evaluate. New varieties are brought from different places in the country or introduced from different country either by post or by personal visit or by plant collecting expedition or with the help of Food and Agriculture Organization. After collection the plants are grown for some generations at different locations and afterwards tested for general suitability. By introduction superior variety is produced. In addition to it this can be included in hybridization programme. Following crop varieties are introduced in India:

(a) **Ridley variety of wheat:** It was introduced from Australia. It is very suitable for hilly tracts of India. It has good yield and resistant to rust disease.

(b) **Tie Shin Tun and FA-17 varieties of potato:** These varieties were obtained from China. FA-17 variety gives high yield and is quite tasty. Tie Shin Tun variety is quite large in size, attractive and has high yield.

(c) **Sioux variety of tomato:** It was obtained from U.S.A. This can be grown in summer as well as winter. Fruits are of large size and gives high yield.

(d) **Philippine early variety of cowpea:** It was introduced from Philippine . It matures early. The pods are straight, green and thick. Seeds are red & white and kidney shaped.

(e) **Early Badger variety of pea:** It was brought from U.S.A. It grows fast and matures early. Its grains are sweet.

(f) **Haryana mulahathi No-1 :** It is the Russian collection number EC 111236 tested and released by CCS Haryana Agricultural University, Hissar after All India Coordinated Trial.

**2. Selection:**

Selection means to choose out the most useful variant from a mixed populaton. One important thing to be taken care is that proper genotype only to be selected. Selection procedure is of following kinds:

**(a) Mass selection:**

From farmer's field (mixed population) good looking useful variants are selected. Seeds of these selected plants are sown next season or next year. Again from that population good plants are selected. This process is repeated for some generations, till the desired improvement is achieved. Mass selection is based on phenotype. Therefore some times good looking plants are not of good genotype.

**(b) Individual plant selection or pure line selection:**

Under this method from the mixed population the best plant is selected and its seeds are obtained. The seeds are sown in next season or next year. Again from those plants the best plant is selected. This process is repeated for some generations till the desired improvement is obtained. By this method qualitative improvement in characters such as amount of protein, amount of oil, amount of sugar, length of filament etc. can be possible. This method is based on pureline theory of Johansan.

**3. Hybridization:**

By introduction and selection, beyond certain limit, improvement can not be made. Therefore plant breeder has to adopt suitable breeding method. In hybridization method good qualities of parents are combined into offsprings. Hybridization can be defined as cross breeding between related organisms differing in one or several characters. The resultant offsprings are called as hybrids.

**Hybridization techniques:**

Before taking up hybridization the plant breeder has to know the breeding behaviour of the plant ie. The plant is monoecious or dioecious, unisexual or bisexual, self pollinated or cross pollinated. In addition to these the plant breeder must know its floral biology and following informations.

(1) Flowering period

(2) If the plant belong to gramineae family then what time panicles emerge.

(3) The sequence of inflorescence.

(4) When the buds bloom.

(5) How many buds bloom each day.

(6) Anthesis time.

(7) Dehiscence time.

(8) Receptivity time of stigma.

(9) For how long pollen remains viable.

Now the methods of hybridization are described below.

**1. Choice of parent:**

In choice of parents care must be taken for objective of breeding. Then the breeder should decide which parent is to be used as female and male.

2. **Culture of parent plant:** The parents should be grown in experimental garden with optimum growth condition.

3. **Protection of pollen:** In order to save contamination of pollen from other plants, anthers should be collected before dehiscence in clean glass vial. In that vial the anther will dehisce and will be free from contamination.

4. **Emasculation:** Removal of anthers before dehiscence is known as emasculation. The procedure differ from crop to crop. A needle and forecep are used in this process. The needle and forecep before use are sterilized by dipping in methylated or rectified sprit. From hermaphrodite flowers stamens before dehiscence are taken out by help of needle and forecep. But in case of monoecious the female flowers have to be covered carefully In inoculation the petals and sepals should not be removed unless it is unavoidable.

There is one more technique for emasculation in sorghum crop. The whole inflorescence is immersed in hot water at 50 centigrade. Due to this all its pollen grains are killed. Thus imasculation is done.

5. **Bagging:** After emasculation the inflorescence is covered by muslin or special paper bag so that outside pollen should not fall on the stigma. Afterwards a label is tied. On the label is written the name of female and male parents and date of pollination. This bag should remain there till seed formation is not complete.

6. **Pollination:** Pollination should be done at a time when stigma is receptive. For this purpose the bag should be some what opened and the collected pollen is applied on the stigma by a brush. After this process the bag is again closed properly.

**Difficulties in plant breeding:**

During hybridization a plant breeder may have to face following difficulties:

(1) Maturity period of the parents may be different.

(2) Failure in fertilization may be due to sterility or non crossability.

**Favorable conditions for hybridization:**

Following circumstances are favorable for hybridization:

(1) Viable pollen

(2) Receptive stigma

(3) Morphological or physiological compatibility between pollen and stigma.

(4) Resistance of flowers to manipulation.

(5) Chromosome similarity between male and female parents.

(6) Favorable environment for flowering and fruiting.

**Hybridization in self pollinated crops:**

Self pollinated crops are many viz. oat, wheat, barly, paddy, cotton, tobacco, peas, beans, etc. In such crops improvement by hybridization has been classified as mentioned below by Hays, Immer and Smith:

(a) Pedigree selection method.

(b) Bulk method.

(c) Back cross method.

(d) Multiple cross method.

These methods are described below:

**(a) Pedigree selection method:**

Under this method selection is done in every generation after F2. In addition to it, breeding behaviour of the selected plant is studied and proper record is maintained. With this method improvement takes less time. However this need more labour, care and funds. Pedigree method is also known as following because of reasons mentioned against each:

1. Pure line selection: because pureline or inbred line is used.
2. Individual plant selection: because individual plants are the basis of pedigree selection.
3. Head to row selection : because in thickly planted and profusely

tillering crops as wheat and barly where individual plant can not be selected, head is selected and its seeds are sown separately in rows.

4. Projeny selection: because on basis of projeny of individual plants selection for desirable plant is done.
5. Single plant selection: because single plants are selected and their progenies are maintained separately.
6. Inbred selection: because inbred or pure line is used .

Differences between clone and pure line are mentioned in following table:

**Table: Differences between clone and pure line.**

| Points | Clone | Pureline |
|---|---|---|
| Parents | Progenies of a simple vegetatively propagated heterozygous plant | Progenies of single self fertilized homozygous plants |
| Production | By vegetative means | Produced by selfing |
| Genetical Constitution | All members of a clone are identical having same genotype but heterozygous | All members of pureline are identical & homozygous |
| Occurrence | Occurs in vegetatively propagated crops | Occurs in self pollinated crops |
| Importance | Utilized directly as improved variety as well as parent for hybridization | Same |

**(b) Bulk method:**

This method matches with mass selection method. Natural hybridization is allowed in F2 generation to get F3 generation. Selection is done in F3. Seeds are removed from the selected plants. These seeds are sown and plants raised. Crossing is again allowed in these plants. This process is repeated for some generations till the desired is obtained.

**(c) Back cross method:**

F1 offsprings when crossed with one of the parent is called backcross. Backcross breeding method is used to transfer certain character such as disease resistance, drought resistance etc. in the desired parent.

**(d) Multiple cross method:**

Cross between more than two parents is called multiple crossing. Its objective is to transfer desirable characters from more than two

parents into one variety. Under this method first of all selected pureline parents are made. Afterwards crossing is done between pairs. The F1 are crossed. As shown in the diagram below the crosses are made so as to combine all the desirable characters in the new variety:

A X B C X D E X F G X H

AB X CD EF X GH

ABCD X EFGH

ABCDEFGH

One of the advantages of this method is that desirable characters of several parents can be transferred into one variety. New combinations are also developed.

In this method there is a defect also. Because several parents are involved in this method, therefore chances are to develop undesirable combination of characters.

**Hybridization in cross pollinated crops:**

In cross pollinated crops viz. maize, rye, cucurbits, bajra, jute, clover, fodder grasses, some fruit plants, etc. as breeding procedure inbreeding is done to produce homozygous lines. The method is described below: The desirable plants are selected. For inbreeding the plant are self fertilized for about 9 - 10 generations. Then they become homozygous (Table below) In inbreeding process some abnormalities may occur eg. Sterility, loss in size, chlorophyll deficiency etc. After some generations the plant become homozygous and then breed true. From the true breeding plants the desired ones are selected. Then they are crossed. The offsprings develop heterosis. Heterotic plants express the characters in vigorous forms.

**Table: Increase in homozygosity in cross pollinated plant by selfing for ten generation.**

| Generations selfed | Homozygosity | Heterozygosity |
|---|---|---|
| 1 | 0 | 100 |
| 2 | 50 | 50 |
| 3 | 75 | 25 |
| 4 | 87.5 | 12.5 |
| 5 | 93.75 | 6.25 |
| 6 | 96.875 | 3.125 |
| 7 | 98.437 | 1.563 |
| 8 | 99.219 | 0.781 |
| 9 | 99.610 | 0.390 |
| 10 | 99.795 | 0.195 |

Following hybridization procedures are followed in cross pollinated crops:

1. **Single cross method:** two good inbred lines are crossed. The hybrid is used for cultivation.
2. **Double cross method:** In this method two single crosses are made. For example A x B and C x D. Afterwards F1 of these crosses are hybridized. Selection is made and the new variety is produced. The variety NP 825 of wheat and Ganga 101 of maize were developed by double cross method.
3 **Triple cross method:** In this method the F1 of two inbred lines (F1 of A x B) is crossed with a good variety. This way several good genes are combined in one variety. Ganga 2 a good variety of maize was developed by triple cross method.

**Kinds of hybridization:**

Depending on parental relationships hybridization can be of following types:

1. Intervarietal cross
2. Interspecefic cross
3. Intergeneric cross

**1. Intervarietal cross:**

Desirable characters can be combined without difficulty. In wheat, tobacco, cotton, tomato etc. by intervarietal crossings high yielding, disease resistant, drought resistant, nutritive and high quality variety can be developed. The variety NP 165 of wheat was developed by crossing Australia Federation with NP 79.

**2. Interspecific cross:**

Hybridization between different species of a genus is called interspecific cross. In several crops such as wheat, mustard, cotton, luffa, tobacco etc. improvement have been done by this method.

Interspecific cross can be done in plants having one type of chromosomes or different kinds of chromosomes. Some examples are mentioned below:

Interspecific cross between *Primula floribunda* (2n=18) and *Primula verticillata* (2n=18) was made. The F1 chromosome number was doubled. The tetraploid developed was named as Tetraploid *Primula kewensis* (2n=36). Both the species have same number of chromosomes.

Example of interspecific crosses with different chromosome number is *Pennisetum typhoidium* (2n=14) and *Pennisetum purpureum* (2n=28). *Pennisetum typhoidium* suffers from rust disease whereas *Pennisetum purpureum* is resistant to rust disease and has good quality of forage. Its F1 hybrid chromosome number is doubled. Thus a hexaploid drought resistant and high yielding new variety is produced. Interspecific cross is usually recommended for vegetatively propagated crops.

**3. Intergeneric cross:**

Crosses between different genus is called intergeneric cross. For crop improvement intergeneric crosses have been attempted in several crops. For example sorghum and sugarcane; sugarcane and bamboo; maize and teosenti; wheat and rye; mustard and radish etc. Success of intergeneric cross is quite difficult. But if success comes results are wonderful. In modern time intergeneric crosses are important. Some intergeneric crosses are described below:

(1) **Intergeneric cross between wheat(Triticum) and rye(Secale):** The hybrid obtained between Triticum and Secale is known as Triticale or *Secale triticum.* It has a lot of economic importance.

(2) **Intergeneric cross between a type of wheat(Triticum) and Aegilops:** Percibal (1935) reported a cross between *Triticum turgidum* (2n=14) and *Aegilops ovata*(2n=14).He has produced a useful hybrid of economic importance.

(3) **Intergeneric cross between sugarcane (Saccharum)and jowar(Sorghum).** Venkatraman and Janki have crossed *Saccharum officinarum* and *Sorghum vulgare* in Coimbatore, Tamil Nadu. The obtained hybrid was early maturing and of economic importance.

**Mutation breeding:**

In modern times for improvement in crops, fruits and flowers mutation breeding is widely attempted. For example in Coimbatore (Tamil Nadu) on a variety GEB 24 of paddy by irradiation with X ray a useful mutant variety 5782 was produced. This mutant variety is dwarf, heavy tillering type and has other economic characters. Similarly in Indian Agricultural Research Institute Jagdishan, Bhatia and Swaminathan (1961) have treated the varieties NP 799 and NP 809 with X rays, phosphorus 32, & sulphur 35 and produced a mutant variety NP 836. In the same way a good mutant variety of groundnut was produced. In case of gram (*Cicer arientinum*) a mutant variety T

87 was evolved. Other chemicals eg. ethyl methane sulphonate and methyl ethane sulphonate have been used with success in several crops. For further information on mutation please consult the chapter on mutation.

**Polyploidy breeding**

Several crop varieties have been developed by induction of polyploidy. To understand the role of polyploidy in plant breeding please consult the chapter on polyploidy.

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