UNIT-I

SYLLABUS

GENETICS: Introduction The Basics: Historical development in the field of genetics, Organisms suitable for genetic experimentation, Prokaryotic genetics, Cell cycle mitosis and meiosis, Control points in cell cycle progression in yeast, Role of meiosis in life cycle of organism

Unit – I

1. INTRODUCTION

Genetics is the study of genes, and tries to explain what they are and how they work. Genes are how living organisms inherit features from their ancestors; for example, children usually look like their parents because they have inherited their parents' genes. Genetics tries to identify which features are inherited, and explain how these features are passed from generation to generation.

SCOPE OF GENETICS

"The experiments which will here be discussed took their origin from artificial fertilizations which have been carried out on ornamental plants in order to obtain new color variants." This opening sentence of Gregor Mendel's paper points to one of the humble origins of genetics: horticulture. Significantly, as late as 1901, it was the Royal Horticultural Society of London that sponsored the English translation of the then newly rediscovered classic. But the practical desires of the lovers of flowers were not the only incentives to the birth of genetics. All through the eighteenth and nineteenth centuries the species problem occupied the minds of biologists. On the one hand, Linnaeus had defined species in a way which lacked operational usefulness: There are as many species as there were diverse forms created at the beginning by the Infinite Being. On the other hand, Linnaeus and other plant hybridizers attempted to probe into the nature of species by experimental means. Were hybridizations between different species possible? Could they be

induced artificially? Did they ever occur in nature? What kind of organism was a species hybrid? Was it sterile like the mule? If fertile, what kind of offspring did it produce? In 1859, Darwin's Origin of Species proved to be the successful break through the confines of a static conception of species within which biology had accumulated so much of its unordered bulk of empirical facts. Then plant hybridization became a potential tool for progress in a new range. Mendel himself, who began his studies before the appearance of Darwin's book but wrote his report six years after that event, by then had recognized clearly the role of genetics in this respect. The detailed analysis of successive generations of hybrids "seems to be the only right way," he wrote, "by which we can finally reach the solution of a question the importance of which should not be underestimated in connection with the history of the evolution of organic forms."' Genetics and evolution were indeed to become intimately and fruitfully associated with one another; but the scope of genetics was destined to embrace many other regions. From variations in color and form, and from the species problem and hybridization, the emphasis soon shifted to the permanent units which underlie the externally visible phenomena. Mendel's essential discovery had been the recognition that the hereditary contributions of two parents were not each undefined wholes which in the offspring mingled into a new whole. On the contrary, he showed that the hereditary makeup consists of many separable elements which recombine in many ways in the sequences of generations and always reappear in unchanged nature. Bateson called these units factors, "makers." Johannsen, in 1909, coined the term "gene." For him, "the word gene was ... completely free from any hypothesis." It was meant "solely to express the fact that characters of the organism are conditioned by special, at least partially separable and thus in a sense independent 'states,' 'factors,' 'units' or 'elements' in the constitution of the germcells."

The scope of genetics as a central biological discipline rests on the fact that genes are those parts of biological systems which give continuity to them. The primitive experiment of cutting an amoeba in two and finding that only the nucleated part survives and multiplies has been refined until we know that it is the genic material on which survival and multiplication of the organism depends. Most of the cellular material may be removed without permanent harm. Regeneration soon takes care of replacement. A gene, however, once removed from a cell, is not regenerated. Within a cell, a gene always comes from a gene. Again, this attribute of the gene is part of the organization of the whole. The material structure of the gene seems to carry within it the

potentiality of its replication, but actual genic multiplication depends on the presence of a whole assembly of structures and processes. The discovery of the cyclical nature of many biochemical sequences in cellular metabolism has been one of the significant events of our times. Genes are agents in these cycles not only because they determine the existence of enzymes, without which the reactions would not proceed sufficiently, but because some of these reactions themselves must lead to the making of more genes. We admire a jongleur who tosses and keeps rotating several rings moving in space. Figuratively speaking, a cell consists of thousands of such rings functionally bound together not by an outside mover but by themselves. The rings are not all of equal rank. Destroy some, and the rest will re-create them. Destroy a single agent in others, and the whole system will disintegrate. These single agents are the genes. Through them, cellular physiology is brought within the scope of genetics. Rightly, more than thirty years ago, Muller called the gene "the basis of life."

Evolution early was close to the mind of the geneticist. Its study is now unthinkable without the framework of genetics. The differences between species are largely if not exclusively due to differences in their genes. Evolution is based on mutability, the origin of new varieties of genes, on the distribution and recombination's of these varieties within the members of populations, and on the effects which these diverse genotypes have on the fitness of individuals and of populations as whole entities. The New Systematic and the Modern Synthesis in evolution, to borrow phrases from Julian Huxley, are witness to the part which genetics has taken in the transformation of these subjects. Evolution is a historical field. Just as the history of the earth, or that of astronomical systems, is based on the properties of elementary physical particles, so the history of life on our planet is based on the properties of its genetic material. These sciences of their own spheres of endeavor. To say-as it has been said that a lion or a palm is only some genes' way of making more genes expresses deep insights which link the lion and the palm to genetics-although little work on the genetics of lions or palms has been carried out. But the lions and palms remain valid topics for biological study as lions and palms. How evolution brought forth these specific species is not explained simply by referring to their genes. The analysis of animal behavior has rightly been re-recognized as another central discipline within biology. The inheritance of behavior patterns and the inheritance of plasticity in the range of possible behavioral phenomena are being rediscovered in general and analyzed in detail. Genes determine

the degree of exploratory behavior in rats, intelligence or dullness in running through a maze, fear reactions in birds to an object resembling in outline a specific predator, or the details of a complex song. This is amazing and intelligible at the same time. If chemicals can change intimately one's reactions, if under the influence of drugs spiders change the pattern according to which they weave their webs, why should not genes as biochemical cellular agents do the same? If genes can help to organize the differential development of a complex nervous system, why should they not be able to incorporate in it the subtleties of inborn reactions? The wonder is that there is behavior. Genetics provides one of the analytical tools for its study.

The scope of genetics, with its enlightenment of all branches of biology, makes it an obvious partner in the pursuit of applied biology. From horticulture to plant and animal breeding in general, the concepts and methods of genetics-be it individual or strain genetics in terms of hybridization and segregation, or population genetics in terms of equilibria and selection-form indispensable parts of procedure. Problems of pest control involve the existence of innately sensitive strains and the origin of resistant ones. Host-parasite relations depend on a balance of genetically determined interactions between the two components of the combating teams.

Human genetics is both a basic and an applied science. Knowledge of the genetic makeup of the species Homo sapiens, in its individuals and varieties, is the latest gain in the long history in which anatomy and physiology, in ever more penetrating modes, have provided insight into the biological nature of Man and anthropology into that of men. Eugenics, the planning toward rational improvement of the genetic makeup of human populations, has been regarded as a utopian dream and a dangerous one in addition. Many of our concerns have been recognized as being excessive or even unnecessary, and our eagerness to act precipitously has rightly decreased. But, while the attempt at improvement beyond the present average could well be relegated to the future, even the most determined opponents of positive eugenics have now realized with the fervor of converts that negative eugenics, the attempt to inhibit the increase of harmful genotypes, is an immediate necessity.

A good understanding of the principles of Mendelian inheritance is a prerequisite to the conceptual understanding of evolutionary theory. Indeed, though Darwin himself subscribed to

the "blending" theory of inheritance, it has since been determined that evolution by natural selection requires discrete genes.

HISTORY OF GENETICS

Year	Scientist(s)	Discovery
1858	Charles Darwin Alfred Russel Wallace	Joint announcement of the theory of natural selection-that members of a population who are better adapted to the environment survive and pass on their traits.
1859	Charles Darwin	Published The Origin of Species.
1866	Gregor Mendel	Published the results of his investigations of the inheritance of "factors" in pea plants.
1900	Carl Correns Hugo de Vries Erich von Tschermak	Mendel's principles were independently discovered and verified, marking the beginning of modern genetics.
1902	Walter Sutton	Pointed out the interrelationships between cytology and Mendelism, closing the gap between cell morphology and heredity.
1905	Nettie Stevens Edmund Wilson	Independently described the behavior of sex chromosomes-XX determines female; XY determines male.
1908	Archibald Garrod	Proposed that some human diseases are due to "inborn errors of metabolism" that result from the lack of a specific enzyme.

1910	Thomas Hunt Morgan	Proposed a theory of sex-linked inheritance for the first mutation discovered in the fruit fly, Drosophila, white eye. This was followed by the gene theory, including the principle of linkage.
1927	Hermann J. Muller	Used x-rays to cause artificial gene mutations in Drosophila.
1928	Fred Griffith	Proposed that some unknown "principle" had transformed the harmless R strain of Diplococcus to the virulent S strain.
1931	Harriet B. Creighton Barbara McClintock	Demonstrated the cytological proof for crossing-over in maize.
1941	George Beadle Edward Tatum	Irradiated the red bread mold, Neurospora, and proved that the gene produces its effect by regulating particular enzymes.
1944	Oswald Avery Colin MacLeod Maclyn McCarty	Reported that they had purified the transforming principle in Griffith's experiment and that it was DNA.
1945	Max Delbruck	Organized a phage course at Cold Spring Harbor Laboratory which was taught for 26 consecutive years. This course was the training ground of the first two generations of molecular biologists
late 1940s	Barbara McClintock	Developed the hypothesis of transposable elements to explain color variations in corn.
1950	Erwin Chargaff	Discovered a one-to-one ratio of adenine to thymine and guanine to cytosine in DNA samples from a variety of organisms.
1951	Rosalind Franklin	Obtained sharp X-ray diffraction photographs of DNA.
1952	Martha Chase	Used phages in which the protein was labeled with 35S and the

	Alfred Hershey	DNA with 32P for the final proof that DNA is the molecule of heredity.
1953	Francis Crick James Watson	Solved the three-dimensional structure of the DNA molecule.
1958	Matthew Meselson Frank Stahl	Used isotopes of nitrogen to prove the semiconservative replication of DNA.
1958	Arthur Kornberg	Purified DNA polymerase I from E. coli, the first enzyme that made DNA in a test tube.
1966	Marshall Nirenberg H. Gobind Khorana	Led teams that cracked the genetic code- that triplet mRNA codons specify each of the twenty amino acids.
1970	Hamilton Smith Kent Wilcox	Isolated the first restriction enzyme, HindII, that could cut DNA molecules within specific recognition sites.
1972	Paul Berg Herb Boyer	Produced the first recombinant DNA molecules.
1973	Joseph Sambrook	Led the team at Cold Spring Harbor Laboratory that refined DNA electrophoresis by using agarose gel and staining with ethidium bromide.
1973	Annie Chang Stanley Cohen	Showed that a recombinant DNA molecule can be maintained and replicated in E. coli.
1977	Fred Sanger	Developed the chain termination (dideoxy) method for sequencing DNA.
1983	James Gusella	Used blood samples collected by Nancy Wexler and her co-

		workers to demonstrate that the Huntington's disease gene is on chromosome 4.
1985	Kary B. Mullis	Published a paper describing the polymerase chain reaction (PCR), the most sensitive assay for DNA yet devised.
1988		The Human Genome Project began with the goal of determining the entire sequence of DNA composing human chromosomes.
1989	Alec Jeffreys	Coined the term DNA fingerprinting and was the first to use DNA polymorphisms in paternity, immigration, and murder cases.
1989	Francis Collins Lap-Chee Tsui	Identified the gene coding for the cystic fibrosis transmembrane conductance regulator protein (CFTR) on chromosome 7 that, when mutant, causes cystic fibrosis.
1990		First gene replacement therapy-T cells of a four-year old girl were exposed outside of her body to retroviruses containing an RNA copy of a normal ADA gene. This allowed her immune system to begin functioning.
1993		FlavrSavr tomatoes, genetically engineered for longer shelf life, were marketed.

DEFINITIONS AND TERMS

- **Allele:** One alternative of a pair or group of genes that could occupy a specific position on a chromosome.
- **Chromosome:** A linear strand of DNA harboring many genes.
- **DNA:** Deoxyribonucleic acid; the molecule in which genetic information is encoded.

- **Dominant:** An allele producing the same phenotypic effect whether inherited heterozygously or homozygously; an allele that "masks" a recessive allele.
- **Gene:** A unit of genetic information that occupies a specific position on a chromosome and comes in multiple versions called alleles.
- **Genotype:** The genetic constitution of an organism.
- **Heterozygous:** Having a genotype with two different and distinct alleles for the same trait.
- **Homozygous:** Having a genotype with two of the same alleles for a trait.
- **Phenotype:** The physical or observable characteristics of an organism.
- **Recessive:** An allele producing no phenotypic effect when inherited heterozygously and only affecting the phenotype when inherited homozygously; an allele "masked" by a dominant allele.

Model organisms

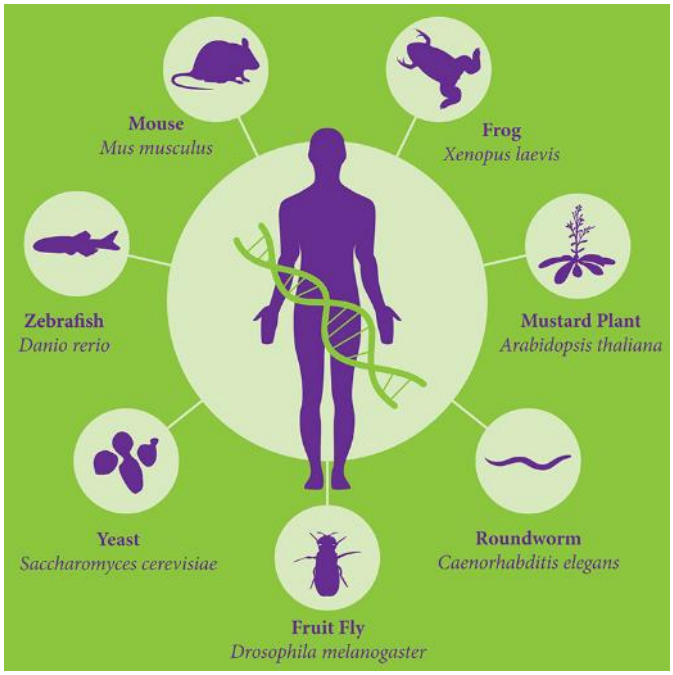
A model organism is a non-human species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made in the organism model will provide insight into the workings of other organisms. Model organisms are in vivo models and are widely used to research human disease when human experimentation would be unfeasible or unethical. This strategy is made possible by the common descent of all living organism, and the conservation of metabolic and developmental pathways and genetic material over the course of evolutionStudying model organisms can be informative, but care must be taken when extrapolating from one organism to another

In researching human disease, model organisms allow for better understanding the disease process without the added risk of harming an actual human. The species chosen will usually meet a determined taxonomic equivalency to humans, so as to react to disease or its treatment in a way that resembles human physiology as needed. Although biological activity in a model organism does not ensure an effect in humans, many drugs, treatments and cures for human diseases are developed in part with the guidance of animal models. There are three main types of disease models: homologous, isomorphic and predictive. Homologous animals have the same causes, symptoms and treatment options as would humans who have the same disease. Isomorphic

animals share the same symptoms and treatments. Predictive models are similar to a particular human disease in only a couple of aspects, but are useful in isolating and making predictions about mechanisms of a set of disease features.

Model organisms in genetics

- Many organisms are used in genetic research. Some of the qualities that make an organism a good model for genetic experimentation include:
    - well known genetic history, allowing extrapolation to other related organisms.
    - short life cycle.
    - matings produce large amount of offspring.
    - easy to handle
    - genetic variation must exist between individuals in a population. Without genetic variation, genetic crosses are uninformative.
- Model organisms include representatives from all the main lineages of the tree of life
    - Eukaryote model organisms: *Saccharomyces cereviceae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, *Mus musculus*, Zebra fish, *Neurospora crassa*, *Tetrahymena*, paramecium, *Chlamydomonas reinhardtii*, *Pisum sativum*, *Zea maize*, and *Gallus*.
    - Prokaryotic model organisms: Most common is *E. coli*, but many others as well, including members of Archaea.
    -



Prokaryotic Genetics

Bacterial plasmids and conjugation

Bacteria are typical representatives of prokaryotes. Prokaryotes have cell structure and their DNA is concentrated in a so-called nucleoid region. However, different from eucaryotes, bacteria have neither meiosis nor mitosis.

There are three principal mechanisms of genetic recombination (genetic material transfer into a HOST organism) in bacteria occurring in nature and also in lab conditions: conjugation, transformation and transduction.

CONJUGATION This is the most important mechanism for widespread transfer of genetic information between bacteria. It is the direct transfer of bacterial DNA between organisms and requires CELL-TO-CELL CONTACT.Most conjugation is PLASMID- MEDIATED.

PLASMIDS are small, circular, ds-DNA molecules, measuring about 2-10 kilobases. They all have the ability to replicate in bacteria. Multiple copies of plasmids can be found in a host cell; however, not all plasmids can transfer themselves.

A CONJUGATIVE plasmid has the codes for the genes allowing transfer between cells.

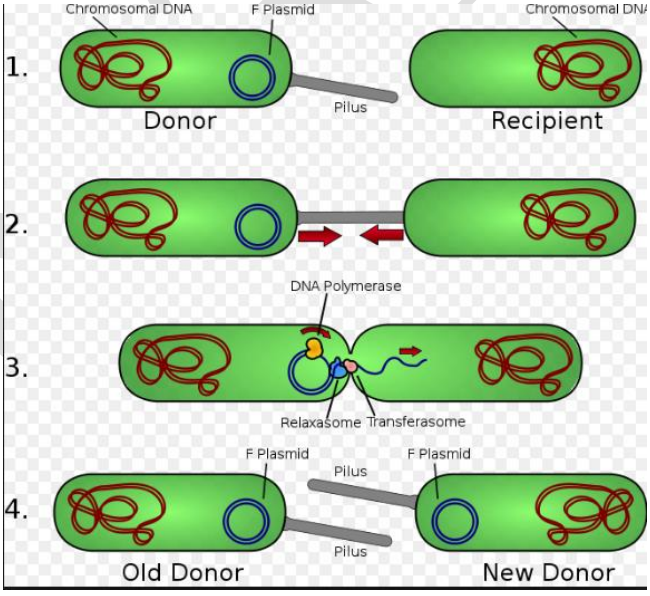
A NONCONJUGATIVE plasmid requires the help of a conjugative plasmid to transmit itself to another bacteria.

NARROW-HOST RANGE PLASMIDS exist only within a single species.

BROAD-HOST RANGE PLASMIDS exist in different genera of organisms.

Plasmids contain the following:

1. 1. An ORI or origin of replication that allows autonomous replication.
2. 2. One or more genes that confer specific antibiotic resistance.
3. 3. Sites for restriction endonucleases which are used for inserting specific DNA fragments.
4. 4. Code for virulence factors such as toxins or adhesions



Plasmids capable of mediating conjugation carry genes coding for the production of a PILUS, a protein appendage which is found on the surface of the donor cells. Bacteria which carry these plasmids are called F(+) cells, making them the donor cells. The tip of the pilus attaches to the surface of the recipient cell, and DNA transfer occurs. It is unclear whether the DNA is transferred through the pilus, or if the pilus serves as the mechanism for holding the two cells together. Either way, this is the reason why it has also been termed BACTERIAL SEX.

Once the recipient cell acquires the F plasmid, it changes from a F(-) to a F (+) cell, thus allowing it to become a donor cell now.

In a very small proportion of cells, the F plasmid is incorporated into the bacterial chromosome. Once inserted, the entire bacterial chromosome acts like a F plasmid. These are then called high frequency recombination strains (Hfr strains). This leads to 2 processes:

1. 1. The F plasmid and the entire bacterial DNA undergoes conjugation with an F(-) cell.
2. 2. The F plasmid is excised along with a portion of the bacterial DNA; hence the F plasmid is termed F prime (F').

Bacterial transformation

Transformation is a process in which genetic material taken in from the environment is added to a part of the bacterial DNA. The DNA may also replace an existing gene or part of it from the genome of the bacteria, thus resulting in loss of the activity of that gene.

For the first time transformation was demonstrated in 1928 by Frederick Griffith, an English bacteriologist searching for a vaccine against bacterial pneumonia. Griffith discovered that a non-virulent strain of Streptococcus pneumoniae could be transformed into a virulent one by exposure to strains of virulent S. pneumoniae that had been killed with heat. In 1944 it was demonstrated that the transforming factor was genetic, when Oswald Avery, Colin MacLeod, and Maclyn McCarty showed gene transfer in S. pneumoniae. Avery, Macleod and McCarty called the uptake and incorporation of DNA by bacteria "transformation."

Bacterial transformation may be referred to as a stable genetic change brought about by taking up naked DNA (DNA without associated cells or proteins), and competence refers to the state of

being able to take up exogenous DNA from the environment. Two different forms of competence should be distinguished: natural and artificial.

Natural competence Some bacteria (around 1% of all species) are naturally capable of taking up DNA under laboratory conditions; many more may be able to take it up in their natural environments. Such species carry sets of genes specifying machinery for bringing DNA across the cell's membrane or membranes. Natural competence Artificial competence is not encoded in the cell's genes. Instead it is induced by laboratory procedures in which cells are passively made permeable to DNA, using conditions that do not normally occur in nature.

Bacterial transduction

As abovementioned, transduction is one of three mechanisms of genetic recombination in bacteria occuring in nature and also in lab conditions. In other words, transduction is a process, in which nucleic acid from one organism can be transfered into another living organim by means of a intermediate virus.

In other words transduction is the phage-mediated transfer of bacterial DNA.

Phages are bacterial viruses with a DNA molecule enclosed in a protein shell. The structure of the protein shell imposes a limitation on the amount of DNA that can be enclosed by the virus, usually about 50 kilobases.

Most phages carry ds-DNA coiled up inside a protein coat. There may be species of phages which have a ss-DNA or ss-RNA as well.

There are two kinds of transductions:

Generalized transduction

When phages enter a bacterial cell and replicate, each phage head usually has a copy of the replicated phage genome. Sometimes, an empty phage head is produced. Bacterial DNA may be mistakenly packaged into that empty phage head, then transferred to another bacterial cell. Thus, the DNA that enters the second bacterial cell is a short segment of the chromosome of the first

bacterial cell. As with transformation, recombination must occur for transduction to be successful.

It is termed generalized since the phage will pick up any portion of the bacterial chromosome at random. (It is not specific for a particular chromosomal segment.)

The genes can be transferred only within a certain species because phages usually attack a limited range of bacteria.

Phages can also pick up and transfer plasmid DNA. For example, the penicillinase gene of Staphlococci is located in a plasmid, and the phages can pick up that plasmid and through transduction, pass it on to another cell, thus transferring the penicillanase gene to other staphylococci species.

Specialized transduction

Bacteriophages that lyse the host cell are known as virulent phages, and cause a lytic cycle of infection. In contrast, phages that can infect a bacterial cell without causing its death are called temperate phages.

The bacterial cell which survives after phage infection is called a lysogen, and the integrated temperate phage inside of it is termed a prophage.

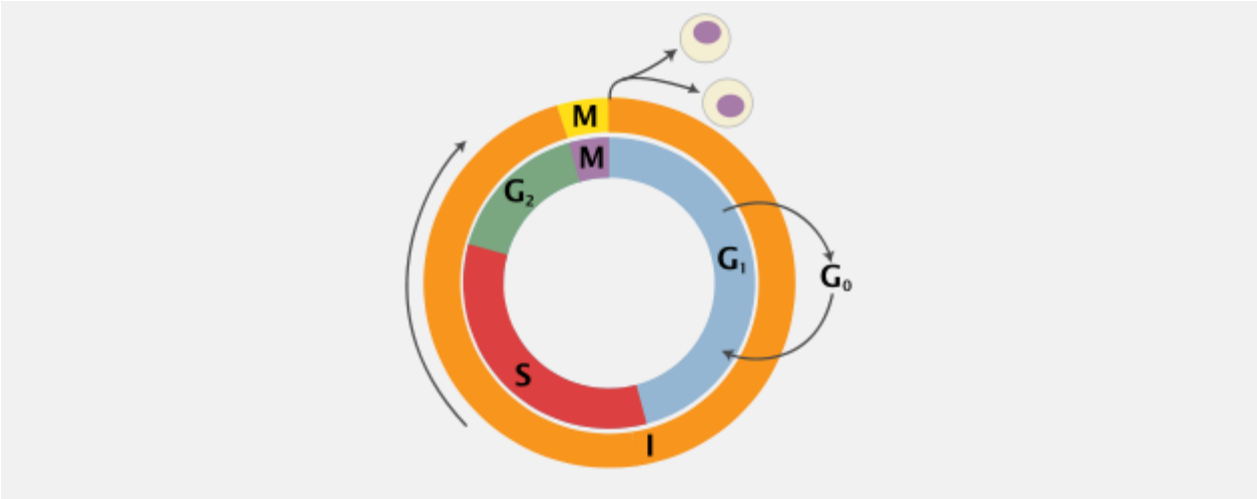
The phage DNA is then inserted into the bacterial cell DNA and is also replicated as part of the host cell chromosome.

When a bacterial cell is infected by one phage, no other phage can attack the bacteria, because once the phage is integrated into the bacterial cell DNA, it imparts immunity against superinfection by other phages.

The lysogen phase is stable but not permanent. If the prophage is excised during the process of replication, the bacterial cell may eventually be lysed. This can be replicated in the lab by exposure of bacteria to UV light. (Eg., hospital sterilization of rooms with UV.)

In contrast to generalized transduction, temperate phages normally have a specific insertion site on the chromosome and can pick up only a short length of DNA containing a few genes on either side of this site. Thus, it is termed specialized or restrictive transduction.

The Cell Division Cycle



Cells that stop dividing exit the G1 phase of the cell cycle into a so-called G0 state.

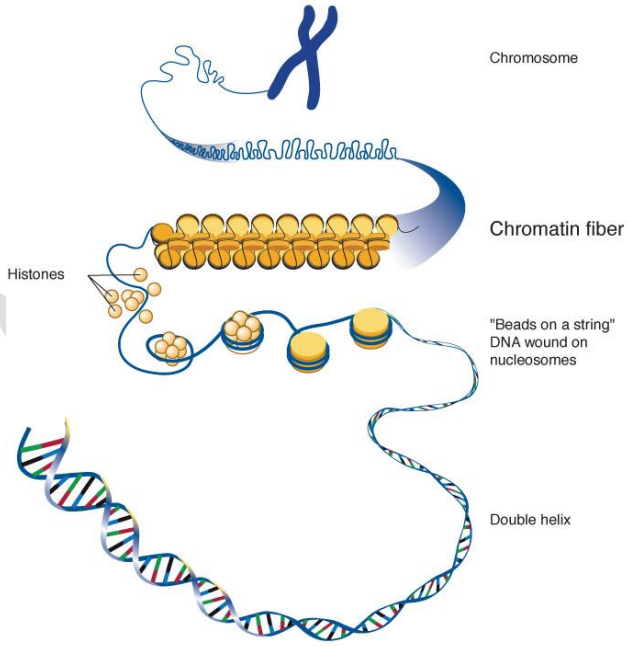
Cells reproduce genetically identical copies of themselves by cycles of cell growth and division. The cell cycle diagram on the left shows that a cell division cycle consists of 4 stages:

- G1 is the period after cell division, and before the start of DNA replication. Cells grow and monitor their environment to determine whether they should initiate another round of cell division.
- S is the period of DNA synthesis, where cells replicate their chromosomes.
- G2 is the period between the end of DNA replication and the start of cell division. Cells check to make sure DNA replication has successfully completed, and make any necessary repairs.
- M is the actual period of cell division, consisting of prophase, metaphase, anaphase, telophase, and cytokinesis.

Chromosomes

Chromosomes were first named by cytologists viewing dividing cells through a microscope. The modern definition of a chromosome now includes the function of heredity and the chemical composition. A chromosome is a DNA molecule that carries all or part of the hereditary information of an organism. In eukaryotic cells, the DNA is packaged with proteins in the nucleus, and varies in structure and appearance at different parts of the cell cycle.

Chromosomes condense and become visible by light microscopy as eukaryotic cells enter mitosis or meiosis. During interphase (G1 + S + G2), chromosomes are fully or partially decondensed, in the form of chromatin, which consists of DNA wound around histone proteins



(nucleosomes).

In G1, each chromosome is a single chromatid. In G2, after DNA replication in S phase, as cell enter mitotic prophase, each chromosome consists of a pair of identical sister chromatids, where each chromatid contains a linear DNA molecule that is identical to the joined sister. The sister chromatids are joined at their centromeres, as shown in the image below. A pair of sister chromatids is a single replicated chromosome, a single package of hereditary information.

Ploidy

Humans are **diploid**, meaning we have two copies of each chromosome. We inherited one copy of each chromosome from other mother, and one copy of each from our father. Gametes (sperm cells or egg cells) are **haploid**, meaning that they have just one complete set of chromosomes.

Chromosomes that do not differ between males and females are called **autosomes**, and the chromosomes that differ between males and females are the sex chromosomes, X and Y for most mammals. Humans most commonly have 22 pairs of autosomes and 1 pair of sex chromosomes (XX or XY), for a total of 46 chromosomes. We say that humans have **2N** = 46 chromosomes, where **N** = 23, or the haploid number of chromosomes.

Cells with complete sets of chromosomes are called **euploid**; cells with missing or extra chromosomes are called **aneuploid**. The most common aneuploid condition in people is variation in the number of sex chromosomes: XO (having just one copy of the X), XXX, or XYY. Having no X chromosome results in early embryonic death.

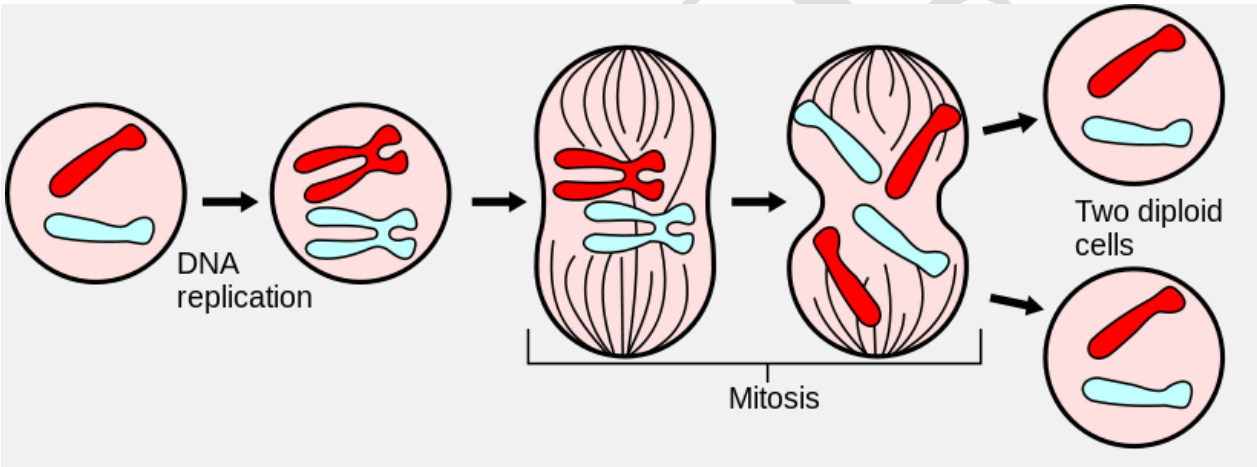
The two copies of a particular chromosome, such as chromosome 1, are called **homologous.** The karyotype image above shows the homologous pairs for all the autosomes. Homologous chromosomes are not identical to each other, unlike sister chromatids. They frequently have different variants of the same hereditary information – such as blue eye color vs brown eye color, or blood type A versus blood type B.

Mitosis

Mitosis produces two daughter cells that are genetically identical to each other, and to the parental cell. A diploid cell starts with 2N chromosomes and 2X DNA content. After DNA replication, the cells is still genetically diploid (2N chromosome number), but has 4X DNA content because each chromosome has replicated its DNA. Each chromosome now consists of a joined pair of identical sister chromatids. During mitosis the sister chromatids separate and go to opposite ends of the dividing cell. Mitosis ends with 2 identical cells, each with 2N chromosomes and 2X DNA content. All eukaryotic cells replicate via mitosis, except **germline** cells that undergo meiosis (see below) to produce **gametes** (eggs and sperm).

- prophase – chromosomes condense; each chromosome consists of a pair of identical sister chromatids joined at the centromere.
- metaphase – chromosomes line up at the middle of the cell, along the plane of cell division, pushed and pulled by microtubules of the spindle apparatus
- anaphase – *sister chromatids separate* and migrate towards opposite ends of the cell
- telophase – chromatids cluster at opposite ends of the cell and begin to decondense
- cytokinesis – the membrane pinches in to divide the two daughter cells

Here is a simplified diagram illustrating the overall process and products of mitosis:



Meiosis

This is a special sequence of 2 cell divisions that produces haploid gametes from diploid germline cells. It starts with a diploid cell that has undergone chromosomal DNA replication: 2N chromosomes, 4X DNA content. Two successive divisions, with no additional DNA replication, results in 4 haploid gametes: 1N chromosomes, 1X DNA content.

NOVA has a good interactive side-by-side comparison of mitosis and meiosis on this page: How cells divide

Meiosis sets the stage for Mendelian genetics. Students need to know that most of the genetics action occurs in the **first** meiotic division:

- homologous chromosomes pair up and align end-to-end (synapsis) in prophase I

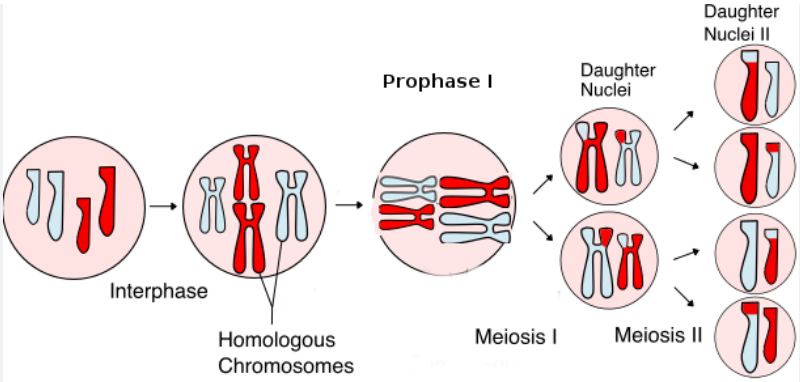
- crossing over occurs between homologous chromosomes in prophase I, *before*chromosomes line up at the metaphase plate
- homologous chromosomes separate to daughter cells (sister chromatids do not separate) in the first division, creating haploid (1N) cells
- the separation of each pair of homologous chromosomes occurs independently, so all possible combinations of maternal and paternal chromosomes are possible in the two daughter cells – this is the basis of Mendel's Law of Independent Assortment
- the **first division** is when daughter cells become functionally or genetically haploid

The last point appears to be the most difficult for students to grasp. Consider the X and Y chromosomes. They pair in prophase I, and then separate in the first division. The daughter cells of the first meiotic division have either an X or a Y; they don't have both. Each cell now has only one sex chromosome, like a haploid cell.

One way of thinking about ploidy is the number of *possible* alleles for each gene a cell can have. Right after meiosis I, the homologous chromosomes have separated into different cells. Each homolog carries one copy of the gene, and each gene could be a different allele, but these two homologs are now in two different cells. Though it looks like there are two of each chromosome in each cell, these are *duplicated* chromosomes; ie, it is one chromosome which has been copied, so there is only one possible allele in the cell (just two copies of it).

The second meiotic division is where sister (duplicated) chromatids separate. It resembles mitosis of a haploid cell. At the start of the second division, each cell contains 1N chromosomes, each consisting of a pair of sister chromatids joined at the centromere.

Here is a simplified diagram illustrating the overall process and products of meiosis:



Meiosis Overview from Wikipedia by Rdbickel. In prophase I, homologous chromosomes pair and separate in the first division (Meiosis I). In Meiosis II, sister chromatids separate.

It is very important that you recognize how and why cells become haploid after meiosis I.

To confirm for yourself that you understand meiosis, work through one or more of these interactive tutorials:

- The U. Arizona Cell Biology Project's Meiosis tutorial has a click-through animation of meiosis, with 10 thought-provoking problem questions.
- Jung Choi's interactive flash tutorial, programmed by Pearson, uses human chromosome 7, with wild type and cystic fibrosis alleles for CFTR, to track segregation through meiosis, with and without crossing over: Meiotic Segregation tutorial

Chromosomes, chromatids, what is the difference and how many chromosomes are there at different times of the cell cycle and after mitosis and meiosis?

Chromosomes by definition contain the DNA that makes up the fundamental genome of the cell. In a prokaryote, the genome is usually packaged into one circular chromosome consisting of a circular DNA molecule of a few million base pairs (Mbp). In eukaryotes, the genome is packaged into multiple linear chromosomes, each consisting of a linear DNA molecule of tens or hundreds of Mbp. Chromosomes exist at all different phases of the cell cycle. They condense and become visible to light microscopy in prophase of mitosis or meiosis, and they decondense during interphase, in the form of chromatin (DNA wrapped around nucleosomes, like "beads on a string").

The chromosome number, N, in eukaryotes, refers to the number of chromosomes in a haploid cell, or gamete (sperm or egg cell). Diploid cells (all the cells in our body except our gametes) have 2N chromosomes, because a diploid organism is created by union of 2 gametes each containing 1N chromosomes. In terms of chromosome number (ploidy), it's useful to think of chromosomes as packages of genetic information. A pair of sister chromatids is one chromosome because it has genetic information (alleles) inherited from only one parent. A pair of homologous chromosomes, each consisting of a single chromatid in a daughter cell at the end of mitosis, has alleles from the father and from the mother, and counts as 2 chromosomes.

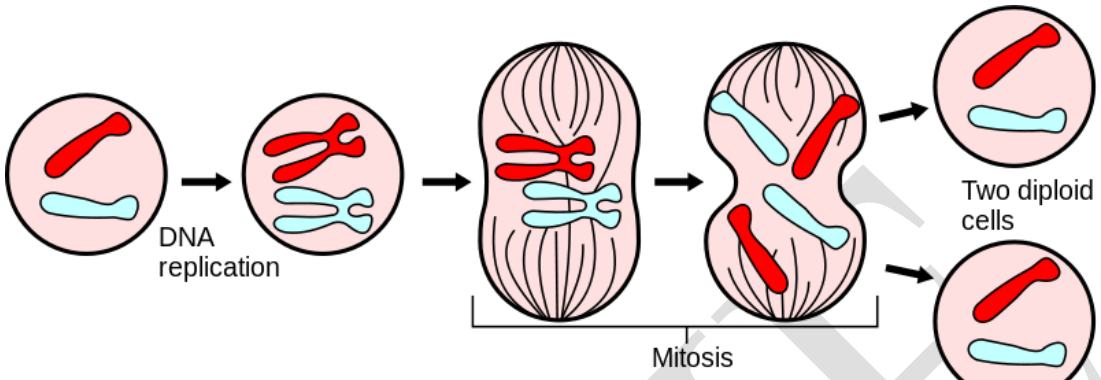
This chromosome number stays the same after chromosome replication during S phase: each chromosome entering cell division now consists of a pair of sister chromatids joined together at the centromere. Then in mitosis, the sister chromatids of each chromosome separate, so each daughter cell receives one chromatid from each chromosome. The result of mitosis is two identical daughter cells, genetically identical to the original cell, all having 2N chromosomes. So during a mitotic cell cycle, the DNA content per chromosome doubles during S phase (each chromosome starts as one chromatid, then becomes a pair of identical sister chromatids during S phase), but the chromosome number stays the same.

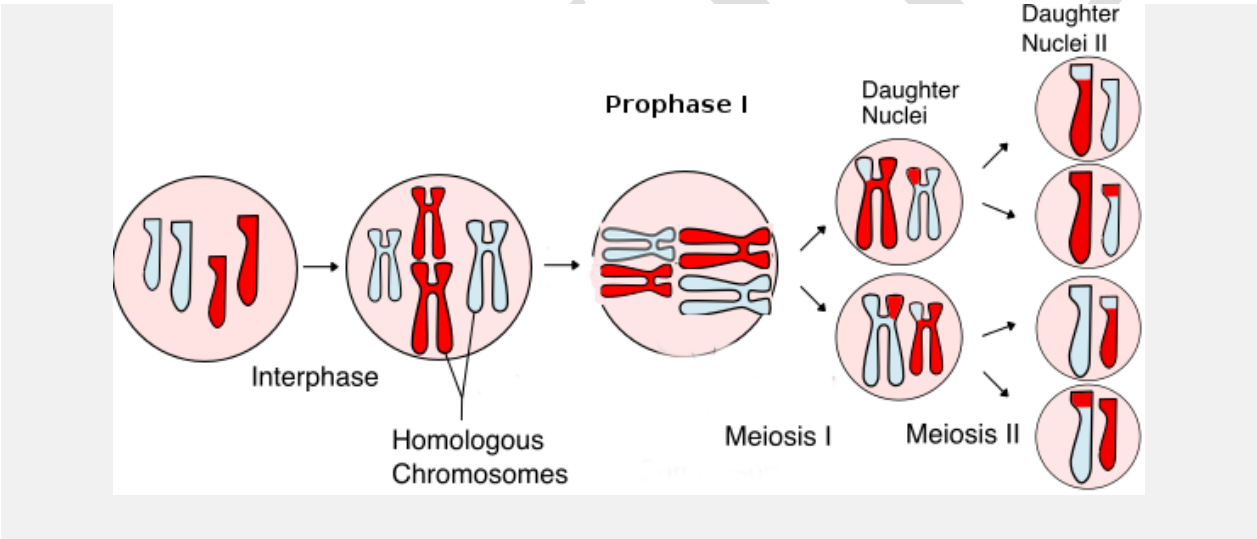
A chromatid, then, is a single chromosomal DNA molecule. The number of chromatids changes from 2X in G1 to 4X in G2 and back to 2X, but the number of chromosomes stays the same.

The chromosome number is reduced from 2N to 1N in the first meiotic division, and stays at 1N in the second meiotic division. Because homologous chromosomes separate in the first division, the daughter cells no longer have copies of each chromosome from both parents, so they have haploid genetic information, and a 1N chromosome number. The second meiotic division, where sister chromatids separate, is like mitosis. **Chromosome number stays the same when sister chromatids separate.**

Using the information above, compare these two simplified diagrams of mitosis and meiosis to visualize why cells are haploid after meiosis I. Specifically, compare the chromosomes in cells at the end of mitosis vs the end of meiosis I, recognizing that the diagram of mitosis tracks just

a *single* pair of homologous chromosomes, whereas the diagram of meiosis tracks *two* pairs of homologous chromosomes (one long chromosome and short chromosome):





Cell cycle and check points

Introduced the cell cycle and the role of microtubules therein. This lecture will discuss the regulatory mechanisms and biochemical checkpoints throughout the cell cycle. Disclaimer: these notes are not my finest work – a lot of this is just a collection of random facts.

cyclins and cyclin-dependent kinases (CDKs)

CDKs are important master regulators of the cell cycle. Their role is to phosphorylate proteins on either S or T amino acids and thereby regulate the activity of those proteins. Yeast have just

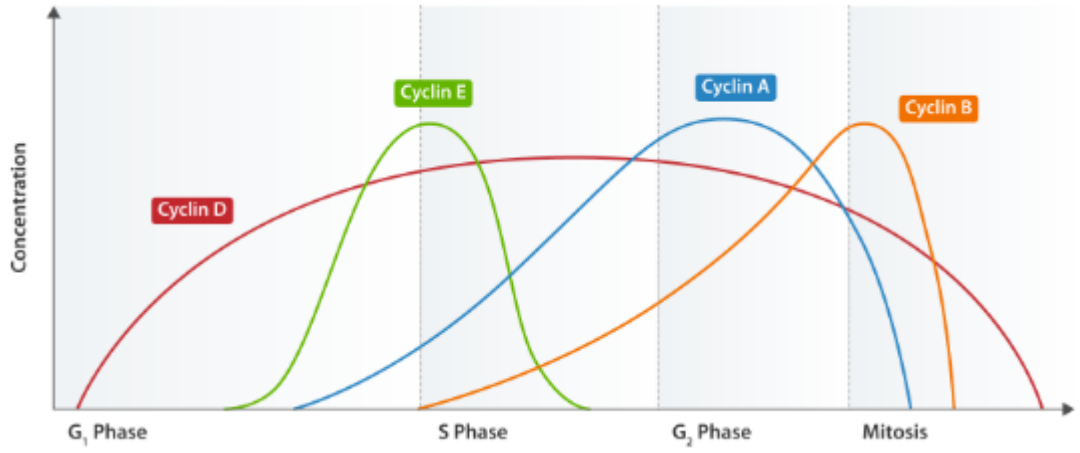
one CDK (Cdk1), while 'metazoans' (animals) like us have nine, of which four are really critical to the cell cycle and will be introduced today.

How are the CDKs themselves regulated? The levels of these proteins remain pretty constant throughout the cell cycle, yet their levels of *activity* rise and fall cyclically. CDKs need to hydrolize ATP for energy in order to perform phosphorylation. They have an ATP binding cleft whose ability to bind ATP is regulated by two mechanisms. First, CDKs have a 'flexible T loop' which contains a threonine (T) residue which normally blocks the ATP binding cleft, but not when the T is phosphorylated. Second, cyclins bind CDKs and induce a conformational change that also helps to expose the ATP binding cleft. Therefore a fully active CDK is one which is both phosphorylated at the T on the T loop *and* is bound to a cyclin.

The various activities of the cell cycle, then, are determined by the combination of cyclins and CDKs that are active at each stage, as shown in the following table:

cell cycle stage	**cyclins**	**CDKs**	**comments**
G1	Cyclin D	CDK4&6	Can react to outside signals such as growth factors or mitogens.
G1/S	Cyclins E & A	CDK2	Regulate centrosome duplication; important for reaching START
S	Cyclins E & A	CDK2	Targets are helicases and polymerases
M	Cyclins A & B	CDK1	Regulate G2/M checkpoint. The cyclins are synthesized during S but not active until synthesis is complete. Phosphorylate lots of downstream targets.

All cyclins contain a conserved 100 amino acid 'cyclin box.' Cyclin/CDK complexes regulate the cell cycle both by promoting activites for their respective stages, and by inhibiting activites for future cell cycle stages that must not yet be reached. Therefore cyclins must be able to be both generated *and* degraded in order for the cell cycle to proceed.



Antibodies against Cyclin D inhibit entry into the S phase, which can be measured by whether BrdU (a fluorescent nucleotide analog) gets incorporated into DNA (which only happens during synthesis).

DNA replication starts at 'prereplication complexes' that get assembled at *origins of replication* during early G1 phase. The S-phase cyclin/CDK complexes phosphorylate these complexes and thereby trigger replication starting from these 'origins'.

Here is a bit more about how the CDKs are regulated through phosphorylation. A CDK-activating kinase (CAK; a trimeric complex composed of CDK-7, Cyclin H and Mat1) phosphorylates amino acid T160 in CDK, located at the T loop, thereby *activating* CDK. CDKs are also regulated by CDK inhibitors p27 (CDKN1B gene), p21 (CDKN1A gene) and p57 (CDKN1C gene), which bind to and inhibit both of the G1 CDKs (CDK4 & CDK6). p27 does this by physically blocking the cyclin/CDK complex's interaction with its targets. p15 (CDKN2B gene) and p16 (CDKN2A gene) are both Ink4s ("inhibitors of kinase 4", though they also inhibit CDK6) control the mid G1 phase by binding to CDK4 and CDK6 and

blocking their binding to cyclin D. This results in decreased phosphorylation of target proteins. Overexpression of p16 arrests the cell cycle by inhibiting CDK4/Cyclin D during early G1.

Another inhibitor called Rb prevents entry into the S phase by binding to E2F transcription factors. E2Fs are transcriptional activators when they act alone but repressors when bound to Rb. The mid-G1 cyclin/CDK complexes partially phosphorylate Rb, reducing its binding to E2Fs; the late G1 complex of CDK2/Cyclin E completely phsophorylates it, preventing its binding to E2F. E2Fs can then act as transcriptional activators for genes needed in the S phase.

S-phase cyclin/CDK complexes accumulate in late G1, but are still bound to an inhibitor called Sic1. But G1 CDK/cyclin complexes polyphosphorylate Sic1 at six sites. When and only when all six sites are phosphorylated (which takes a while), Sic1 is released, whereupon it gets polyubiquitinated and therefore degraded by the proteasome. The S-phase cyclin/CDK complexes are then free to induce DNA replication. This mechanism allows the cell to accumulate many of these complexes 'ahead of time' (starting in G1) but then post-translationally activate them all at once in order to suddenly start massive-scale DNA replication in the S phase.

Targeting of proteins such as Sic1 to the proteasome is mediated by two complexes SCF (Skp, Cullin & F-Box) and APC/C (anaphase promoting complex / cyclosome), each composed of ubiquitin and a protein ligase. These two control three major transiations in the cell cycle:

1. the onset of S phase by degradation of Sic1
2. beginning of anaphase by degradation of securin
3. exit from mitosis by degradation of Cyclin B.

APC/C is composed of several proteins including but not limited to cullin (APC2), Ring (APC11) and SCF-like protein. APC/C's role is to ubiquitinate (add ubiquitin tags to) proteins, thus flagging them for degradation by the proteasome. Cdc20 casues APC/C to polyubiquitinate the anaphase inhibitor securin in point (2) above. Cdh1 causes APC/C to polyubiquitinate Cyclin B in point (3) above. Cdh1 is itself regulated by phosphorylation – the G1/S cyclin/CDK complexes phosphorylate it, preventing it from acting too soon; Cdc14 then later activates Cdh1.

SCF has no binding partner – rather, its ability to ubiquitinate targets (substrates) is determined by the latter's phosphorylation state. SCF is active throughout the cell cycle; the cell regulates its activity by regulating the phosphorylation of SCF's targets. An example is Sic1 (above).

MPF (Maturation or Mitosis Promoting Factor depending on who you ask) is a fancy word for the cyclin B / CDK1 complex, which is active in the M phase – actually, required in order for entry to the M phase. Here's how MPF itself is regulated. CDK1 has two phosphorylation sites (in vertebrates; only one in yeast) at amino acids Y15 and T161. In animals, phosphorylation of Y15 is inhibitory, while phosphorylation of T161 is activating. The cyclin/CDK2 complex begins its life wholly unphosphorylated (thus inactive), then Wee1 phosphorylates Y15, CAK then phosphorylates T161 too, then finally Cdc25 removes the Y15 phosphorylation, allowing the complex to be active (about 100x more activity than with neither site phosphorylated). Cdc25 overexpression results in premature removal of this Y15 phosphorylation so that the cell enters the M phase before it has had enough time to grow during the G2 phase. This results in 'wee cells' which are literally small because the cell growth was inhibited, so that the daughter cells after mitosis are smaller than the mother cell was. (Hence the name Wee1, which is of course backwards since Wee1 acts *against* the 'wee cell' phenotype).

Once a cell passes the restriction point (R) in late G1, it is committed to passing through the S phase.

Many cancers involve a loss of p16 leading to cyclin D1 overexpression. Other cancers (esp. late onset cancers – breast, lung, bladder) often have hyperphosphorylation of Rb leading to release of E2F to promote the cell cycle. This hyperphosphorylation can be caused by loss or 'misexpression' of one or more things

Regulatory steps in the cell cycle

Here is an overview of what are considered to be the 9 fundamental steps of cell cycle regulation.

Cell starts in phase G0 or G1.

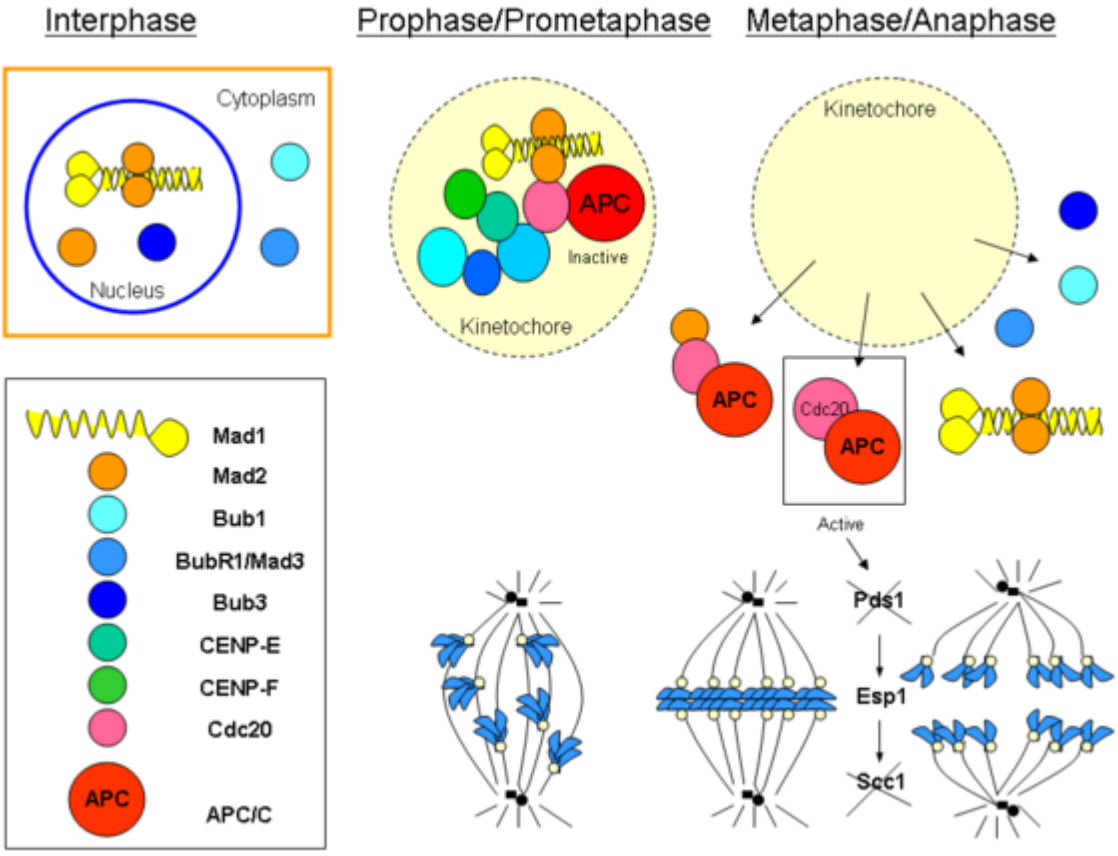
1. DNA replication machinery begins to assemble at *origins of replication*
2. G1 cyclin-CDK complexes inactivate Cdh1
3. G1 cyclin-CDK complexes activate the S-phase cyclin-CDK expression

4. G1 cyclin-CDK complexes phosphorylate and thereby inactivate S-phase inhibitor(s?)
5. SCF polyubiquitinates the phosphorylated S-phase inhibitor, targeting it for proteasome degradation. ***Cell enters S phase.***
6. S-phase cyclin/CDK activates the preplication complexes that began to assemble in step 1. ***S phase and G2 phase happen.***
7. Cdc25 phosphatase activates M-phase cyclin/CDKs. ***Cell enters M phase and gets to metaphase.***
8. APC/C and Cdc20 target securin for proteasomal degradation. ***Cell advances from metaphase to anaphase.***
9. CdcA phosphatase activates Cdh1, making it possible for APC/C and Cdh1 to target the M-phase cyclins for proteasomal degradation. ***Cell returns from M phase to G0 or G1 phase.***

Step 7 relies on a mechanism to recognize unreplicated DNA and stalled replication forks in order to ensure that mitosis does not proceed before all DNA has been replicated. Two proteins are involved in this mechanism: ATR and Chk1 (pronounced 'check one'). Both are protein kinases that 'sense' the state of DNA. ATR is located at the replication fork and activates other protein kinases, leading to phosphorylation and therefore activation of Chk1 which is also a kinase. Chk1 then phosphorylates and *in*activates Cdc25, which would otherwise activate MPF. ATR thus continues to prevent M phase entry until replication is complete (= when replication forks are gone?).

Step 8 involves an even more complicated mechanism called the mitotic spindle assembly checkpoint which prevents entry into anaphase until each and every kinetochore is attached to a microtubule. Sister chromatids are held together at the centromeres by a multi-protein complex called cohesin (composed of smc1, smc3 and kleisin). Separase is an enzyme that cleaves **kleisin**, physically releasing the chromatids. Securin inhibits separase to prevent separation of the chromatids. Once all kinetochores are attached, securin is targeted for degradation, thus freeing separase from inhibition and causing entry into anaphase. The mechanism for 'knowing' when to target securin for degradation is the really complicated part. It involves Mad1, Mad2, Cdc20, APC/C. Mad2 can have an 'open' or 'closed' conformation.

'closed' Mad2 inactivates Cdc20, thus preventing its association with APC/C. This pretty Wikimedia commons graphic by Dawn08 gives some sense of how complicated it all is:



Proper segregation of the daughter chromosomes is monitored by the 'mitotic exit network'. At this stage, MPF is inactviated, Cdh1 is dephosphorylated, and the cell can enter telophase.

There is also a 'spindle position checkpoint'. In yeast, Tem1 becomes associated with the spindle pole body at the centrosome.

'DNA damage checkpoints' detect whether DNA has been damaged (e.g. by UV light, chemicals, etc.). The cell cycle arrests in G1 or S, preventing the copying of damaged bases until they can be repaired. Arrest in G2 allows double-stranded breaks to be repaired before mitosis. Tumor suppressor genes are important in preventing replication of damaged DNA.

Cells can sense UV or gamma radiation damage through a protein called ATM/R. ATM/R phosphorylates and activates Chk2, which then phosphorylates Cdc25, marking it for degradation. ATM/R also stabilizes the tumor suppressor p53 (gene: TP53), which is a transcription factor that activates p21, which (inhibits the G1 CDKs? and) arrests the cell cycle in G1. p53 undergoes loss-of-function mutations in perhaps 50% of cancers, and many of the mutations are dominant negative so only one allele need be mutated. Mdm2 also regulates p53, and ATM/R prevents Mdm2′s binding to p53. p53 is regulated by multiple means, and also has multiple roles in promoting DNA repair, cell cycle arrest, and apoptosis

UNIT-II

SYLLABUS

GENETICS: Mendels experiment design, Mono hybrid, di-hybrid and tri-hybrid crosses, Law of segregation & principle of independent assortment, Verification of segregates by test and back crosses, Chromosomal theory of inheritance, allelic interaction concepts of dominance, recessiveness, Concepts of incomplete dominance , co-dominance, Concepts of semi dominance, pleiotropy, Concepts of multiple allele, pseudo-allelic,

MENDELIAN GENETICS

Mendel's Laws of Inheritance

Gregor Mendel is a Father of Genetics. His work, entitled "Experiments on Plant Hybrids" was published in 1866. Like many great scientific discoveries, it was ignored for 34 years. In 1900, Mendel's work was rediscovered by three botanists working independently

– Hugo de Vriies of Holand

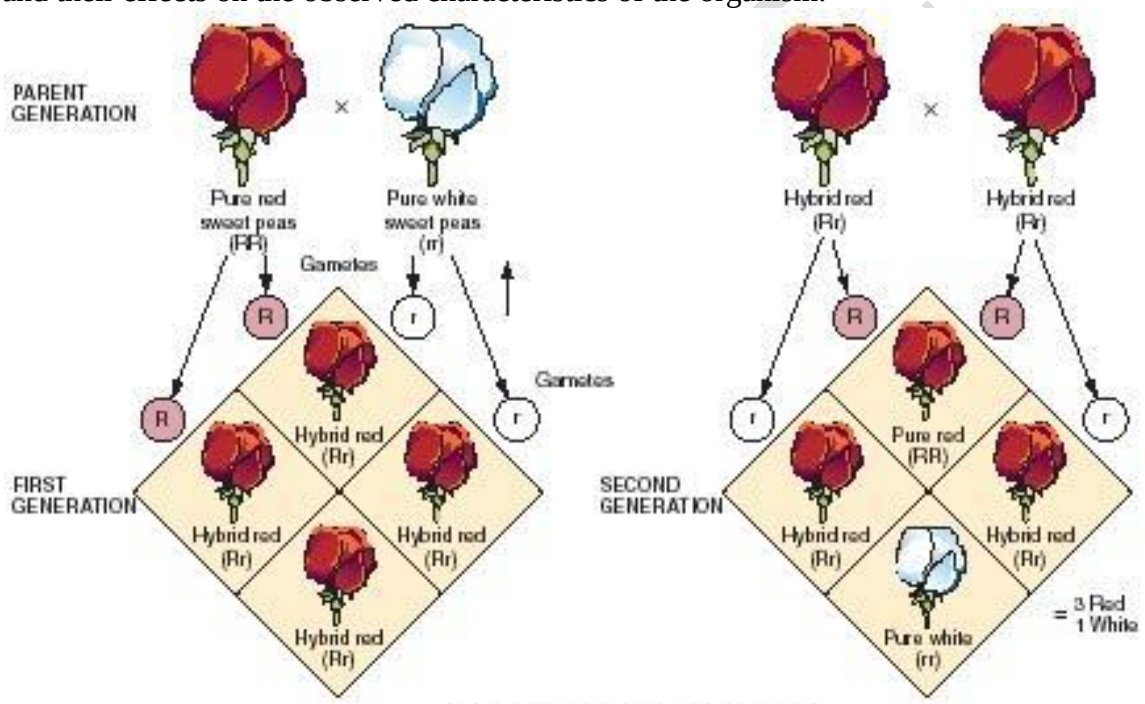
– Carll Correns off Germany
– Eriich von Tschermak of Austria.

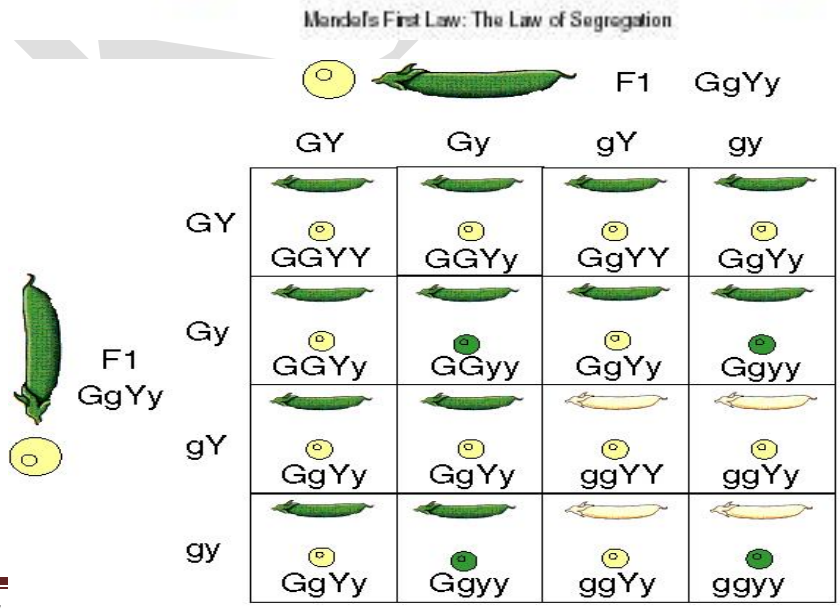
Mendel's Experiments

Gregor Mendel, the Austrian monk famous for his experiments with pea plant characteristics, was the first to identify discrete units of heredity and thus discredit the blending theory. Mendel used characteristics of pea plants and four o'clock flowers to analyze the hereditary patterns of these traits. His historic experiments led him to the conclusion that inherited characteristics were carried in discrete, independent units (later named genes). In Mendel's interpretation, hereditary characteristics occurred in pairs of factors that had specific relationships. Mendel devised two fundamental principles of inheritance:

- **Mendel's Principle of Segregation:** The factors of inheritance (genes) normally are paired, but are separated or segregated in the formation of gametes (eggs and sperm).
- **Mendel's Principle of Independent Assortment:** Each factor's distribution in the gametes is not related to the distribution of any other factor. (This principle is not strictly true due to the organization of genes on chromosomes.)

Mendel also defined and described the relationships between the different factors of inheritance and their effects on the observed characteristics of the organism.





Independent assortment

Mendel's Observations

Mendel made numerous important observations in his exhaustive study of pea plants' characteristics. He elaborated an important distinction between dominant and recessive traits through his work with pea plants.

By studying the characteristics of pea plants, such as their height, seed shape, seed color, flower position, and other traits this discussion will use height as a primary example. Mendel first crossbred one tall, true-breeding plant with one short, true-breeding plant. Contrary to the blending theory, all the offspring were tall. In terms of genotype, the original tall plant was TT (two dominant alleles; homozygous), the short plant was tt (two recessive alleles; homozygous), and the second-generation plants were Tt (one dominant and one recessive allele; heterozygous).

When Mendel next allowed these plants to self-fertilize, he found that the short trait reappeared in the third generation. The ratio of short to tall plants was almost exactly 3:1. Their genotypes were as follows - 1 short (tt) : 2 tall (Tt) : 1 tall (TT).

Punnett Squares: Simulation

These results can be simulated with a Punnett square, a calculation device used to determine the possible genotypes of offspring given the genotypes of the parents. The parents' genotypes are represented by letters, one allele in each cell of the upper row (traditionally the mother) and rightmost column (traditionally the father). The offspring's genotypes are then calculated by observing the intersection of the mother's and father's individual alleles (mush like a multiplication table). Use the interactive Punnett square below to simulate Mendel's results or experiment with combinations of your own. (If you do not select a genotype, the simulation will assume Mendel's original cross: a true-breeding short plant with a true-breeding tall plant.)

Keys to Mendel’s Experiments

The garden pea was an ideal organism for study because:

– Vigorous growth

– Self fertilization

– Easy to cross fertilize

– Produces large number of offspring each generation

Mendel analyzed traits with discrete alternative forms (one of two options)

– purple vs. white flowers

– yellow vs. green peas

– round vs. wrinkled seeds

– long vs. short stem length

Mendel established pure/true breeding lines to conduct his experiments.

(traits remain constant from generation to generation)

Monohybrid Crosses and Mendel's Principle of Segregation

Terminology used in breeding experiments:

– Parental generation is the P generation.

– True breeding unless stated otherwise

– Progeny of P generation is the first filial generation,

" designated F1.

– When F1 interbreed or are "selfed",

– the second filial generation, F2, is produced.

– F2 is always F1 x F1

Subsequent interbreeding produces F3, F4, and F5 generations

Mendel's First Three Postulates:

Using the consistent pattern of results in the monohybrid crosses, Mendel derived the following three postulates or principles of inheritance. The Monohybrid cross reveals how one trait is transmitted from generation to generation.

Unit Factors in Pairs: *Genetic characters are controlled by unit factors that exist in pairs in individual organisms.*

.**Dominance/Recessiveness:** *When two unlike unit factors responsible for a single character are present in a single individual, one unit factor is dominant to the other, which is said to be recessive.*

.**Segregation:** *During the formation of gametes, the paired unit factors separate or segregate randomly so that each gamete receives one or the other with equal likelihood.*

Monohybrid Crosses and Mendel's Principle of Segregation:

A monohybrid cross involves true-breeding strains that differ in a single trait. To determine whether both parents contribute equally to the phenotype of a particular trait in offspring a set of reciprocal crosses is performed. In Mendelian genetics, offspring of a monohybrid cross will exactly resemble only one of the parents.

Mendel noted that traits that disappear in the F1 reappear in the F2. The F2 has a ratio of about three individuals with the phenotype of the F1 to one individual with the

"reappearing" phenotype

– 3:1 ratio

– 3/4 to 1/4

Mendel reasoned that information to create the trait was present in the F1 in the form of "factors". now called genes.

By convention, letters may be used to designate alleles,

– with the dominant a capital letter (S)

– and the recessive in lowercase (s).

Individuals with identical alleles are said to be homozygous for that gene

– (e.g., genotypes SS and ss)

– because all their gametes will have the same allele for this trait.

Individuals with different alleles are heterozygous (e.g., Ss).

– because 1/2 of their gametes will contain one allele, and 1/2 the

Other.When Mendel had conducted experiments for the seven different traits in garden peas he made these conclusions:

1. Results of reciprocal crosses are always the same.

2. The F1 resembled only one of the parents.

3. The trait missing in the F1 reappeared in about ¼ of the F2 individuals.

Punnett Squares

A Punnett square is a grid that enables one to predict the outcome off simple genetic crosses proposed by the English geneticist, Reginald Punnett.

1. Write down the genotypes of both parents

– Malle parentt = *Ttt*

– Femalle parentt = *Ttt*

2. Write down the possible gametes each parent can make.

– Malle gamettes: *T* or *tt*

– Femalle gamettes: *T* or *tt*

3. Fill in the Punnett square with the possible genotypes of the offspring. Determine the relative proportions of genotypes and phenotypes of the offspring

– Genotypic ratio

"TT : Tt : tt

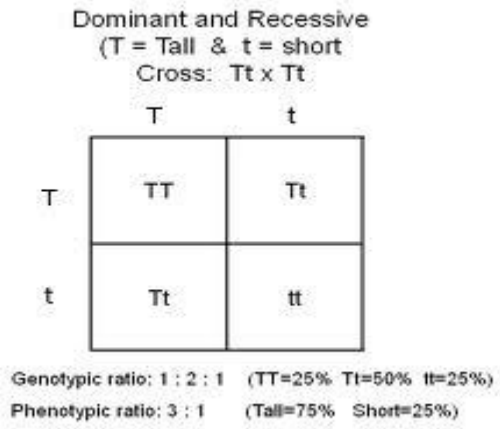
" 1 : 2 : 1

– Phenotypic ratio

" Tallll :: dwarff

" 3 :: 1

Monohybrid cross



The Principle of Segregation

The first Mendelian law, the principle of segregation, states:

– "Recessive characters, which are masked in the F1 from a cross between two true-breeding strains, reappear in a specific proportion in the F2." This is because alleles segregate during anaphase I of meiosis and progeny are then produced by random combination of the gametes.

Test cross:

A **test cross** is a cross of the F1 plant to a homozygous recessive plant. Test crosses help establish genotype.

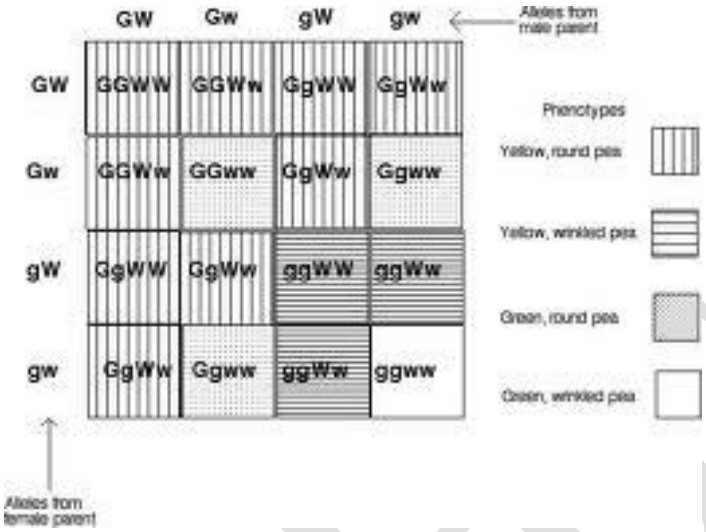
Mendel's Dihybrid Cross Generated a Unique F2 Ratio:

Independent assortment: *During gamete formation, segregating pairs of unit factors assort independently of each other.*

As a natural extension of the monohybrid cross, Mendel also designed experiments in which he examined two characters simultaneously. Such a cross, involving two pairs of contrasting traits, is a **dihybrid cross** or *two-factor cross.*

For example, if pea plants having yellow seeds that are round are bred with those having green seeds that are wrinkled, the results shown in will occur: the F1 offspring will all be yellow and round. It is therefore apparent that yellow is dominant to green and that round is dominant to wrinkled. When the F1 individuals are selfed, approximately of the F2 plants express yellow and round, express yellow and wrinkled, express green and round, and express green and wrinkled. Instead of crossing one P1 parent with both dominant traits (yellow, round) and one with both recessive traits (green, wrinkled), plants with yellow, wrinkled seeds are crossed with those with green, round seeds. In spite of the change in the P1 phenotypes, both the F1 and F2 results remain unchanged.

Dihybrid cross



APPLICATIONS OF MENDEL'S PRINCIPLES:

The reason for these laws is found in the nature of the cell nucleus. It is made up of several chromosomes carrying the genetic traits. In a normal cell, each of these chromosomes has two parts, the chromatids. A reproductive cell, which is created in a process called meiosis, usually contains only one of those chromatids of each chromosome. By merging two of these cells (usually one male and one female), the full set is restored and the genes are mixed. The resulting cell becomes a new embryo. The fact that this new life has half the genes of each parent (23 from mother, 23 from father for total of 46 in the case of humans) is one reason for the Mendelian laws. The second most important reason is the varying dominance of different genes, causing some traits to appear unevenly instead of averaging out (whereby dominant doesn't mean more likely to reproduce—recessive genes can become the most common, too).

There are several advantages of this method (sexual reproduction) over reproduction without genetic exchange:

1. Instead of nearly identical copies of an organism, a broad range of offspring develops, allowing more different abilities and evolutionary strategies.
2. There are usually some errors in every cell nucleus. Copying the genes usually adds more of them. By distributing them randomly over different chromosomes and mixing the genes, such errors will be distributed unevenly over the different children. Some of them will therefore have only very few such problems. This helps reduce problems with copying errors somewhat.
3. Genes can spread faster from one part of a population to another. This is for instance useful if there's a temporary isolation of two groups. New genes developing in each of the populations don't get reduced to half when one side replaces the other, they mix and form a population with the advantages of both sides.
4. Sometimes, a mutation can have positive side effects. For example, sickle cell anemia is a mutation that can cause the benefit of malaria resistance. The mechanism behind the Mendelian laws can make it possible for some offspring to carry the advantages without the disadvantages until further mutations solve the problems

NON-MENDELIAN GENETICS

Non-Mendelian inheritance is a general term that refers to any pattern of inheritance in which traits do not segregate in accordance with Mendel's laws. These laws describe the inheritance of traits linked to single genes on chromosomes in the nucleus. In Mendelian inheritance, each parent contributes one of two possible alleles for a trait. If the genotypes of both parents in a genetic cross are known, Mendel's laws can be used to determine the distribution of phenotypes expected for the population of offspring. There are several situations in which the proportions of phenotypes observed in the progeny do not match the predicted values.

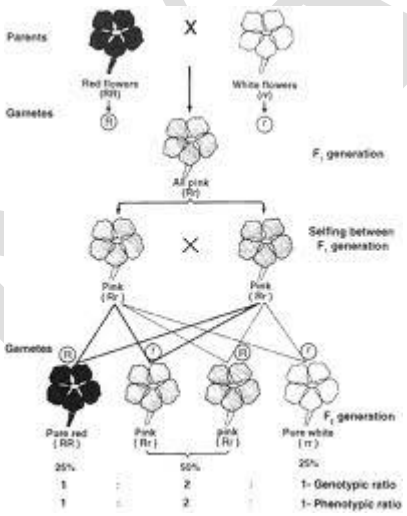
Although inheritance of traits in fungi, viruses, and bacteria are all non-Mendelian, the phrase "non-Mendelian inheritance" is usually only used to describe the exceptions which occur in eukaryotic reproduction

Incomplete Dominance:

Definition: Incomplete dominance is a form of intermediate inheritance in which one allele for a specific trait is not completely dominant over the other allele. This results in a combined phenotype.

Incomplete dominance:

Incomplete dominance occurs when the phenotype of the heterozygous genotype is an intermediate of the phenotypes of the homozygous genotypes. For example, the snapdragon flower color is either homozygous for red or white. When the red homozygous flower is paired with the white homozygous flower, the result yields a pink snapdragon flower. The pink snapdragon is the result of incomplete dominance. A similar type of incomplete dominance is found in the four o'clock plant where in pink color is produced when true bred parents of white and red flowers are crossed. When plants of F_1 generation is self pollinated the phenotypic and genotypic ratio of the F_2 generation will be same and is 1:2:1



Over-dominance:

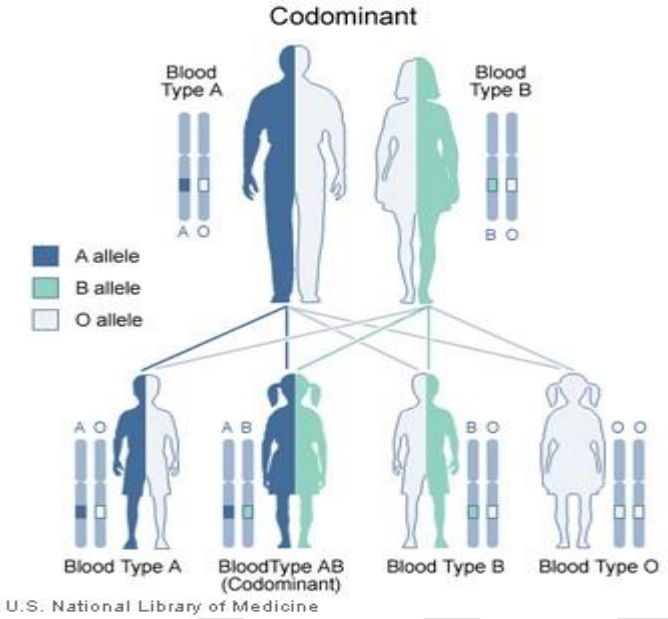
It is a condition in genetics where the phenotype of the heterozygote lies outside of the phenotypical range of both homozygote parents. Over dominance can also be described as heterozygote advantage, wherein heterozygous individuals have a higher fitness than homozygous individuals.

An example in humans is sickle cell anemia. This condition is determined by a single polymorphism. Possessors of the deleterious allele have lower life expectancy, with homozygotes rarely reaching 50 years of age. However, this allele also yields some resistance to malaria. A thus in region where malaria exerts or has exerted a strong selective pressure, sickle cell anemia has been selected for its conferred partial resistance to the disease. While homozygotes will have either no protection from malaria or a dramatic propensity to sickle cell anemia, heterozygotes enjoy a partial resistance to both

Codominance:

It is a situation in which two different alleles for a genetic trait are both expressed. Codominance is a relationship between two versions of a gene. Individuals receive one version of a gene, called an allele, from each parent. If the alleles are different, the dominant allele usually will be expressed, while the effect of the other allele, called recessive, is masked. In codominance, however, neither allele is recessive nor are the phenotypes of both alleles expressed.

A slightly more complicated multiple-allele system determines blood type in humans. The three alleles are: A, B, and O, corresponding to A, B, and O type blood respectively. The A and B alleles are dominant over the O allele, which is always recessive. However, there is an additional twist: the A and B alleles exhibit incomplete dominance and, when inherited together, give rise to AB blood type. Yet another catch is waiting, however: humans also have the Rh factor, a special type of protein found in most (but not all) human blood. Rh factor's presence is inherited in the ordinary Mendelian fashion, with Rh-positive dominant over Rh-negative. In total, this means humans have 8 simple blood types.



Epistasis: it is an interaction between genotypes at two *different* gene loci, which sometimes resembles a dominance interaction at a single locus. Epistasis modifies the characteristic 9:3:3:1 ratio expected for two non-epistatic genes. Most genetic systems involve complex epistatic interactions among multiple gene loci.

1. **Recessive epistasis**
2. **Dominant epistasis**
3. **Supplementary epistasis**

Multiple alleles

Although any individual has at most two different alleles, most genes exist in a large number of allelic forms in the population as a whole. In some cases, the alleles have different effects on the phenotype, and their dominance interactions with each other can be described as a series.

For example, the best known human blood groups, the ABO system, comprises three sets of alleles at the ***I*** locus, I^A, I^B, and I^O. The first two are dominant to the latter: that is, the **AA** and

AO genotypes produce indistinguishable blood group phenotypes, called "*Type A*", as do **BB** and **BO**, which produce "*Type B*" blood. In another example, coat color in siamese cats and related breeds is determined by a series of alleles at the albino gene locus (***c***) that produce different levels of pigment and hence different levels of color dilution. Four of these are c^+, c^b, c^s, and c^a (standard, Birman, siamese, and albino, respectively), where the first allele is completely dominant to the last three, and the last is completely recessive to the first three.

PLEIOTROPY AND LETHAL ALLELES

Introduction

From Mendel's experiments, you might imagine that all genes control a single characteristic and affect some harmless aspect of an organism's appearance (such as color, height, or shape). Those predictions are true for some genes, but definitely not all of them! For example:

- A human genetic disorder called Marfan syndrome is caused by a mutation in one gene, yet it affects many aspects of growth and development, including height, vision, and heart function. This is an example of **pleiotropy**, or one gene affecting multiple characteristics.
- A cross between two heterozygous yellow mice produces yellow and brown mice in a ratio of 2:12:12, colon, 1, not 3:13:13, colon, 1. This is an example of **lethality**, in which a particular genotype makes an organism unable to survive.

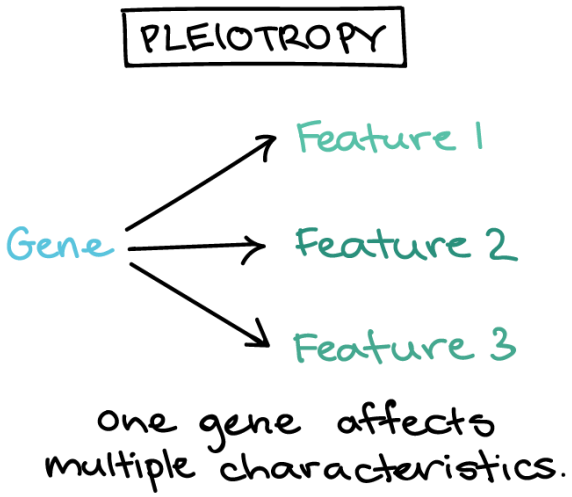
In this article, we'll take a closer look at pleiotropic genes and lethal alleles, seeing how these variations on Mendel's rules fit into our modern understanding of inheritance.

Pleiotropy

When we mentioned Mendel's experiments with purple-flowered and white-flowered plants, we didn't discuss any other phenotypes associated with the two flower colors. However, Mendel noticed that the flower colors were always correlated with two other features: the color of the seed coat (covering of the seed) and the color of the axils (junctions where the leaves met the stem)^{1,2}1,2start superscript, 1, comma, 2, end superscript.

In plants with white flowers, the seed coats and axils were colorless. In plants with purple flowers, on the other hand, the seed coats were brown-gray and the axils were reddish. Thus, rather than affecting just one characteristic, the flower color gene actually affected three.

Genes like this, which control multiple, seemingly unrelated features, are said to be **pleiotropic** (*pleio-* = many, *-tropic* = effects)^11start superscript, 1, end superscript. We now know that Mendel's flower color gene specifies a protein that causes colored particles, or pigments, to be made^22start superscript, 2, end superscript. This protein works in several different parts of the pea plant (flowers, seed coat, and leaf axils). In this way, the seemingly unrelated phenotypes can be traced back to a defect in one gene with several jobs.



Simple schematic illustrating pleiotropy.

In pleiotropy, one gene affects multiple features (feature 1, feature 2, feature 3.

Caption: One gene affects multiple characteristics.

Importantly, alleles of pleiotropic genes are transmitted in the same way as alleles of genes that affect single traits. Although the phenotype has multiple elements, these elements are specified as a package, and the dominant and recessive versions of the package would appear in the offspring of two heterozygotes in a ratio of 3:13:13, colon, 1.

Pleiotropy in human genetic disorders

Genes affected in human genetic disorders are often pleiotropic. For example, people with a hereditary disorder called Marfan syndrome may have a set of seemingly unrelated symptoms, including the following^{1,3}1,3start superscript, 1, comma, 3, end superscript:

- Unusually tall height
- Thin fingers and toes
- Dislocation of the lens of the eye
- Heart problems (in which the aorta, the large blood vessel carrying blood away from the heart, bulges or ruptures).

These symptoms don't seem directly related, but as it turns out, they can all be traced back to the mutation of a single gene. This gene encodes a protein that assembles into chains, making elastic fibrils that give strength and flexibility to the body's connective tissues^44start superscript, 4, end superscript. Mutations that cause Marfan syndrome reduce the amount of functional protein made by the body, resulting in fewer fibrils.

How does the identity of this gene explain the range of symptoms? Our eyes and the aortas normally contain many fibrils that help maintain structure, which is why these two organs are affected in Marfan syndrome^55start superscript, 5, end superscript. In addition, the fibrils serve as "storage shelves" for growth factors. When there are fewer of them in Marfan syndrome, the growth factors cannot be shelved and thus cause excess growth (leading to the characteristic tall, thin Marfan build)^44start superscript, 4, end superscript.

Lethality

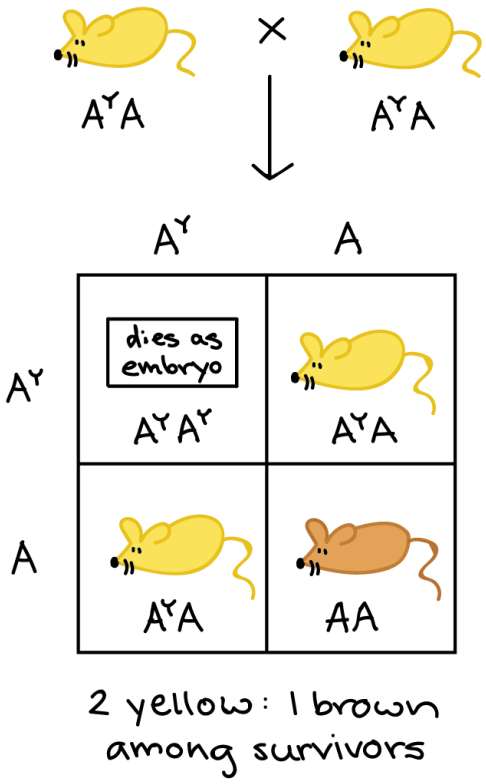
For the alleles that Mendel studied, it was equally possible to get homozygous dominant, homozygous recessive, and heterozygous genotypes. That is, none of these genotypes affected the survival of the pea plants. However, this is not the case for all genes and all alleles.

Many genes in an organism's genome are needed for survival. If an allele makes one of these genes nonfunctional, or causes it to take on an abnormal, harmful activity, it may be impossible to get a living organism with a homozygous (or, in some cases, even a heterozygous) genotype.

Example: The yellow mouse

A classic example of an allele that affects survival is the lethal yellow allele, a spontaneous mutation in mice that makes their coats yellow. This allele was discovered around the turn of the 20th century by the French geneticist Lucien Cuenót, who noticed that it was inherited in an unusual pattern^{6,7}6,7start superscript, 6, comma, 7, end superscript.

When yellow mice were crossed with normal agouti (brown) mice, they produced half yellow and half brown offspring. This suggested that the yellow mice were heterozygous, and that the yellow allele, A^Y*AY*A, start superscript, Y, end superscript, was dominant to the agouti allele, A*A*A. But when two yellow mice were crossed with each other, they produced yellow and brown offspring in a ratio of 2:12:12, colon, 1, and the yellow offspring did not breed true (were heterozygous). Why was this the case?



Two yellow mice (A^YA*AYA*A, start superscript, Y, end superscript, Agenotype) are crossed to one another. The Punnett square for the cross is:

	A^Y*AY***A, start superscript, Y, end superscript**	*AA***A**
A^Y*AY*A, start superscript, Y, end superscript	A^Y*AY*A, start superscript, Y, end superscriptA^Y*AY*A, start superscript, Y, end superscript(dies as embryo)	A^Y*AY*A, start superscript, Y, end superscriptA*AA* (yellow)
AAA	A^Y*AY*A, start superscript, Y, end superscriptA*AA*(yellow)	*AAAAA*(agouti/brown)

There is a phenotypic ratio of 2:1 yellow:brown among the mice that survive to birth.

As it turned out, this unusual ratio reflected that some of the mouse embryos (homozygous A^YA^Y*AYAY*A, start superscript, Y, end superscript, A, start superscript, Y, end superscript genotype) died very early in development, long before birth. In other words, at the level of eggs, sperm, and fertilization, the color gene segregated normally, resulting in embryos with a 1:2:11:2:11, colon, 2, colon, 1 ratio of A^YA^Y*AYAY*A, start superscript, Y, end superscript, A, start superscript, Y, end superscript, A^YA*AYA*A, start superscript, Y, end superscript, A, and AA*AA*A, A genotypes. However, the A^YA^Y*AYAY*A, start superscript, Y, end superscript, A, start superscript, Y, end superscript mice died as tiny embryos, leaving a 2:12:12, colon, 1 genotype and phenotype ratio among the surviving mice^{7,8}7,8start superscript, 7, comma, 8, end superscript.

Alleles like A^Y*AY*A, start superscript, Y, end superscript, which are lethal when they're homozygous but not when they're heterozygous, are called **recessive lethal alleles**.

PENETRANCE and EXPRESSIVITY

Egg colour of the endangered Hawai'ian *Oo'Aa* bird is controlled by a single locus, *BLU*, where the B allele is dominant to the b allele. As part of the recovery strategy, a pure-breeding blue-egg bird (BB) from one island is crossed with a pure-breeding white-egg bird (bb) from another island. Because all the offspring are Bb heterozygotes, they are all expected to show a uniform, blue phenotype like that of the BB parent. However, if penetrance and / or expressivity vary, three patterns of variation that differ from expectation are possible.

If penetrance varies [left], the Bb genotype sometimes does not *penetrate through to the phenotype*: some Bb eggs are blue just like those of their BB parents, others are white like those of their bb parents, despite presence of the B allele. "*Lack of penetrance*" is sometimes used to explain individuals whose phenotypes do not reflect their inferred genotypes, e.g., a dominant trait that appears to "*skip*" a generation in a pedigree

If expressivity varies [middle], the Bb genotype will not be *expressed uniformly in the phenotype*: all eggs are blue, but the exact shade of blue *varies among individuals with the same genotype*. Variable expressivity is frequently attributable to environmental factors or variation at

gene loci elsewhere in the genome.

If expressivity *and* penetrance both vary [right], there may be a continuous gradient of phenotypes between white and blue eggs. Note that *lack of penetrance can be considered as an extreme from of expressivity*, in which the range of expression includes non-expression.

UNIT-III

SYLLABUS

GENETICS: Eukaryotic nuclaear genome, Nucleotide sequence composition-unique & repetitive DNA, Satellite DNA, Centromere and telomere DNA, Respective transposed sequences, Genetic organization of prokaryotic and viral genome, Structure and characteristics of bacterial and eukaryotic chromosomes

CHROMOSOME STRUCTURE

During nuclear division, the DNA (as chromatin) in a Eukaryotic cell's nucleus is coiled into very tight compact structures called chromosomes. These are rod-shaped structures made of protein and DNA, which are visible (when stained) only during nuclear division.

The DNA in Eukaryotic cells is coiled tightly around proteins called histones, which help in the tight packing of DNA. During interphase, the DNA is not tightly coiled into chromosomes, but exists as chromatin.

When preparing for nuclear division, during the S phase of interphase, the chromosomes copy themselves (i.e. DNA replication occurs). Each half of the chromosome is now called a chromatid. Note that there is still only one chromosome; it consists of two chromatids but has only one centromere. The function of this is to hold the two chromatids together until they separate during anaphase.

Chromosomes are simpler in Prokaryotes. Their DNA is in a single chromosome, and exists as a loop (ccc DNA).

Eukaryotic Genomes

The survival strategy for most bacteria is centered on metabolic adaptability and a short doubling time. Many bacteria can double in less than twenty minutes under ideal conditions. Bacterial genomes, then, must contain enough information to specify a broad array of biosynthetic and catabolic systems, but must also be small enough for rapid duplication. Multicellular eukaryotes are not as dependant on rapid doubling as are bacteria. For most, the

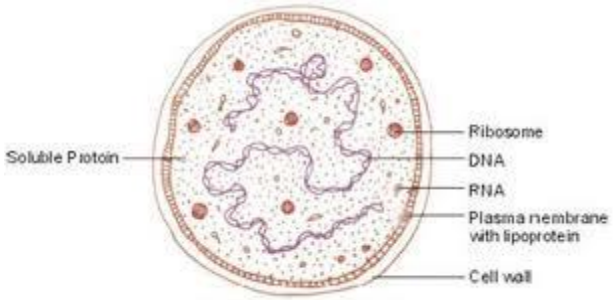
generation time is much longer than the time required to replicate the genome, so that this step is not rate-limiting as it is in bacteria. Consequently, there is less selective pressure for the control of genome size in most eukaryotes. We have already seen that introns represent a part of the genome that does not specify protein sequence. Other **non-coding** genomic elements include **satellite DNA, telomeres, transposons**, and **pseudogenes**.

The moderately repetitive DNA includes those sequences which have multiple copies in the genome, designed to increase the rate or amount of gene product, and some regulatory sequences found scattered throughout the genome. Sequences such as the ribosomal RNA and transfer RNA, which are required in large amounts for protein synthesis have many copies on the genome. The histone sequences also have large copy numbers in the genome. Another group of moderately repetitive DNA sequences are those scattered throughout the genome; known as SINES (short interspersed elements) and LINES (long interspersed elements). Some famous SINES and LINES: Alu repeats are the major SINE in mammalian genomes. They are ~300 bp long and about a million of such sequences exist scattered throughout the genome. They account for ~10% of the genome. They are transcribed into RNA but have no known function?? They are known as Alu repeats because they contain the recognition sequence for the restriction enzyme Alu. The most common LINE in the human genome is L1, a 6 000 bp sequence which is repeated some 50 000 times in the human genome. L1 sequences are also transcribed and some even encode proteins! Their function in the cell is unknown. Both the Alu and L1 sequences are transposable elements, capable of moving to different sites in the genome. The group of sequences with a small number of copies on the genome include such sequences as the globins. This family of genes contains a number of closely related sequences, varying by only a few bases in the code, will cross hybridise. These are also known as gene clusters. The final group, the single copy sequences make up the vast majority of genes on the genome (gene being a functional unit which codes for a single polypeptide chain). This group is the most complex and takes the longest to re-anneal, hence the log scale on the time. The highly repetitive DNA re-anneals in seconds while the most complex single copy group takes hours or days to re-anneal.

Chromosome Structure of Prokaryotes: (Bacteria)

In contrast to the linear chromosomes found in eukaryotic cells, the strains of bacteria initially studied were found to have single, covalently closed, circular chromosomes. The circularity of

the bacterial chromosome was elegantly demonstrated by electron microscopy in both Gram negative bacteria (such as *Escherichia coli*) and Gram positive bacteria (such as *Bacillus subtilis*). Bacterial plasmids were also shown to be circular. In fact, the experiments were so beautiful and the evidence was so convincing that the idea that bacterial chromosomes are circular and eukaryotic chromosomes are linear was quickly accepted as a definitive distinction between prokaryotic and eukaryotic cells. However, like most other distinctions between prokaryotic and eukaryotic cells, it is now clear that this dichotomy is incorrect. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids.



DNA in Prokaryotic Cell

Experimental evidence for multiple chromosomes and linear chromosomes initially came from studies using pulsed field gel electrophoresis (PFGE), an approach that uses alternating electric fields to separate large DNA molecules on an agarose gel. Subsequently genome sequencing projects have added to the list of bacteria with multiple or linear chromosomes.

Some examples of bacterial genome organization

Bacteria	Chromosome(s)	Plasmid(s)
Agrobacterium tumefaciens	one linear (2.1 Mb) + one circular (3.0 Mb)	two circular (450 + 200 Kb)
Bacillus subtilis	one circular (4.2 Mb)	
Bacillus thuringiensis	one circular (5.7 Mb)	six (each >50 Kb)
Borrella	one linear (0.91 Mb)	multiple circular + linear (5-200 Kb)
Bradyrhizobium japonicum	one circular (8.7 Mb)	
Brucella melitensis	two circular (2.1 + 1.2 Mb)	
Brucella suis biovars 1, 2, 4	two circular (1.0 + 2.0 Mb)	
Brucella suis biovar 3	one circular (3.1 Mb)	
Buchnera sp. strain APS	one circular (640 Kb)	two circular (< 7.8 Kb each)
Deinococcus radiodurans	two circular (2.6 + 0.4 Mb)	two circular (177 + 45 Kb)
Escherichia coli K-12	one circular (4.6 Mb)	
Leptospira interrogans	two circular (4.7 + 0.35 Mb)	
Paracoccus denitrificans	three circular (2.0 + 1.1 + 0.64 Mb)	
Pseudomonas aeruginosa	single circular (6.3 Mb)	
Rhizobacterium meliloti	two circular (3.4 + 1.7 Mb	one circular megaplasmid (1,400 Kb)
Rhodobacter sphaeroides	two circular (3.0 + 0.3 Mb)	
Ureaplasma urealyticum	one circular (0.75 Mb)	
Vibrio cholerae	two circular (2.9 + 1.1 Mb)	
Vibrio parahaemolyticus	two circular (3.2 + 1.9 Mb)	
Xylella fastidiosa	one circular (2.7 Mb)	two circular (51 + 1.3 Kb)

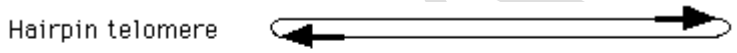
The first convincing evidence that some bacteria have multiple chromosomes came from studies on Rhodobacter sphaeroides. Both molecular and studies clearly demonstrated that R. sphaeroides has two large circular chromosomes. One of the chromosomes is 3.0 Mb and the other is 0.9 Mb. Genes encoding rRNAs and tRNAs required for translation, and metabolic enzymes are distributed between the two chromosomes. Multiple chromosomes have also been found in many other bacteria, including Agrobacterium tumefaciens, Rhizobium, Brucella, Paracoccus denitrificans, Ochrobactrum anthropi, Leptospira interrogans, Burkholderia, Vibrio cholerae, Deinococcus radiodurans, and many others from diverse groups of bacteria.

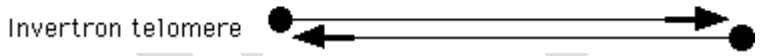
Furthermore, some bacteria have linear chromosomes. Borrelia have linear chromosomes and most strains contain both linear and circular plasmids; most of the bacteria in the genus Streptomyces have linear chromosomes and plasmids and some have circular plasmids as well. In addition, in some cases there may be a dynamic equlibrium between linear and circular forms

of a DNA molecule. There is some evidence that linearization may be due to integration of a linear phage genome into the circular DNA molecule.

Linear chromosomes and plasmids were not discovered in bacteria until relatively recently. The first published evidence for linear chromosomes was in 1979, but because the techniques used at that time were limited and because the dogma that all bacterial chromosomes are circular was so entrenched, few people believed that linear chromosomes and plasmids occured in bacteria until 1989. By that time pulsed field gel electrophoresis had been developed, and this new technique provided convincing evidence that the chromosome of Borrelia burgdoferi was linear.

The ends of linear DNA molecules (called telomeres) pose two problems that do not apply to circular DNA molecules. First, since free double-stranded DNA ends are very sensitive to degradation by intracellular nucleases, there must be a mechanism to protect the ends. Second, the ends of linear DNA molecules must have a special mechanism for DNA replication. These problems are solved by features of the telomeres. Two different types of telomeres have been observed in bacteria: hairpin telomeres and invertron telomeres.





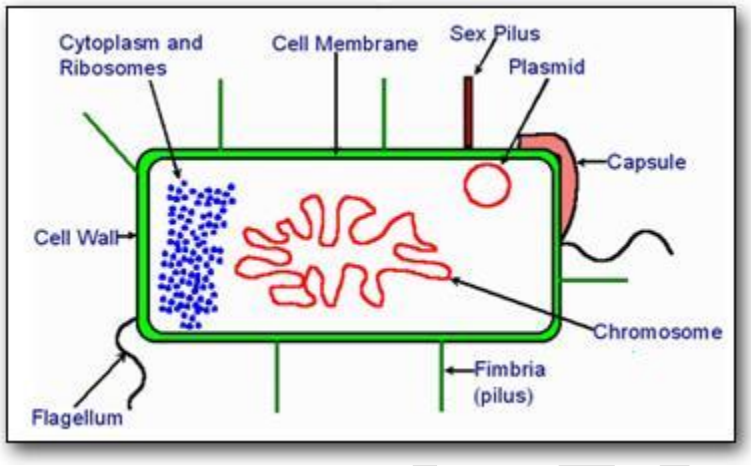
There are examples of linear DNA molecules in bacteria that are protected by both types of telomeres: palindromic hairpin loops are protected by the lack of free double-stranded ends, and invertron telomeres are protected by proteins that bind to the 5'-ends. Both of these mechanisms are also used by some phage, eukaryotic viruses, and eukaryotic plasmids.

The two types of telomeres also solve the problem of DNA replication differently. Invertron telomeres have a protein covalently attached to the 5' ends of the DNA molecule (called the 5'-terminal protein or TP for short). DNA polymerase interacts with the TP at the telomere and catalyzes the formation of a covalent bond between the TP and a dNTP. The dNTP bound to the

TP has a free 3'-OH group which acts as the primer for chain elongation. Replication of hairpin telomeres is less well understood. Apparently multiple hairpin sequences can pair to form concatemers that are replication intermediates.

The important take-home point is that we are just beginning to appreciate the similarity of many processes once thought to be completely different between bacteria and eukaryotes, partly because we now have better tools for studying these processes and partly because most of the earlier studies focused on relatively few types of bacteria. The more we study a wider diversity of bacteria, phages, and plasmids, the more obvious it becomes that E. coli is an excellent model for dissecting broad features of molecular and cell biology, but not all bacteria do everything the same way. Furthermore, we have only recently begun to attack the molecular genetics of the Archae, and what we have learned so far suggests that this diverse group of prokaryotes share even more common features with the eukaryotes.

1. The circular genomes of mitochondrial and chloroplast are a notable exception to the rule that eukaryotic chromosomes are linear. However, this nicely fit into the dichotomy that eukaryotic chromosomes are linear and bacterial chromosomes are circular because these organelles seem to have evolved from entrapped bacteria.
2. Other examples include the presence of introns, and poly-A tails on mRNA.
3. This genus includes B. burgdorferi, the causative agent of Lyme disease.
4. Streptomyces make a wide variety of useful antibiotics, including streptomycin.
5. For example, linear DNA was precipitated in the most commonly used procedures for purifying bacterial plasmids, and the procedures for purifying chromosomal DNA relied upon the differential binding of ethidium bromide to "sheared DNA fragments" compared to circular DNA.
6. It is not intuitively obvious how the ends of a linear DNA molecule could be completely replicated. All known DNA polymerases require a pre-existing primer for initiation of DNA replication. The primer is usually a short RNA molecule with a free 3'-OH group that can be extended by DNA polymerase. If a linear DNA molecule was primed at one end, DNA synthesis could continue to the other end. However, once the primer is removed, the DNA corresponding to the primer could not be replicated.

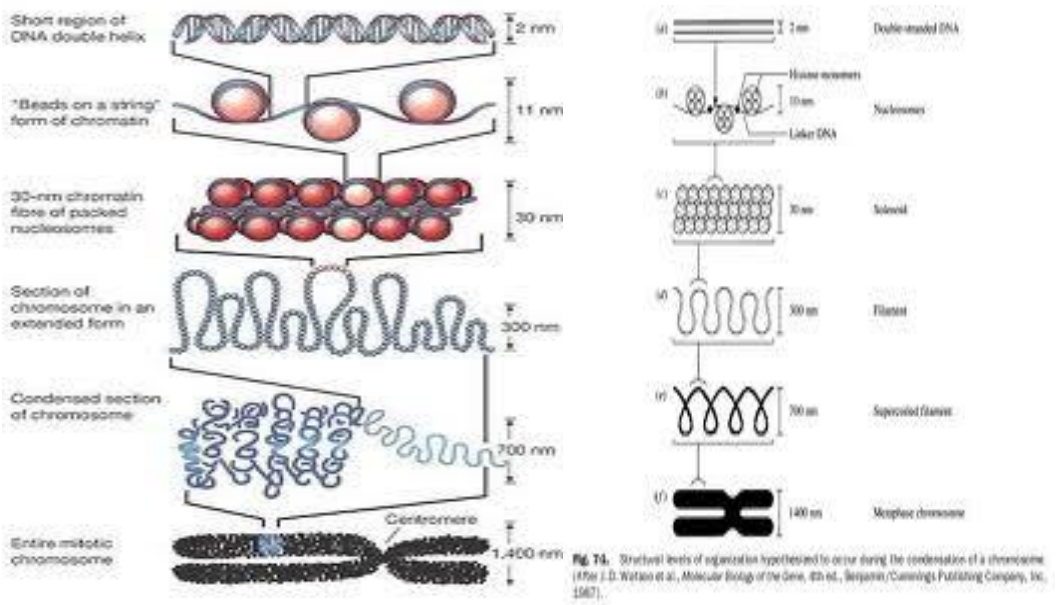


The telomers at the end of chromosomes of most eukaryotic cells are replicated by a different mechanism: most telomeres are short GC-rich repeats that are added in a 5' to 3' direction by the enzyme telomerase.

Eukaryotic Chromosome Structure (Plants and Animals)

In eukaryotes the chromosomes are found packaged within a nuclear membrane, unlike the case in prokaryotes. This membrane consists of a DNA double helix bound to an octamer of core histones (2 dimers of H2A and H2B, and an H3/H4 tetramer). Together, the DNA bound around this histone core forms what is known as the nucleosome. About 147 base pairs of DNA coil around 1 octamer, and ~20 base pairs are sequestered by the addition of the linker histone (H1), and various length of "linker" DNA (~0-100 bp) separate the nucleosomes.

Packaging of DNA is facilitated by the electrostatic charge distribution: phosphate groups cause DNA to have a negative charge, whilst the histones are positively charged. Most eukaryotic cells contain histones (with a few exceptions) as well as the kingdom Archaea, a protist group. Histones are positively charged molecules as they contain lysine and arginine in larger quantities and DNA is neatively charged. So they make a strong ionic bond in between them to form nucleosome.



Other variations in numbers of chromosomes—Polyploidy

Polyploidy is a term used to describe cells and organisms containing more than two paired (homologous) sets of chromosomes results from having **additional whole haploid sets** of chromosomes. If a normal diploid individual is said to have **2n** chromosomes, and a normal gamete is said to have **n** chromosomes, then polyploid individuals can be represented as: 3n, 4n, 6n, 8n, etc. Polyploidy is rare in many groups of animals, but we do observe it in fish, amphibians, and lizards.

Autotriploid. Many domestic plants are autopolyploids. **Usually sterile**. Problems at meiosis if there are odd numbers of chromosome sets (3,5, etc.). Unbalanced gametes. Some "seedless" varieties of fruit are polyploidy.

Allotetraploid. Non-homologous sets of chromosomes cannot synapse at meiosis. But if both sets are doubled (**mitotic failure**), all chromosomes now have a homolog to pair with, meiosis can take place normally.

Chromosomal Mosaics

It is when different cells within an individual, who has developed from a single fertilized egg, have a different chromosomal makeup. Most commonly there will be some cells with a typical number of chromosomes (46 chromosomes) and other cells with an altered number or structure of chromosomes. The most common kind of chromosomal mosaicism found at prenatal diagnosis involves trisomy, where the abnormal cells contain 47 chromosomes. Down syndrome mosaicism is an example of trisomy mosaicism. These individuals have some cells with the typical number of chromosomes (46) and some cells with an extra chromosome 21, for a total of 47 chromosomes. Mosaicism may exist for all kinds of chromosome abnormalities (monosomy, triploidy, structural changes, etc). Although more rare, there may even be mosaicism where both different cell types are abnormal in structure or number, and there are no normal cells involved.

Chromosomal mosaicism describes a **group of disorders**. Any chromosomal change, in number or structure, can be present in a mosaic form. However, trisomy mosaicism is the most common kind ascertained during prenatal diagnosis.

All of the cells in our body come from a single cell, the fertilized egg or zygote. In order for the zygote to develop into a baby, this single cell must grow and divide. Before cells can divide each chromosome must make an identical copy of itself. At cell division each chromosome and its identical copy pull apart into two separate cells. Now the resulting cells, also called daughter cells, have the same chromosome make-up as the original cell. The two new cells will repeat this process. In each cell, the chromosomes will duplicate and divide into two new cells. The result, is 4 cells identical to the first original cell. This process of cell division is called mitosis. Sometimes a mistake can occur when the chromosomes are separating into the two daughter cells. An extra chromosome may travel into the wrong cell or a chromosome may get lost in the separation of the cells. The result would be two daughter cells with different chromosomal make-up.

In this illustration, the green cell represents a cell with an abnormal chromosome make-up. All cells that come from the green cell will share the same chromosome change. We say that all cells originating from that cell are in the same cell line. The baby that develops from this embryo will have some cells in his/her body which have the typical number of chromosomes and some that have the chromosome change.

a) Normal and abnormal cells are found in most tissues

b) Normal and abnormal cells are confined to specific tissues

Trisomy mosaicism can occur in one of two ways**:**

- In an abnormal fertilized egg with 47 chromosomes, one of the cells may lose the extra chromosome at cell division, leaving 46 chromosomes in that cell. All cells that are derived from that cell will have 46 chromosomes. The rest of the cells will have 47 chromosomes.

In a typical zygote with 46 chromosomes, at cell division one of the cells may retain a duplicated copy of one of the chromosomes. This produces a cell with 47 chromosomes. All cells that are

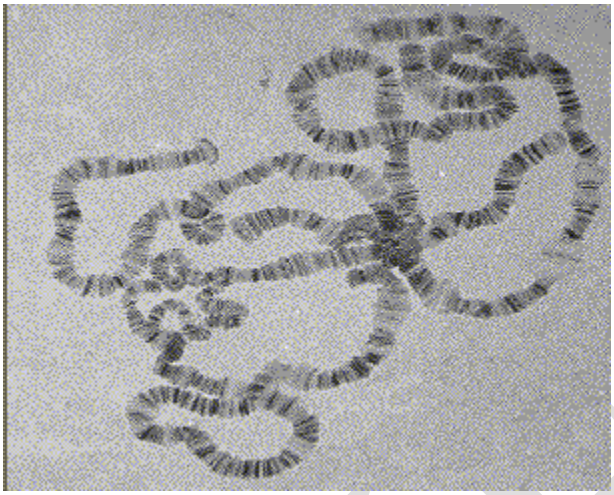
derived from that cell also have 47 chromosomes. The rest of the cells will have 46 chromosomes.

Polytene Chromosome:

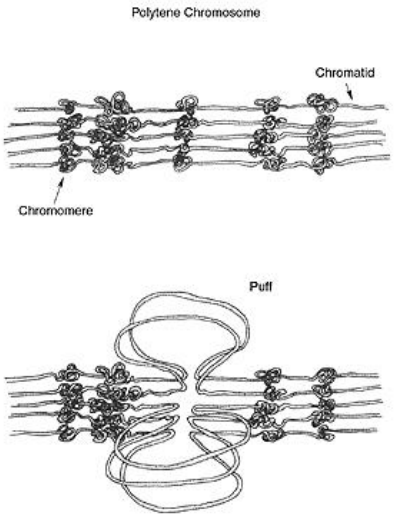
To increase cell volume, some specialized cells undergo repeated rounds of DNA replication without cell division (endomitosis), forming a giant **polytene chromosome**. Polytene chromosomes form when multiple rounds of replication produce many sister chromatids that remain synapsed together.

In addition to increasing the volume of the cells' nuclei and causing cell expansion, polytene cells may also have a metabolic advantage as multiple copies of genes permits a high level of gene expression. In *Drosophila melanogaster*, for example, the chromosomes of the larval salivary glands undergo many rounds of endoreplication, to produce large amounts of glue before pupation.

Polytene chromosomes have characteristic light and dark banding patterns that can be used to identify chromosomal rearrangements and deletions. Dark banding frequently corresponds to inactive chromatin, whereas light banding is usually found at areas with higher transcriptional activity. The banding patterns of the polytene chromosomes of *Drosophila melanogaster* were sketched in 1935 by Calvin B. Bridges, in such detail that his maps are still widely used today. The banding patterns of the chromosomes are especially helpful in research, as they provide an excellent visualization of transcriptionally active chromatin and general chromatin structure.



Chromosome puffs are diffused uncoiled regions of the polytene chromosome that are sites of RNA transcription. A **Balbiani ring** is a large chromosome puff.



Polytene chromosomes were originally observed in the larval salivary glands of *Chironomus* midges by Balbiani in 1881, but the hereditary nature of these structures was not confirmed until they were studied in *Drosophila melanogaster* in the early 1930s by Emil Heitz and Hans Bauer. They are known to occur in secretory tissues of other dipteran insects such as the Malpighian tubules of *Sciara* and also in protists, plants, mammals, or in cells from other insects. Some of the largest polytene chromosomes described thus far (see scale bar in figure below) occur in

larval salivary gland cells of the Chironomid genus *Axarus*. Polytene chromosomes are about 200micron in length. The chromonema of these chromosomes divide but do not separate. Therefore, they remain together to become large in size. Another form of chromosomal enlargement that provides for increased transcription is the lampbrush chromosome.

Polytene chromosomes are also used to identify the species of Chironomid larvae that are notoriously difficult to identify. Each morphologically distinct group of larvae consists of a number of morphologically identical (sibling) species that can only be identified by rearing adult males or by cytogenetic analysis of the polytene chromosomes of the larvae. Karyotypes are used to confirm the presence of specific species and to study genetic diversity in species with a wide range.

GENETIC code

As we learned in Structure of Nucleic Acids, DNA and RNA are made up by sequences of nitrogen bases-pairs: adenine, thymine, guanine, and cytosine. Scientists have long understood that these nitrogen bases somehow contained the information that coded for specific amino acids. However, it took some time before they figured out how the base pairs accomplished this coding. Scientists main problem lay in the fact that while there were only 4 nitrogen bases (nucleotides), there were 20 amino acids for which those nucleotides had to code. If adenine, thymine, guanine, and cytosine each coded for a particular amino acid, then the DNA/mRNA information system would only be able to code for 4 amino acids. If, however, groups of two nucleotides coded for a single amino acid, the story is somewhat different. Given four nucleotides looked at in groups of two, there are sixteen possible combinations (AA, AT, AG, AC, TA, TT, TG, TC, GA, GT, GG, GC, CA, CT, CG, CC); that sixteen is still not enough to code for twenty amino acids. But if the nucleotides code for amino acids in groups of three then there are sixty-four possible combinations. Scientific experiments have verified that nucleotides code for amino acids in successive groups of threes. These groups of threes are called codons.

Degeneracy of the Genetic Code

As we know, since the genetic code is read in triplets and there are four possible bases that can occupy each position, the number of possible codons is 4 X 4 X 4, or 64 codons. However, there are only 20 known amino acids. Experiments have shown that three codons function also function stop codons, acting as termination signals in translation. Yet that brings the count up to only twenty-three necessary codons. The vast difference between possible codon variations and needed codon variations means, as seen in the figure below, that each amino acid is specified by more than one codon. Because the genetic code therefore does not code to its capacity, it is called "degenerate".

First position (5' end)

	U	C	A	G	
U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G
A	AUU AUC Ile AUA AUG	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G

Third position (3' end)

Amino acid names:

Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartate
Cys = cysteine
Gln = glutamine
Glu = glutamate
Gly = glycine
His = histidine
Ile = Isolevcine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = Tyrosine
Val = valine

UNIT-IV

SYLLABUS

GENETICS: Definition and types of mutation, Causes of mutations, Ames test for mutagenic agents, Screening procedure for isolations of mutants and use of mutants, Variations in chromosomes structure deletion, duplication, Inversion and translocation, Sex determination, Sex linkage, sex linked diseases

Gene Mutation

Mutation = a process that produces a gene or chromosome that differs from the wild type 2. Mutation = the gene or chromosome that results from a mutational process 3. a mutant is the organism or cell whose changed phenotype is attributed to a mutation B. General Types 1. Gene mutation = the allele of a gene changes. 2. Chromosome mutation = segments of chromosomes, whole chromosomes, or entire sets of chromosomes change C. What does wild type (wt) mean? Wild type is an arbitrary standard for what "normal" is for an organism. Please remember that what is considered wild type today may have been a mutant in the evolutionary past. D. Direction of the mutation 1. Forward mutations are changes away from the wt 2. Reverse mutations (reversions) are changes from the mutant allele back to the wt allele E. Mechanisms for gene mutation 1. Errors in DNA replication 2. Errors in DNA repair 3. Environmental mutagen causes DNA damage that is not repaired correctly 4. Transposons and insertion sequences (a mobile DNA elements that can move from one location in the chromosome to another; the element may "jump" into a gene thereby mutating it) F. Why study gene mutation? 1. Variants in genes (which are caused by mutations) are needed to study the transmission of traits 2. Mutations can tell the researcher about the function of a gene product in a biological system 3. Mutations are the basis for cancer and other genetic diseases 4. Gene mutations serve as the source for most alleles in a population and is therefore the origin of genetic variation within a population 5. Mutations drive evolution: mutations are the raw material upon which natural selection acts. II. Classification of mutations A. General info 1. Various schemes for classification depending upon which aspect of mutation is being examined 2. Classes are not mutually exclusive B. Point of origin 1. Somatic mutations a) mutations that are in the somatic tissues of the body b) mutations are NOT transmitted to progeny c) the extent of the phenotypic

effect depends upon whether the mutation is dominant or recessive (dominant mutations generally have a greater effect) d) the extent of the phenotypic effect depends upon whether it occurs early or late in development (early arising mutations have a greater effect) (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) e) sectoring phenotypes may be seen when the mutation occurs during embryonic development f) cancer caused by somatic mutations 2. Germinal mutations a) mutations that are in the germ tissues of the body b) mutations MAY BE transmitted to progeny c) dominant mutations are seen in first generation after the mutation occurs d) if a female gamete containing an X-linked mutation is fertilized, the males will show the mutant phenotype e) recessive mutations will only be seen upon the chance mating with an individual carrying the recessive allele too; thus, the recessive mutation may remain hidden for many generations C. Phenotypic effects 1. Morphological mutations are mutations that affect the outwardly visible properties of an organism (i.e. curly ears in cats) 2. Lethal mutations are mutations that affect the viability of the organism (i.e. Manx cat). 3. Conditional mutations are mutations in which the mutant allele causes the mutant phenotype only in certain environments (called the restrictive condition). In the permissive condition, the phenotype is no longer mutant. (i.e. Siamese cat – mutant allele causes albino phenotype at the restrictive temperature of most of the cat body but not at the permissive temperature in the extremities where the body temperatures is lower). 4. Biochemical mutations are mutations that may not be visible or affect a specific morphological characteristic but may have a general affect on the ability to grow or proliferate. a) Most microorganisms are prototrophs which means that they can grow on a simple growth medium including an energy source and inorganic salts. Biochemical mutations include those that affect proteins or enzymes required to grow on various nutrients or to synthesize various components. Thus, these mutations cause the microorganisms to become auxotrophs (they must be supplied with additional nutrients if they are to grow). For example, the bacterium Escherichia coli does NOT require the amino acid tryptophan for growth because they can synthesize tryptophan. However, there are E. coli mutants that have mutations in the trp genes. These mutants are auxotrophic for tryptophan, and tryptophan must be added to the nutrient medium for growth. b) Humans can also have biochemical mutations (also called inborn errors in metabolism). Such examples include hemophilia, phenylketonuria, and galactosemia. D. Loss of function vs. gain of function

mutations 1. Loss of function mutations are those that destroy the function of the gene product. Many times in diploid organisms, these are recessive mutations because the other wild type allele still encodes a functional gene product. However, it is possible to have a dominant loss of function mutation in which the mutant gene product interferes with the activity of the gene product from the wild type allele. a) Null mutation = loss of function mutation where gene product is completely inactive (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) b) Leaky mutation = loss of function mutation where gene product is not completely inactive (partially active still) (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) 2. Gain of function mutations are those that produce a new function for the gene product. Gain of function mutations are dominant. (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) III. The occurrence of mutations A. Frequencies of mutations 1. Mutation frequency = # of times mutation appears in the population / # of individuals in the population where a population can be bacterial cells, people, gametes 2. Mutation rate = # of mutations / unit time where unit time can be per cell division, cell generation (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) 3. Mutations are relatively rare. 4. Different genes have different mutation frequencies (Table 7-1) 5. Different organisms have different overall mutation frequencies (Table 7-2) B. Detection of mutations in humans 1. Detection of germinal dominant mutations by human pedigree analysis (shows up in the pedigree as the sudden appearance of a novel phenotype) 2. Detection of germinal recessive mutations are more difficult because they remain masked by the dominant allele until the union of two heterozygotes 3. Detection of germinal X-linked mutations arising in female gametes appear in some of the males in the generation after the mutation occurred. C. Detection of mutations using the specific-locus test, a system for detecting recessive mutations in diploids. Heterozygote individual for gene(s)A that give phenotype A is crossed with a homozygous recessive individual for gene(s)a that gives phenotype a. The frequency of the mutant phenotype (a) is quantitated. (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) D. Detection of X-linked mutations in Drosophila using the ClB chromosome The ClB chromosome is the X chromosome bearing the C allele which prevents crossover, the l allele which is a recessive lethal, and the Bar allele which is a dominant eye

mutation. (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) E. Detection of mutations in microorganisms 1. General info on microorganism growth and manipulation a) Growth is rapid (some bacterial populations double every 30 minutes) b) Growth can occur in liquid media to cell density of approx 109 bacteria/ml c) Growth can occur on solid media (agar plate). Organisms are spread across a plate of solid media (plating). Single cells are deposited randomly and will grow into a colony of cells that is visible to the naked eye. 2. Microorganisms allow for the use of selective systems for mutation detection vs. the screening systems used for higher organisms. A selective system is one in which the experimenter can DEMAND that the only individuals that grow or survive are the ones that have the mutation of interest. On the other hand, a screening system is one in which the experimenter must examine each individual to see if it has the mutation of interest. 3. Microbial selective systems a) Selection for reversion of an auxotroph to a prototroph: Plate 109 adenine auxotrophs on agar plate with no adenine. The only bacteria that grow are those that have a random mutation in the mutant ad gene that now reverts it back to the wild type allele. (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) b) Selection for resistance to an environmental factor (bacteriophage, antibiotics). Example, Luria-Delbruck experiment: Tested whether mutations arose in response to plating on selective media (in this case bacteriophage resistant mutants were selected for by plating on media containing bacteriophage which normally kill the bacterium). Two hypotheses: (a) physiological change where mutations arose after plating because the bacteria sensed the phage and altered themselves so that they may become resistant to the phage or (b) random mutation where mutations arose randomly before plating and all plating did was select for the resistant bacteria (from An Introduction to Genetic Analysis, 6th ed. By Griffiths et al. W. H. Freeman and Company) Versus Luria and Delbruck tested the above hypothesis by starting 20 very small cultures (0.2 ml) and 1 large culture (10 ml) with low numbers of bacteria. The cultures were allowed to grow for numerous generations and them 0.2 ml aliquots were plated on phage plates (all of the small cultures and aliquots of the large culture) and the # of resistant bacteria were counted. If the physiological hypothesis was correct, then one would expect that each plate would contain the same number of phage resistant bacteria. However, if the random mutation hypothesis was correct, some of the small cultures may have had the random phage resistance mutation occur

early and thus almost the entire population is phage resistant while other small cultures may have had the random phage resistance mutation occur very late early and thus almost the entire population is not phage resistant. They found a large fluctuation in the number of phage resistant bacteria on plates from the small 0.2 ml cultures (Table 7-3), and thus random mutation was the correct hypothesis. In the larger culture, the fluctuations are averaged out and so you do not see the large variation in #s. 4. Microbial screening systems a) Screening for forward mutations from wild type to auxotrophy: Plate bacteria on complete media to form colonies. Replica plate many, many colonies to plates with and without the nutrient you are testing for auxotrophy. Compare the plus and minus nutrient X plates to look for a colony that appears on the plus nutrient plate but not the minus nutrient plate. If you expect the mutation frequency to be 1 in 10,000, then you will need to replica plate at least 10,000 colonies to get the 1 mutant. b) Enrichments are important in screening because they reduce the number of organisms that you have to screen. Enrichment for auxotrophs works by selectively removing or killing growing microorganisms while they are in medium that allows only the prototrophs to grow. (1) Filtration enrichment (Figure 7-18) (2) Penicillin enrichment IV. Mutations and cancer A. Cancer is a group of diseases characterized by rapid, uncontrolled proliferation of cells within a tissue resulting in the formation of a tumor. Cancer has many causes and phenotypes but the fundamental mechanism underlying all cancers is genetic. B. There are two types of genes that are involved in cancer formation. 1. Tumor suppressor genes are genes that encode a product that normally stops cell division. Mutations in these genes result in uncontrolled activation of cell division and therefore tumor formation. Mutations are generally recessive and thus you need mutations in both alleles to have cancer. A mutation in one allele predisposes the carrier to cancer. a) Rb gene - retinoblastoma (retinal cancer) b) BRCA1 - hereditary breast cancer gene c) p53 gene mutations are found in a variety of cancers including breast, lung, bladder, and colon cancers. Over 1/2 of all cancers are associated with p53. 2. Proto-oncogenes are genes that encode a product that normally controls cell division (kind of like an on/off switch). Mutations in these genes make the gene product permanently in the on position which results in uncontrolled activation of cell division and therefore tumor formation. a) N-ras – neuroblastoma (tumor formed of embryonic ganglion cells), leukemia b) N-myc – neuroblastoma c) man – mammary carcinoma V. Mutagens in genetic dissection A. Mutagens are agents that cause mutations at a rate higher than the

spontaneous rate. B. Mutagens can be chemical (i.e. cigarette smoke, mustard gas) or radiation (i.e. UV, X-rays,)

CHOMOSOME MUTATION:

CHANGES IN CHROMOSOME STRUCTURE)

Chromosomal mutations are processes that result in rearranged chromosome parts, abnormal numbers of individual chromosomes, or abnormal numbers of chromosome sets. The resulting products are also known as chromosomal mutations. B. For our purposes here, we will be talking about alterations in large regions of the chromosome spanning numerous genes C. Abnormalities from chromosomal mutations are frequently due to: 1. change in gene number (balance) 2. change in gene location 3. break internal to a gene D. Can occur in somatic cells, germinal cells, and gametes E. How can you detect a chromosomal mutation? cytogenetics 1. Cytological examination of the actual chromosomes 2. Genetic analysis of inheritance II. Physical features of the chromosomes A. Size B. Centromere position (appears constricted under microscope) 1. Telocentric (at one end of the chromosome) 2. Acrocentric (off center on the chromosome) 3. Metacentric (in the center of the chromosome) B. Arm length: centromere to the end defines an arm (p = short arm and q = long arm) C. Nucleolar organizer position 1. Nucleoli = intranuclear organelles that contain rRNA (appears as dark spot under microscope) 2. Number of nucleoli ranges from 1 to many per chromosome set 3. Position of nucleolus is next to constriction of the chromosomes called nucleolar organizers which themselves have specific chromosome locations D. Chromomere patterns (beadlike, localized thickening along the chromosomes during prophase) E. Heterochromatin patterns 1. Constitutive heterochromatin is highly condensed chromosomal regions that are for the most part genetically inert and are found in particular chromosomal regions 2. Detected by staining with chemicals (such as Feulgen) that react with DNA as very dense regions of staining F. Banding patterns due to special chromosome staining procedures 1. Specific stains reveal specific bands at specific chromosomal sites a) Quianacrine hydrochloride ‡ Q bands b) Giemsa stain ‡ G bands c) Reversed giemsa ‡ R bands 2. Specialized case of polytene chromosomes in Drosophila E. Karotype = the entire metaphase chromosome complement of individual F. Physical characteristics of chromosomes are useful for detecting chromosome mutations such as chromosome rearrangements including deletions, duplications,

inversions, and translocations. III. Deletions (loss of a chromosomal segment) _A_B_C___o___D_E_F___ ‡ _A_C___o___D_E_F___ A. Types 1. Interstitial (Not at the end of the chromosome) 2. Terminal (Near the end of the chromosome) B. Deletions require two breaks in the chromosome followed by loss of the chromosomal segment and rejoining of the ends. C. The deleted fragment is acentric (without a centromere) and will be lost upon multiple rounds of cell division. D. In meiosis I cells heterozygous for the deletion, chromosomes line up normally except that region corresponding to deletion forms a "loop formed to get proper alignment of the homologous chromosomes.. E. Effect of multigenic deletions 1. Homozygous for multigenic deletion = usually lethal. 2. Heterozygous for mutigenic deletion = frequently lethal because a) Unmasking of lethal allele on homologous chromosome without the deletion b) Upset balance of gene number F. Detection 1. Genetic a) Failure of homozygotes to survive b) Inability of the mutation to revert back to wild type c) Recombination frequency between the genes flanking the deletion is lower than in the wild type d) Unmasking of a recessive allele present on the homologous chromosome without the deletion (pseudodominance of the recessive allele) 2. Cytological a) Deletion loop in meiosis b) Change in banding patterns G. Using deletions to map genes 1. Deletion mapping a) Cross a new recessive mutant with a set of deletion mutants of known map location. The new mutation will show pseudodominance when heterozygous for the deletion that contains the region to which the new mutation maps. See figure 8-8 in text. b) Cross a new mutant suspected of containing a deletion with a set of recessive mutants of known map location. Look for the pseudodominance effect which indicated that your putative deletion is in the same area as the gene that is showing pseudodominance 2. In situ hybridization Add a radioactively labeled gene of interest to a chromosome deletion set and look for the presence or absence of radioactivity for each deletion. The chromosome deletions that do no hybridize to the radioactive gene probe are deleted for the region that contains the gene of interest. IV. Duplications (presence of two copies of a chromosomal region) _A_B_C___o___D_E_F___ ‡ _A_B_C_B_C___o___D_E_F___ OR _A_B_C___o___D_E_F___ ‡ _A_B_C_C_B___o___D_E_F___ A. Types 1. tandem (adjacent to each other in same order) 2. reverse tandem (adjacent to each other in reverse order) 3. nontandem (at a different chromosomal location) B. Tandem duplications can occur due to unequal crossing over where homologous chromosomes pair inaccurately during meiosis I

(Deletion) (Duplication) C. Nontandem duplications may result from crossing over during meiosis within segments of the chromosome that contain inversions or translocations (see later). D. In meiosis I cells heterozygous for the duplication, chromosomes line up normally except that region corresponding to duplication forms a "loop" formed to get proper alignment of the homologous chromosomes. Also, asymmetric pairing within the duplicated region may occur during meiosis I, leading to unequal crossover. (from An Introduction to Genetic Analysis, 6th ed. by Griffiths et al. (W. H. Freeman and Company) E. Effect of duplications 1. Duplications are rare. 2. Heterozygous for duplication = can be lethal because of imbalance generated by extra copies of the duplicated region. 3. Homozygous for duplications is unknown in medical genetics. F. Detection is difficult. Cytology is the main tool (change in bands and duplication loop in meiosis I) G. Duplications have been proposed to be important for evolution because they provide an extra copy of genes that can then undergo mutation while still leaving the original gene unaffected. V. Inversions (flipping of chromosomal segment relative to the rest of the chromosome) _A_B_C D E___o___F_G H__ ‡ _A_D_C B E___o___F_G H__ OR _A_B_C___o___D_E_F___ ‡ _A_E_D___o___C_B_F___ A. Types 1. Paracentric – centromere outside the inversion 2. Pericentric – centromere inside the inversion B. Inversions require two breaks in the chromosome followed by a 180° rotation of the chromosomal segment and rejoining of the ends. C. In meiosis I cells heterozygous for the inversion, inversion loop is formed to get proper alignment of the homologous chromosomes. 1. Paracentric (from An Introduction to Genetic Analysis, 6th ed. by Griffiths et al. (W. H. Freeman and Company) 2. Pericentric (from An Introduction to Genetic Analysis, 6th ed. by Griffiths et al. (W. H. Freeman and Company) D. In meiosis I cells homozygous for the inversion, crossover is normal but linkage map shows inverted gene order. E. Effects of inversions are negligible because there is no change in the genetic material. However, those heterozygous for inversions produce abnormal meiotic crossover products and possibly abnormal progeny (if they survive at all). F. Detection 1. Genetic – recombinant progeny are usually not recovered from heterozygotes 2. Cytological a) If the duplication is pericentric, the arm lengths change. b) Change in the banding pattern c) Dicentric bridge in meiosis for paracentric inversion IV. Translocations (movement of a chromosomal segment from one location to another) _A_B_C___o___D_E__ and _G_H_I___o___J_K__ ‡ _A_B_C___o___J_K__ and _G_H_I___o___D_E__ A. Types 1.

Reciprocal (exchange of segments) 2. Nonreciprocal (1 segment moves to a new location without an exchange) B. Reciprocal translocations require two breaks in two different chromosomes followed by rejoining of the ends. C. In meiosis I cells heterozygous for the deletion, cross conformation forms to get proper alignment of the homologous chromosomes. Showing only meiosis I on the next page: A B C D E G H I J K A B C J K G H I D E A B C D E I H G D E A B C J K J K I H G Lining up during Meiosis I Alternate Segregation A B C D E G H I J K A B C J K G H I D E Adjacent-1 Segregation A B C D E G H I J K A B C J K G H I D E wild type translocated Deleted for JK Duplicated for DE Deleted for DE Duplicated for JK OR Heterozygous for translocation (In most cases, these gametes form nonviable progeny even when mated with wild type.) D. Effect of translocations 1. Semisterility due to adjacent segregation in meiosis 2. Position effects: altered expression of a gene when it is moved to a new location E. Detection 1. Genetic a) Semisterility b) Apparent linkage of genes on separate chromosomes c) Position effects 2. Cytological a) Can change the location of the centromere b) Change in the size of the chromosome c) Cross formation in meiosis I

VARIATION IN CHROMOSOME NUMBER & STRUCTURE

Changes in chromosome number can occur by the addition of all or part of a chromosome (**aneuploidy**), the loss of an entire set of chromosomes (**monoploidy**) or the gain of one or more complete sets of chromosomes (**euploidy**). Each of these conditions is a variation on the normal diploid number of chromosomes. As you would expect each of these can have drastic effects on phenotypic expression.

Aneuploidy - the abnormal condition were one or more chromosomes of a normal set of chromosomes are missing or present in more than their usual number of copies

Monoploidy - the loss of an entire set of chromosomes

Euploidy - an entire set of chromosomes is duplicated once or several times

Chromosome Number in Different Species

In "higher" organisms (**diploids**), members of same species typically have identical numbers of chromosomes in each somatic cell. **Diploid chromosome number** (2n). Nearly all chromosomes will exist in pairs (identical wrt length and centromere placement) except the sex chromosomes. Members of pair are **homologous chromosomes**. **Haploid number** (n) is the number of chromosome pairs.

	2n	n
Human	46	23
Horse 64	32	
Cat	38	19
Geometrid moth	224	112
Tomato	24	12
Pink bread mold	14	7

Autosomal monosomy and trisomy

Occasionally, one finds an organism that has an extra copy of a particular chromosome. This is known as **trisomy**--because there are now 3 copies of an autosome. Some trisomies are viable in animals, but the condition usually has severe effects. These effects are presumably related to the fact that there are 3 copies of every gene on the trisomic chromosome, but only 2 copies of all the genes on the other chromosomes. We will see later, that organisms with three or more copies of **all** the chromosomes are often perfectly viable.

Trisomy of human chromosome 21 is the cause of the disorder known as **Down syndrome.**

(Remember, humans have 23 pairs of chromosomes--the pairs are numbered 1 through 22, plus the X and Y). It is characterized by multiple physical defects, including epicanthal fold, furrowed tongue, characteristic palm and finger print patterns, and lowered IQ. About 1 in 750 live births produces a child with this condition. It results from the **non-disjunction** of chromosome 21

during meiotic anaphase I or anaphase II, when the paired homologs (or paired chromatids) normally migrate to opposite poles of the cell.

Nondisjuction: Nondisjunction ("not coming apart") is the failure of chromosome pairs to separate properly during meiosis stage 1 or stage 2. This could arise from a failure of homologous chromosomes to separate in meiosis I, or the failure of sister chromatids to separate during meiosis II or mitosis. The result of this error is a cell with an imbalance of chromosomes. Such a cell is said to be aneuploid. Loss of a single chromosome (2n-1), in which the daughter cell(s) with the defect will have one chromosome missing from one of its pairs, is referred to as a monosomy. Gaining a single chromosome, in which the daughter cell(s) with the defect will have one chromosome in addition to its pairs is referred to as a trisomy.The members of a chromosome pair (homologs) line up at the metaphase plate during meiotic metaphase I, then separate to opposite poles of the cell during anaphase I--review this material in Klug and Cummings or any introductory Genetics text if you are not thoroughly familiar with it!). If the pair fails to separate, and both migrate to the same pole, half of the resulting gametes will have two copies of chromosome 21, rather than one. When this gamete unites with a normal gamete (bearing one copy of chromosome 21) during fertilization, the resulting gamete has 3 copies of chromosome 21, rather than the normal 2. Nondisjunction of chromosome 21 seems to occur more often in the production of eggs than sperm, and the frequency increases with the age of the parent. Older individuals are often encouraged to test for trisomy 21 by amniocentesis at 15 to 16 weeks after conception. Nondisjunction can happen to other chromosomes in addition to chromosome 21. But human embryos that are trisomic for any other chromosome do not survive to birth. It should be obvious that the other half of the gametes resulting from a non-disjunction event at anaphase 1 will have **0 copies** of the chromosome. When a gamete with 0 copies of a chromosome unites with a normal gamete, the result is a zygote that has only one copy of that chromosome. This is **monosomy. Monosomy is not well tolerated in animals**—usually lethal. Some plants can survive (observed in maize, tomato, Oenothera, and Datura) but they have low viability and are usually sterile. Nondisjunction can also occur at anaphase II, when sister chromatids fail to separate and migrate to opposite poles. Nondisjuntion at anaphase one results in half the gametes being normal, and half being abnormal (see diagram in text).

If one surveys karyotypes of embryos that spontaneously abort, trisomies for all the autosomes are seen, and other forms of chromosomal abnormalities as well, but these conditions are apparently fatal early in development. Normal embryonic development requires a precise diploid complement of chromosomes.

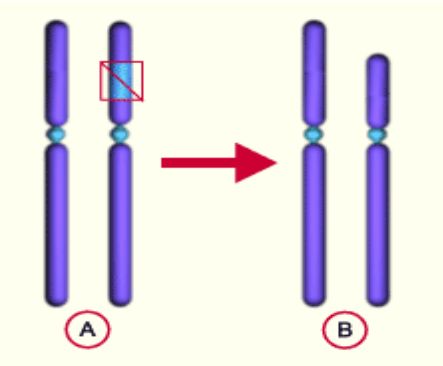
2n / \ n+1 n-1 / \ / \ n+1 n+1 n-1 n-1	2n / \ n n / \ / \ n n n+1 n-1
Schematic of nondisjunction in meiosis I. Duplicated chromosomes in diploid cell (2n). All gametes are affected by nondisjunction in meiosis I. Two gametes have a single extra chromosome; two gametes are missing a single chromosome.	Schematic of nondisjunction in meiosis II. Duplicated chromosomes in diploid cell (2n). Half of the gametes are affected by nondisjunction in meiosis II. One gamete has a single extra chromosome; one gamete is missing a single chromosome.

Chromosomal Rearrangements:

Variations in chromosome structure also exist, and also cause characteristic genetic effects. We will consider deletions and duplications of parts of whole chromosomes, and inversions and translocations of sections of chromosomes.

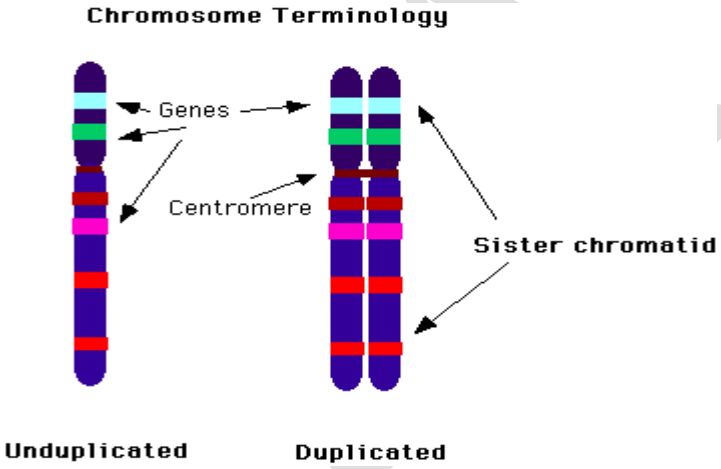
Deletion: Sometimes a chromosome will arise in which a segment is missing. These chromosomes are said to have **deletions.** Deletions are generally harmful, and typically, the larger the deletion, the more harmful it is. Small deletions are often viable if the deletion is heterozygous, because the other chromosome contains copies of the genes missing in the chromosome with the deletion. But even small deletions are usually lethal if they are

homozygous. A deletion on one homologue can "unmask" recessive alleles on the other homologue--this effect is called pseudo-dominace.



Duplications

Duplication often results in reduction of viability, but in general duplications are less severe in their effects than are deletions.

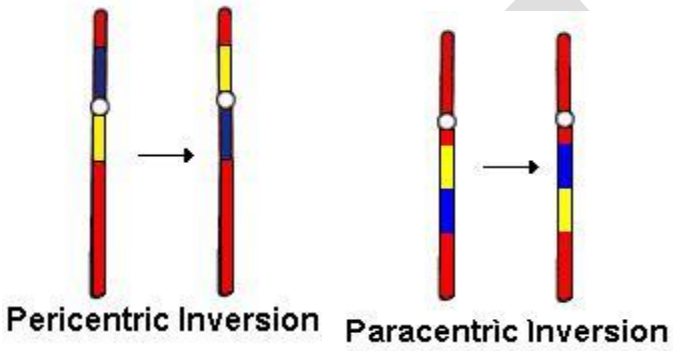


Heterozygotes and homozygotes for small duplications can be viable, although often exhibit phenotypic effects (Bar eye in *Drosophila*).

Duplication may be important in generating multiple copies of genes that require very high levels of expression (**gene amplification**)--**rDNA** codes for genes producing RNA for construction of ribosomes. Also may be source of evolutionary origin and divergence of genes coding for similar (but not identical) proteins (**alpha and beta globin components of hemoglobin**).

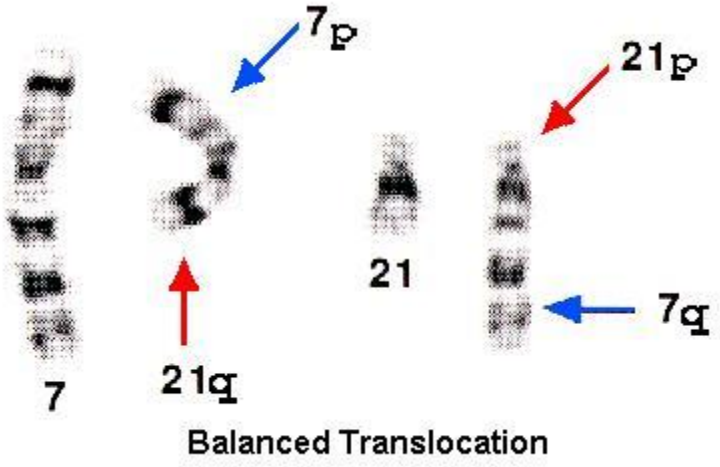
Inversions

Chromosomes can sometimes break and rejoin in a different orientation. If this happens in a germ cell, the gametes will contain a **rearrangement or inversion.** The area between the breaks is inverted (turned around), and then reinserted and the breaks then unite to the rest of the chromosome. If the inverted area includes the centromere it is called a **pericentric inversion.** If it does not, it is called a **paracentric inversion.**



Translocations

5% of people with Down Syndrome have one parent who is heterozygous for a translocation. Chromosome 14 is translocated onto chromosome 21. Half the time, meiosis produces a normal set of balanced translocated chromosomes. But half the time, unbalanced chromosomes are produced, either a 14 w/out the translocated 21 segment or a translocated 14 with the attached 21 plus a normal 21.



Balanced Translocation

Chromosome rearrangements in evolution

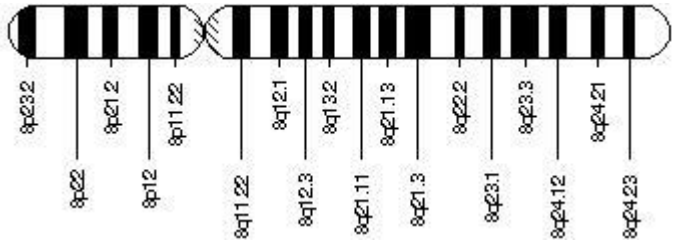
Chromosomal rearrangements are not just rare abnormalities. Many kinds of rearrangements have no deleterious effects, and some may be beneficial (e.g., duplications are the source of novel gene functions and allow evolutionary increase in gene number). Comparisons among species show that chromosomal rearrangements have occurred repeatedly throughout evolutionary history.

Humans normally have 46 chromosomes in each cell, divided into 23 pairs. Two copies of chromosome 8, one copy inherited from each parent, form one of the pairs. Chromosome 8 spans more than 146 million DNA building blocks (base pairs) and represents between 4.5 and 5 percent of the total DNA in cells.

Identifying genes on each chromosome is an active area of genetic research. Because researchers use different approaches to predict the number of genes on each chromosome, the estimated number of genes varies. Chromosome 8 likely contains about 700 genes that provide instructions for making proteins. These proteins perform a variety of different roles in the body.

Geneticists use diagrams called ideograms as a standard representation for chromosomes. Ideograms show a chromosome's relative size and its banding pattern. A banding pattern is the characteristic pattern of dark and light bands that appears when a chromosome is stained with a

chemical solution and then viewed under a microscope. These bands are used to describe the location of genes on each chromosome.



Deletion

Synonym(s)

- gene deletion

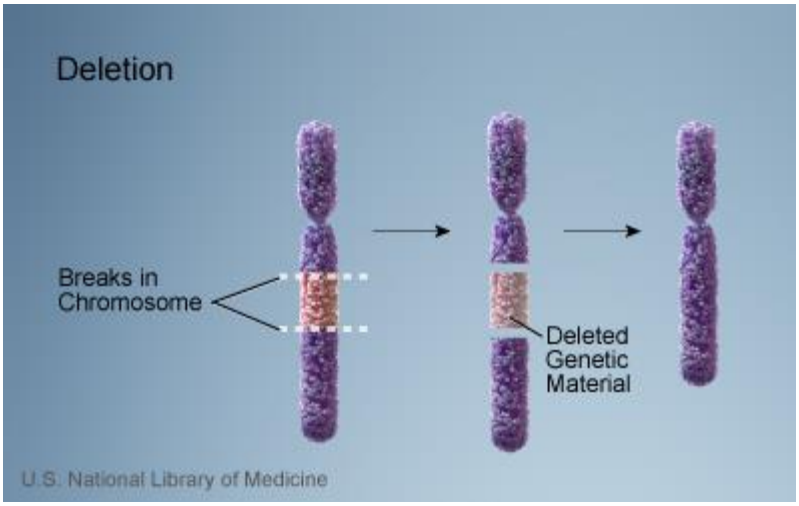
Definition(s)

Absence of a segment of DNA; may be as small as a single base or large enough to encompass one or more entire genes. Large deletions involving a whole segment of a chromosome may be detected by routine examination of the chromosomes; intermediate deletions involving a few genes may be detected by using fluorescent in situ hybridization (FISH); smaller deletions involving a portion of a gene may only be detected by analyzing the DNA.

A loss of part of the DNA from a chromosome; can lead to a disease or abnormality.

Deletion is a type of mutation involving the loss of genetic material. It can be small, involving a single missing DNA base pair, or large, involving a piece of a chromosome.

A deletion occurs when a chromosome breaks and some genetic material is lost.



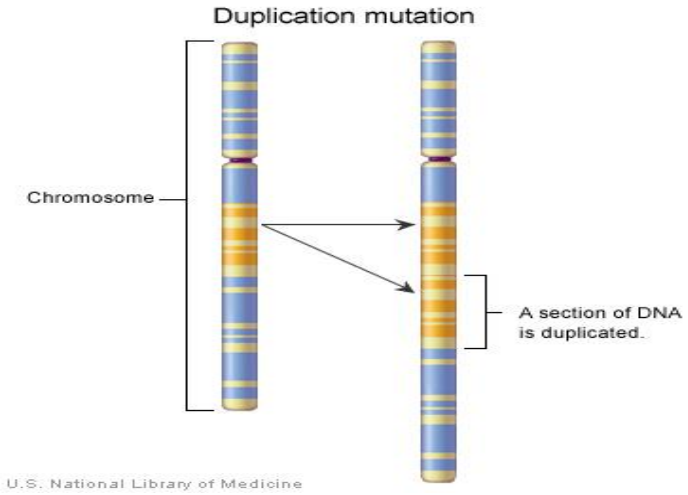
Duplication

Definition(s)

The presence of an extra segment of DNA, resulting in redundant copies of a portion of a gene, an entire gene, or a series of genes, usually caused by unequal crossing-over during gene replication when gametes are formed in meiosis

Duplication is a type of mutation that involves the production of one or more copies of a gene or region of a chromosome. Gene and chromosome duplications occur in all organisms, though they are especially prominent among plants. Gene duplication is an important mechanism by which evolution occurs.

A section of DNA is accidentally duplicated when a chromosome is copied.

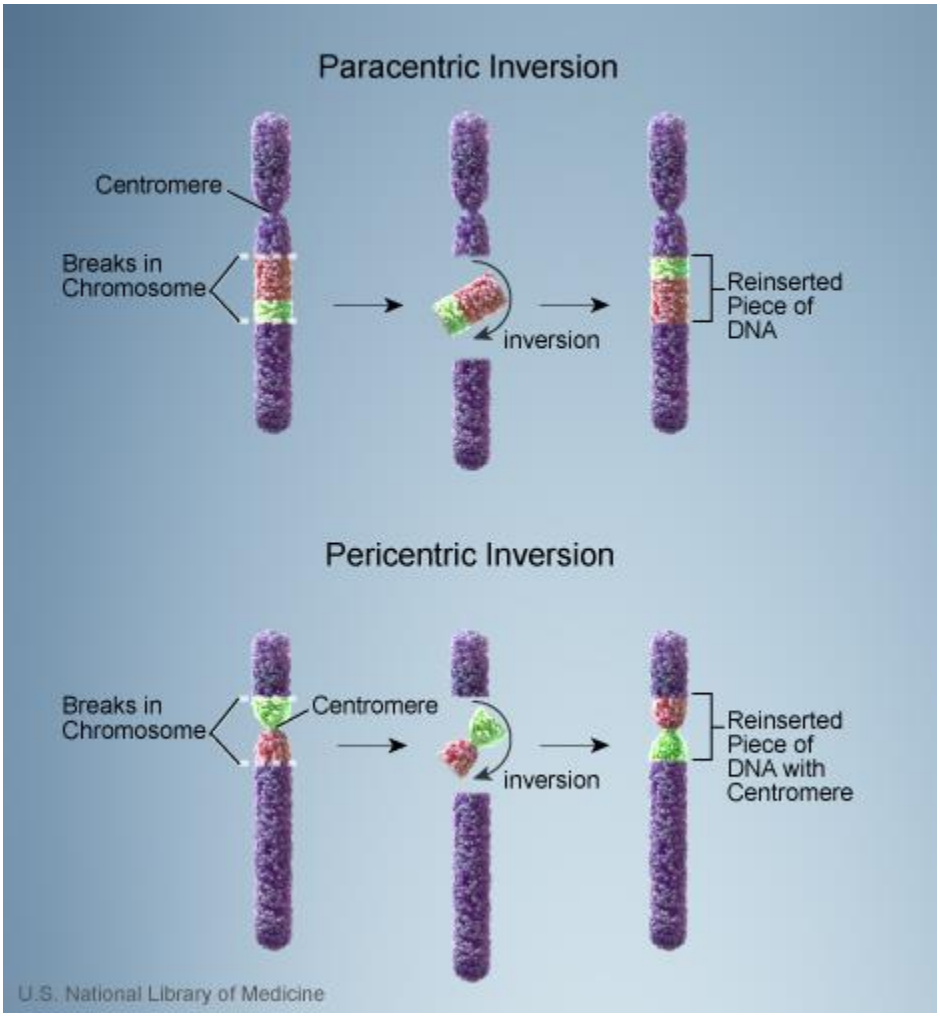


Inversion

Definition(s)

A chromosomal rearrangement in which a segment of genetic material is broken away from the chromosome, inverted from end to end, and re-inserted into the chromosome at the same breakage site. Balanced inversions (no net loss or gain of genetic material) are usually not associated with phenotypic abnormalities, although in some cases gene disruptions at the breakpoints can cause adverse phenotypic effects, including some known genetic diseases; unbalanced inversions (loss or gain of chromosome material) nearly always yield an abnormal phenotype.

Inversions occur when a chromosome breaks in two places and the resulting piece of DNA is reversed and re-inserted into the chromosome. Inversions that involve the centromere are called pericentric inversions; those that do not involve the centromere are called paracentric inversions.



Translocation

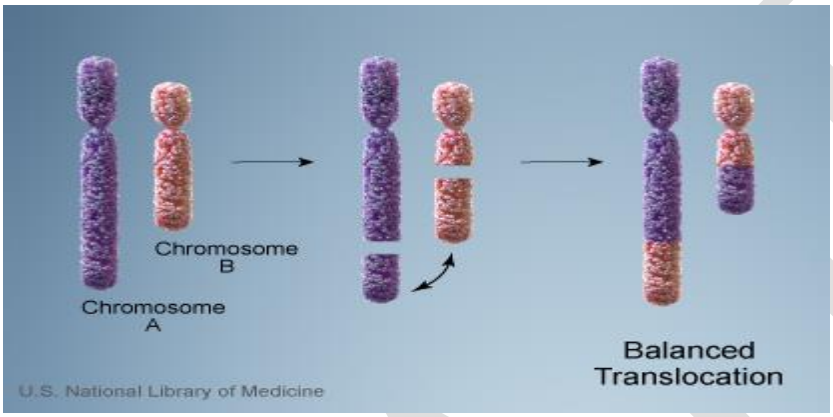
Definition(s)

A chromosome alteration in which a whole chromosome or segment of a chromosome becomes attached to or interchanged with another whole chromosome or segment, the resulting hybrid segregating together at meiosis; balanced translocations (in which there is no net loss or gain of chromosome material) are usually not associated with phenotypic abnormalities, although gene disruptions at the breakpoints of the translocation can, in some cases, cause adverse effects,

including some known genetic disorders; unbalanced translocations (in which there is loss or gain of chromosome material) nearly always yield an abnormal phenotype

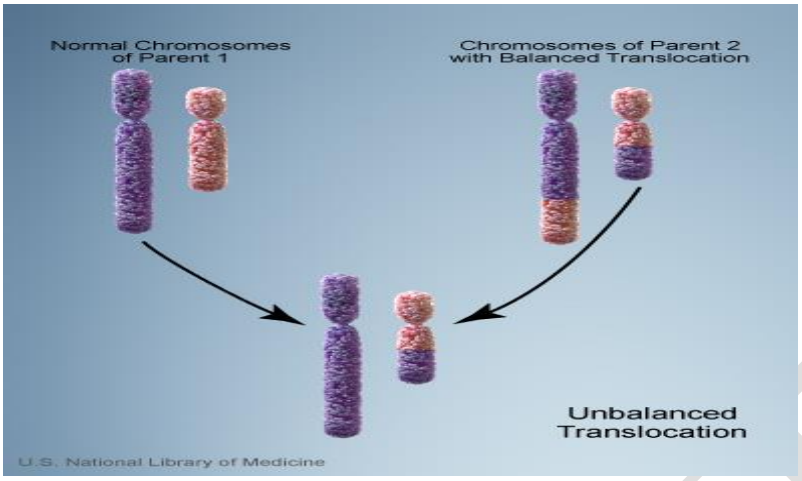
Balanced translocation

In a balanced translocation, pieces of chromosomes are rearranged but no genetic material is gained or lost in the cell.



Unbalanced translocation

An unbalanced translocation occurs when a child inherits a chromosome with extra or missing genetic material from a parent with a balanced translocation.



Can changes in the structure of chromosomes affect health and development?

Changes that affect the structure of chromosomes can cause problems with growth, development, and function of the body's systems. These changes can affect many genes along the chromosome and disrupt the proteins made from those genes.

Structural changes can occur during the formation of egg or sperm cells, in early fetal development, or in any cell after birth. Pieces of DNA can be rearranged within one chromosome or transferred between two or more chromosomes. The effects of structural changes depend on their size and location, and whether any genetic material is gained or lost. Some changes cause medical problems, while others may have no effect on a person's health.

Changes in chromosome structure include:

Translocations

A translocation occurs when a piece of one chromosome breaks off and attaches to another chromosome. This type of rearrangement is described as balanced if no genetic material is gained or lost in the cell. If there is a gain or loss of genetic material, the translocation is described as unbalanced.

Deletions

Deletions occur when a chromosome breaks and some genetic material is lost. Deletions can be large or small, and can occur anywhere along a chromosome.

Duplications

Duplications occur when part of a chromosome is copied (duplicated) too many times. This type of chromosomal change results in extra copies of genetic material from the duplicated segment.

Inversions

An inversion involves the breakage of a chromosome in two places; the resulting piece of DNA is reversed and re-inserted into the chromosome. Genetic material may or may not be lost as a result of the chromosome breaks. An inversion that involves the chromosome's constriction point (centromere) is called a pericentric inversion. An inversion that occurs in the long (q) arm or short (p) arm and does not involve the centromere is called a paracentric inversion.

Isochromosomes

An isochromosome is a chromosome with two identical arms. Instead of one long (q) arm and one short (p) arm, an isochromosome has two long arms or two short arms. As a result, these abnormal chromosomes have an extra copy of some genes and are missing copies of other genes.

Dicentric chromosomes

Unlike normal chromosomes, which have a single constriction point (centromere), a dicentric chromosome contains two centromeres. Dicentric chromosomes result from the abnormal fusion of two chromosome pieces, each of which includes a centromere. These structures are unstable and often involve a loss of some genetic material.

Ring chromosomes

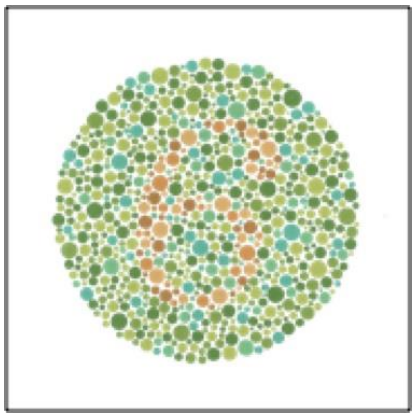
Ring chromosomes usually occur when a chromosome breaks in two places and the ends of the chromosome arms fuse together to form a circular structure. The ring may or may

not include the chromosome's constriction point (centromere). In many cases, genetic material near the ends of the chromosome is lost.

SEX-Linked Inheritance

X-Linked Inheritance

Traits that are determined by alleles carried on the **X chromosome** are referred to as **X-linked**. X-linked alleles require a specific notation: X^c or X^+ where the "+" represents the **dominant** allele and the lowercase letter the **recessive** allele. Females will have two X-linked alleles (because females are XX), whereas males will only have one X-linked allele (because males are XY). Most X-linked traits in humans are recessive.



An Ishihara plate can be used to test for red-green colorblindness. Affected individuals may not see the number 6. Image courtesy of Wikimedia Commons

One example of an X-linked trait is red-green colorblindness. Let (X^c) represent the recessive allele that causes colorblindness and (X^+) represent the normal dominant allele. Females that are X^+X^+ or X^+X^c have normal color vision, while X^cX^c females are colorblind. Males that are X^+Y have normal color vision, while X^cY males are colorblind.

Punnett Squares

To determine the inheritance of red-green colorblindness (or any other X-linked trait), the genotypes of the parents must be considered.For example, if a mother is a **carrier** for colorblindness (X^+X^c), and a father has normal vision X^+Y, then their sons have a 50% chance of

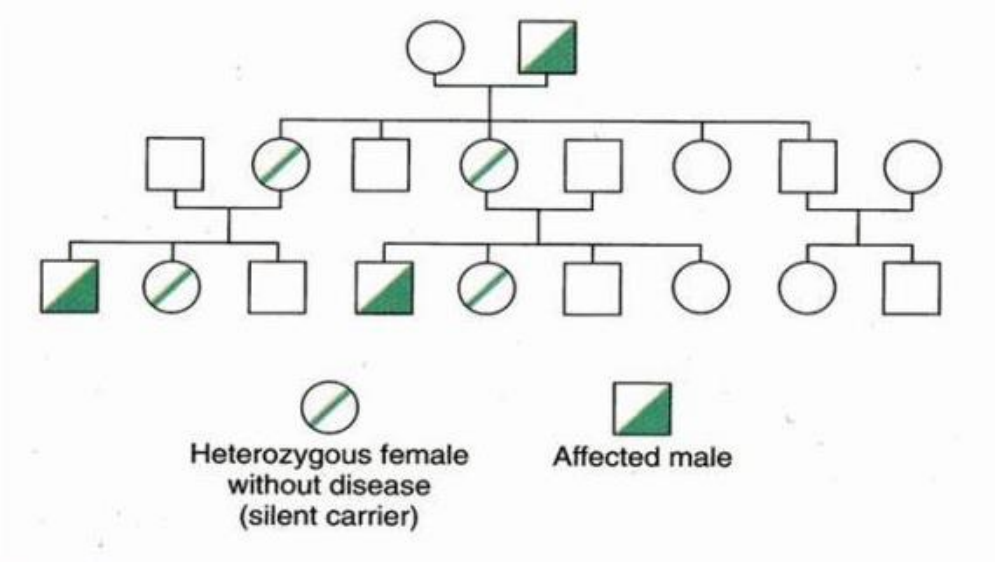
colorblindness because they inherit their X chromosome from their

	X^c	X^+
X^+	$X^c X^+$	X^+X^+
Y	X^cY	X^+Y

mother and their Y chromosome from their father. Their daughters will have a 50% chance of being a carrier (X^+X^c) and a 50% chance of being completely normal (X^+X^+) (see figure). A Punnett square can be used to determine any possible genotypic combinations in the parents.

Pedigree

Here is a pedigree depicting X-linked recessive inheritance. These traits are often passed from a carrier mother to an affected son. X-linked traits are never passed from father to son. Males are more likely to be affected than females. In this pedigree, the carrier (**heterozygous**) females are indicated; however, they do not express the trait being tracked in this pedigree.



UNIT-V

SYLLABUS

GENETICS: Linkage and recombination of genes in a chromosome crossing, Rules of extra nuclear inheritance, Cytoplasmic inheritance, organism heredity, genomic imprinting, Evolution and population genetics, inbreeding and out breeding, Hardy Weinberg law, Alleic and genotype frequency

Unit – V

Linkage and Crossing over

CHROMOSOME THEORY OF LINKAGE

The chromosome theory of linkage of Morgan and Castle states that:

1. The genes which show linkage, are situated in the same pair of chromosomes.
2. The linked genes remain arranged in a linear fashion on the chromosome. Each linked gene has a definite and constant order in its arrangement.
3. The distance between the linked genes determines the degree of strength of linkage. The closely located genes show strong linkage then the widely located genes which show weak linkage.
4. The linked genes remain in their original combination during the course of inheritance.

Kinds of Linkage:

Linkage is generally classified on the basis of three criteria viz., (i) Crossing over, (ii) Genes involved and (iii) Chromosomes involved.

Based on crossing over: Linkage may be classified into (a) complete and (b) incomplete / partial depending up on absence or presence of recombinant phenotypes in test cross progeny.

(a) Complete linkage: It is known in case of males of *Drosophila* and females of silkworms, where there is complete absence of recombinant types due to absence of crossing over.

(b) Incomplete / partial linkage: If some frequency of crossing over also occurs between the linked genes, it is known as incomplete / partial linkage. Recombinant types are also observed besides parental combinations in the test cross progeny. Incomplete linkage has been observed in maize, pea, *Drosophila* female and several other organisms.

Types of linkage

The types of linkage are two types.

1) Complete linkage
2) Incomplete linkage

Complete linkage

Linked genes inherit together for more generation in a continuous and regular fashion on the same chromosome. This phenomena of inheritance is called complete linkage.

Eg: linkage in *Drosophila melanogaster*.

1. Complete Linkage

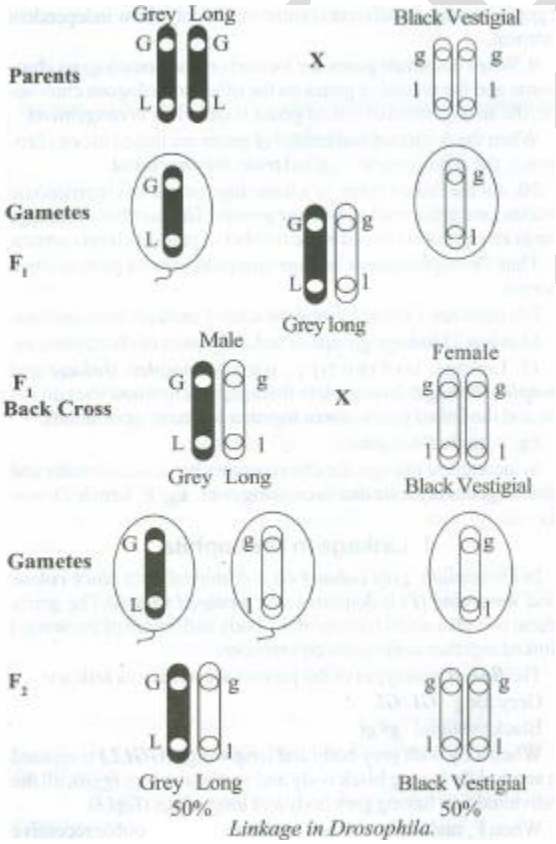
In complete linkage, linked genes inherit together for many generations. Here crossing over does not occur. In complete linkage the genes are closely situated.

Eg. 1. Body colour and shape of wings in male *Drosophila*.

2. Bent wings and shaven bristles in the 4^{th} chromosome of *Drosophila*.

Complete linkage is *the phenomenon in which two or moregenes or characters are inherited together Jor a number of generation.* In this, genes are closely associated and tend to inherit together. Complete linkage is due to the fact that there occurs no break in the

chromosomes. As a result of complete linkage, the young ones inherit only the parental characters. New characters do not appear among the young ones. So complete linkage produces only parental combination; new combinations do not arise. This phenomenon is very rare. It is found only in male *Drosophila*. The F 1 male hybrid is back crossed with recessive female parent. The F 1 male hybrid produces only two types of gametes in which the linked genes (G and L or g and 1) are inherited together. So only two types of offspring are produced in the F2 generation in equal numbers.



Linkage in Drosophila.

Incomplete linkage

The linked genes which are widely located in chromosome and have changes (meiotic prophase) of separation by crossing over. This phenomena are incompletely linked genes and their inheritance is called incomplete linkage.

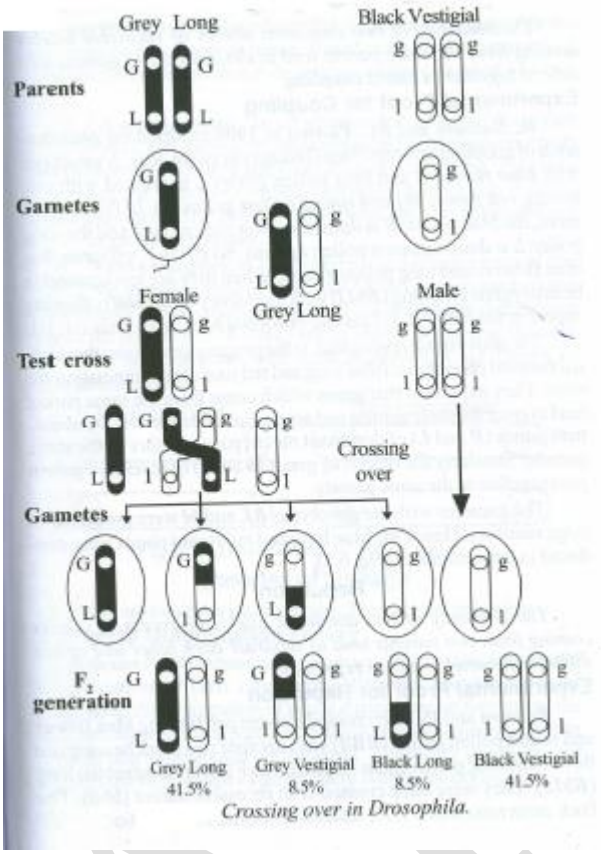
The separation of linked genes during inheritance is called *incomplete linkage.* The linked genes are separated due to crossing over, chromosomal breaks, etc.

Eg. 1. *Body colour and wing shape in female Drosophila.*

2. Seed colour and seed shape in maize.

3. Flower colour and pollen grain shape in sweet pea.

- In incomplete linkage, the linked genes on certain occasions separate. This leads to the formation of new combinations among the young ones.
- Incomplete linkage is due to the breakage of chromosomes during gametogenesis. Incomplete linkage is found in *female Drosophila.*
- This breakage of chromosomes leads to the separation of linked genes and new combinations appear. Because of this new genetic combination the offspring produced in the F2 generation are different from their parent in their phenotype and genotype.
- So the incomplete linkage involves the accidental breakage of chromosomal segments or linked genes, resulting in new combination of genes.
- In the below experiment, the F 1 female hybrid produces four types of gametes. Among four types, two types of gametes carry new combinations due to the separation of linked genes. The gene G is separated from *L* and joins with *I.* In the same way the *L* joins with *g.*
- These combinations are different from the original combination. This type of inheritance is different from the *independent assortment.*
- If the genes are assorted independently the four types of offspring produced in the F2 generation of the above experiment should be in the 1:1:1:1 ratio.



Crossing over in Drosophila.

Importance of linkage

Morgan and *Castle* proposed the chromosomal theory of linkage. The main features of the chromosomal theory of linkage are the following:

1. The genes are arranged in a linear fashion on the chromosomes.

2. The genes on a chromosome are linked

3. Genes showing linkage are located on the same chromosome.

4. Linked genes will remain together during inheritance.

5. The distance between the genes will determine the strength of linkage. The closely located genes show strong linkage. Distantly located genes show weak linkage.

Factors effecting linkage

Linkage is affected by the following factors:

1. Distance: Closely located genes show strong linkage while genes widely located show weak linkage.

2. Age: With increasing age the strength of linkage decreases.

3. Temperature: Increasing temperature decreases the strength of linkage.

4. X-rays: X-ray treatment reduces the strength of linkage.

Based on genes involved: Depending on whether all dominant or some dominant and some recessive alleles are linked together, linkage can be categorized into (a) Coupling phase and (b) Repulsion phase.

(a) Coupling phase: All dominant alleles are present on the same chromosome or all recessive alleles are present on same chromosome.

(b) Repulsion phase: Dominant alleles of some genes are linked with recessive alleles of other genes on same chromosome.

Based on chromosomes involved: Based on the location of genes on the chromosomes, linkage can be categorized into (a) autosomal linkage and (b) X-chromosomal linkage / allosomal linkage / sex linkage.

(a) Autosomal linkage: It refers to linkage of those genes which are located in autosomes (other than sex chromosomes).

(b) X-chromosomal linkage / allosomal linkage / sex linkage: It refers to linkage of genes which are located in sex chromosomes i.e. either 'X' or 'Y' (generally 'X').

LINKAGE GROUPS:

Linkage group refers to a group of genes which are present in one chromosome. In other words, all those genes which are located in one chromosome constitute one linkage group. The number of linkage groups is limited in each individual. The maximum number of linka ge groups is equal to the haploid chromosome number of an organism. For example there are ten linkage groups in corn (2n = 20), seven in garden pea (2n = 14), seven in barley (2n = 14), four in *Drosophila melanogaster* (2n = 8) and 23 in man (2n = 46).

CROSSING OVER

The term crossing over was first used by Morgan and Cattell in 1912. The exchange of precisely homologous segments between non-sister chromatids of homologous chromosomes is called crossing over.

Types of crossing over:

Depending upon the number of chiasmata involved, crossing over is of three types.

Single crossing over: It refers to the formation of single chiasma between non-sister chromatids of homologous chromosomes. It involves two linked genes (Two point test cross).

Double crossing over: It refers to the formation of two chiasmata between non-sister chromatids of homologous chromosomes. It involves three linked genes (Three point test cross).

Multiple crossing over: Occurrence of more than two crossing overs between non-sister chromatids of homologous chromosomes is known as multiple crossing over. However, the frequency of such type of crossing over is extremely low.

Mechanism of Meiotic crossing over:

It is responsible for recombination between linked genes and takes place during pachytene stage of meiosis i.e. after the homologous chromosomes have undergone pairing and before they begin to separate. It occurs through the process of breakage and reunion of chromatids. During pachytene, each chromosome of a bivalent (chromosome pair) has two chromatids so that each bivalent has four chromatids or strands (four-strand stage). Generally one chromatid from each of the two homologues of a bivalent is involved in crossing over. In this process, a segment of one of the chromatids becomes attached in place of the homologous segment of the nonsister chromatid and vice-versa. It is assumed that breaks occur at precisely homologous points in the two nonsister chromatids involved in crossing over; this is followed by reunion of the acentric segments. This produces a cross (x) like figure at the point of exchange of the chromatid segments. This figure is called chiasma (which is seen in diplotene stage of meiosis) (plural-chiasmata).

Obviously, each event of crossing over produces two recombinant chromatids (involved in the crossing over) called cross over chromatids and two original chromatids (not involved in crossing over) referred to as noncrossover chromatids. The crossover chromatids will have new combinations of the linked genes, i.e. will be recombinant; gametes carrying them will produce the recombinant phenotypes in test-crosses, which are called crossover types. Similarly, the noncrossover chromatids will contain the parental gene combinations and the gametes carrying them will give rise to the parental phenotypes or noncrossover types. Therefore the frequency of crossing over between two genes can be estimated as the frequency of recombinant progeny from a test-cross for these genes. This frequency is usually expressed as percent. Thus, the frequency of crossing over (%) can be calculated using the formula;

$$\text{Frequency of crossing over(\%)} = \frac{\text{No. of recombinant progeny from a test cross}}{\text{Total number of progeny}} \times 100$$

Kinds of crossing over

Single cross over

- When only one chiasma occurs at one point of the chromosome pair is called single cross over.
- It produces two known cross over chromatid and two cross over chromatid.

Double cross over

- When the crossing over occurs at two point between any points in the same chromosome pair is called double crossing over.
- It produces 4 cross overs

Triple cross over

- When the cross over occur at 3 point between any 3 points in the same chromosome pair is called triple cross over.
- It produces 6 cross over.

Multiple cross over

- When crossing over occur at more than 3 or 4 point between any two or more points in the pair of chromosomes is called multiple cross over.
- It produces multiple cross over.

Factors effecting crossing over

1) High temperature to increase the frequency of crossing over.
2) X – ray
3) Age

4) Some genetical mutations decrease the frequency of crossing over.

5) Inversion of chromosome segments suppress the crossing over.

Theories about the mechanism of Crossing over

Important Theories That Can Explain the Mechanism of Crossing Over are listed below:

1. Janssen's partial chiasma type theory.

2. Belling's copy choice theory.

3. Darlington's breakage and reunion theory.

1. Breakage and reunion theory:

This theory is based on the assumptions that:

1. Prior to crossing over each chromosome of each bivalent get duplicated to form tetrad.

2. Crossing over occurs only between non-sister chromatids.

3. Crossing over involves the mechanical breaks in non-sister chromatids due to twisting around each other and reunion or recombination of chromatids take place.

According to this theory first of all, chromatids break and then form chiasmata. Crossing over does not produce chiasmata but it is caused by chiasmata.

2. Copy choice theory:

This theory was proposed by Belling in 1933. According to this theory, the paired chromosomes duplicate their genes before the fibres join them to form strands.

When the chromosomes are twisted around each other, reciprocal exchange of the chromatids take place during pachytene or just before.

There may be some recombination during the period of DNA synthesis affecting short, unpaired segments of the chromosomes.

A small part of new DNA helix being synthesized may copy a non-sister helix rather than a sister helix to produce recombination in a very short period.

There are two main objections:

1. Only two chromatids out of four involved in crossing over thus newly formed chromatids would be altered by recombination.

2. Duplication should occur during late meiotic prophase but now it is clear that DNA duplication occurs even before syn-apsis.

Hence, these theories assume that crossing over occurs in the interphase rather than pachytene.

3. Partial chiasma type theory:

According to this theory breaks occur only in two chromatids out of the four at the pachytene stage. These four again rejoined and the chiasmata are formed i.e. chiasmata is the result of cross-ing over.

Cytological detection of crossing over.

- The genetical detection of crossing over in Drosophila melanogaster we will know using their (f1 off springs) phenotype characters. But we unable to see under microscopic examination whether the crossing over occur or not, because the chromosomes are in homologous strains or condition.

- This crossing over or blocks had changed places cannot observed until visible marker may be incorporated on the chromosome.

Stern's experiment for cytological detection of cross over

1) Crossing over is the interchange of chromosome parts between homologous chromosome, and this crossing over is proved by Stern, in 1931, on Drosophila melanogaster.

2) The female Drosophila carries XX chromosome and the male Drosophila carries one X chromosome and one Y chromosome.

3) In a type of female Drosophila the two X chromosomes are different from each other.

4) An X chromosome has a piece of Y chromosome attach to it, the other X chromosome has been broken into two unequal segments and it is shorter than the unbroken X chromosome. Thus the two X chromosome are structurally different from the normal X chromosome.

5) In Drosophila red eye (C) is dominant and carnation eye (c) is recessive. Similarly bar eye (B) is dominant and round eye (b) is recessive.

6) The broken X chromosome contains a recessive gene (c) for carnation eye colour and a dominant eye (B) for bar eye, while it is homologous contains C & b.

7) This female having red bar eyes is crossed with a double recessive male, having carnation round eyes.

8) In the absence of crossing over only two types of female gamates are produced, one type having broken X chromosome containing c & B genes, the other type X chromosome having with a piece of Y chromosome attached and contain C & b genes.

9) If crossing over occurs two more type of gamates are produced. One type having C & B on a broken X chromosome with a piece of Y chromosome. So these 4 types of gamates after fertilization will produce 4 types of off spring that are,

- Carnation colour & bar shape eyes
- Red colour & round shape eyes
- Carnation colour & round shape eyes
- Red colour & bar shape eyes

10) The X chromosome of above said 4 types we can identified under the microscopic examination. This experiment proves that inter change of chromosomal material takes place between the homologous chromosomes.

Creighton & Mc Clintock's experiment

1) Creighton & Mc Clintock proved experimentally the exchange of chromatids during crossing over in maize.

2) They used to strains of maize which showed difference in the 9th chromosome. Because the 9th chromosome has knob at one end and a cell maker at the other end.

3) The other strain has no knob and no cell maker.

4) In additional to genetical characters are selected they are, colour of Kernal and nature of endosperm. Coloured kernel is dominant (C) & the colourless kernel is recessive (c).

5) Starchy endosperm (WX) is dominant and waxy endosperm (wx) is recessive.

6) A maize with knobbed chromosome, coloured kernel and waxy endosperm is crossed with another maize having knobless chromosome colourless kernel and starchy endosperm.

7) Hybrid maize having heterozygous chromosomes and heterozygous genotype are produced in the F1 generation.

8) The F1 hybrid is test crossed with a double recessive knobless chromosome.

9) The outcome F2 generations have examine genetically and cytologically.

10) The outcome F2 off springs have the following characterestics features.

- Knobbed coloured waxy
- Knobbed coloured starchy
- Knobless coloured waxy
- Knobless coloured starchy

11) This result shows the knobbed chromosomes were transformed in meosis process through the crossing over.

Significance of crossing over

a. It produces new individuals having new combinations of traits.

b. Crossing over has helped in establishing the concept of linear arrangement of genes.

c. The frequency of crossing over helps in the mapping of chromosomes. i.e., determining the location of the genes in the chromosomes.

d. Selection of useful recombination by geneticists has brought about green revolution in our country.

CYTOPLASMIC INHERITANCE

Inheritance due to genes located in cytoplasm (plasmagenes) is called cytoplasmic inheritance. Since genes govering traits showing cytoplasmic inheritance are loc ated outside the nucleus and in the cytoplasm, they are refered to as plasmagenes. The sum total of genes present in the cytoplasm of a cell or an individual is known as plasmon. The plasmagenes are located in DNA present in mitochondria (mt DNA) and in chloroplasts (cp DNA). Together both the DNAs are called organelle DNA. Therefore, this type of inheritance is often referred to as organellar inheritance, plastid inheritance or mitochondrial inheritance. In this, generally, the character of only one of the two parents (usually female) is transmitted to the progeny. Hence such inheritance is usually referred to as extra - nuclear or extra-chromosomal or maternal or uniparental inheritance. The cytoplasmic inheritance is of two types: 1) Plastid inheritance and 2) mitochondrial inheritance.

Plastidial or Chloroplast Inheritance: Plastids self duplicated and have some amount of DNA and plays an important role in cytoplasmic inheritance. Plastids have green pigments called chloroplasts. Chloroplasts contain a unique circular DNA (cp DNA) in the stroma that is completely different from the nuclear genome. Some examples of plastid inheritance are given below.

a) Leaf variegation in *Mirabilis jalapa* : The conclusive evidence for cytoplasmic inheritance was first presented by C. Correns in *Mirabilis jalapa* (Four 'O' clock plant) in 1909. He studied inheritance of leaf variegation in *M. jalapa.* Variegation refers to the presence of white or yellow spots of variable size on the green background of leaves. In *M. jalapa*, leaves may be green, white or variegated. Some branches may have only green, only white or only variegated leaves. Correns made crosses in all possible combinations among the flowers produced on these three types of branches. When flowers from green branch were used as female parent, all the progeny were green irrespective of the phenotype (green, white or variegated) of male parent. Similarly, progeny from crosses involving flowers bloomed on white branches as female parent were all white irrespective of the phenotype of male parent. But in progeny from all crosses involving flowers born on variegated branches as female parent, all the three types i.e. green, white and variegated individuals were recovered in variable proportions.

Female Parent	x	Male Parent	
Green	x	Green	
	X	White	Green
	x	Variegated	
White	x	Green	
	X	White	pale Green
	x	Variegated	
Variegated	x	Green	

X	White	Green, white and variegated
x	Variegated	in variable ratio in each of the cases.

The green leaf branches have normal chloroplasts, white branches have mutant chloroplasts and variegated have a mixture of both normal and mutant chloroplasts. The above results indicated that the inheritance is governed by chloroplasts. Since the cytoplasm is contributed to the zygote mainly by female parent, the plastids are transmitted to the zygote from the female parent. Thus the plastids are responsible for variation in the crosses of green, white and variegated leaves.

b) Mitochondrial inheritance: The inheritance of some characters, such as cytoplasmic male sterility in plants, pokyness in *Neurospora* etc., is governed by mitochondrial DNA (mtDNA).

a) **Cytoplasmic Male Sterility (CMS) in maize :** In several crops, cytoplasmic control of male sterility is known. In maize, cytoplasmic male sterility (CMS) is governed by mitochondrial DNA. In such cases, if female parent is male sterile, F1 progeny also will be male sterile, because cytoplasm is mainly derived from female parent.

b) **Pokyness in *Neurospora*:** *Neurospora*, which is a breadmold has two strains i.e. wild and poky. The wild strain has normal growth. While the poky which is a mutant has very slow growth. A cross betw een a poky female and a wild male produce only poky progeny. In reciprocal cross (a cross between wild female and poky male) all the progeny would be wild. This suggests the presence of cytoplasmic inheritance because only difference between the reciprocal crosses is in the main contributor of cytoplasm.

Characteristic Features of Cytoplasmic Inheritance

a. Reciprocal difference: Reciprocal crosses show marked differences for characters governed by plasmagenes. In most cases, plasmagenes from only female parent are transmitted and hence this phenomenon is also called uniparental inheritance.

b. Lack of segregation: In general, F1, F2, F3 and subsequent generations do not show segregation for a cytoplasmically inherited trait, as F1 individuals receive plasmagenes from female parent only.

c. Somatic segregation: Plasmagenes generally show the features in somatic tissues such as leaf variegation features which is of rare occurrence in case of nuclear genes.

d. Association with organelle DNA: Several plasmagenes have been shown to be associated either with chloroplast or mitochondrial DNA. For example: Cytoplasmic Male Sterility (CMS) in sorghum and maize is associated with mitochondrial DNA.

e. Nuclear transplantation: Nuclear transplantation means nucleus of a cell is removed and replaced by nucleus of another genotype from a different cell. If nuclear transplantation reveals a trait to be governed by genotype of cytoplasm and not by that of nucleus, it clearly indicates that the trait or character is governed by cytoplasmic inheritance.

f. Mutagenesis: Some mutagens are highly specific mutagens which act only on the plasmagenes and do not affect nuclear genes Eg; ethidium bromide, Induction of mutations by such agents or chemicals in a gene clearly indicates that it is a plasmagene.

g. Lack of chromosomal location: In many organisms extensive linkage maps of nuclear genes are available. If a gene is shown to be located in one of these linkage groups, obviously it cannot be a plasmagene.

h. Transfer of nuclear genome through back crosses: Nucleus of a variety or species may be transferred into cytoplasm of another variety or species through repeated back crossing with former, which is used as recurrent male parent. Lines produced in this way are called alloplasmic lines, since they have cytoplasm and nucleus from different species.

i. Lack of association with a parasite or symbiont or virus: Only those cytoplasmically inherited traits which are not associated with parasites, symbionts or viruses can be regarded to be governed by plasmagenes.

Extranuclear Inheritance

It is the transmission of genes that occur outside the nucleus. It is found in most eukaryotes and is commonly known to occur in cytoplasmic organelles such as mitochondria and chloroplasts or from cellular parasites like viruses or bacteria.

Extranuclear Inheritance of Organelles

Mitochondria are organelles which function to produce energy as a result of cellular respiration. Chloroplasts are organelles which function to produce sugars via photosynthesis in plants and algae. The genes located in mitochondria and chloroplasts are very important for proper cellular function, yet the genomes replicate independently of the DNA located in the nucleus, which is typically arranged in chromosomes that only replicate one time preceding cellular division. The extranuclear genomes of mitochondria and chloroplasts however replicate independently of cell division. They replicate in response to a cells increasing energy needs which adjust during that cells lifespan. Since they replicate independently, genomic recombination of these genomes is rarely found in offspring contrary to nuclear genomes, in which recombination is common. Mitochondrial disease are received from the mother, sperm does not contribute for it.

Extranuclear Inheritance of Parasites

Extranuclear transmission of viral genomes and symbiotic bacteria is also possible. An example of viral genome transmission is perinatal transmission. This occurs from mother to fetus during the perinatal period, which begins before birth and ends about 1 month after birth. During this time viral material may be passed from mother to child in the bloodstream or breastmilk. This is of particular concern with mothers carrying HIV or Hepatitis C viruses. Examples of cytoplasmic symbiotic bacteria have also been found to be inherited in organisms such as insects and protists.

Types of Extranuclear Inheritance

Three general types of extranuclear inheritance exist. These are vegetative segregation, uniparental inheritance and biparental inheritance.

• **Vegetative segregation** results from random replication and partitioning of cytoplasmic organelles. It occurs with chloroplasts and mitochondria during mitotic cell divisions and results in daughter cells that contain a random sample of the parent cell's organelles. An example of vegetative segregation is with mitochondria of asexually replicating yeast cells.

Maternal inheritance

- The determination of the phenotype of offspring by the genotype of female parent is called *maternal inheritance* or *uniparental inheritance or maternal effect*.
- Example: Pattern of shell coiling in snail.
- oHere the shell coiling is determined by the genotype of the mother and not by the individual's own genotype.

Examples for cytoplasmic inheritance

Plastid inheritance in Mirabilis

Shell-coiling in snail

Kappa particles in Paramecium

Cytoplasmic male sterility in maize

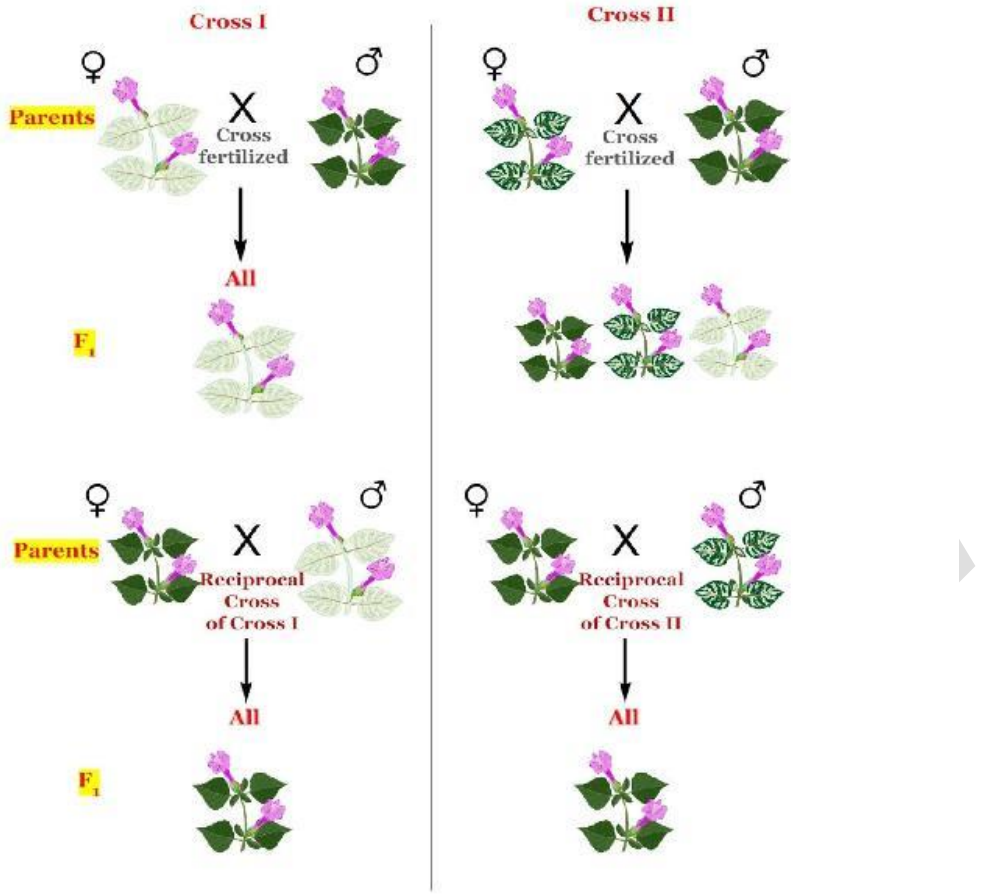
Sigma virus in *Drosophila melanogaster*

Milk factor in mice

LEAF VARIEGATION IN PLANTS

- The first example of cytoplasmic inheritance was reported by Correns (1909) in a variegated variety of the four-o'clock plant *Mirabilis jalapa.*
- Variegated plants have some branches which carry normal green leaves, some branches with variegated leaves (mosaic of green and white patches) and some branches which have all white leaves.
- Flowers on wholly green branches produce seeds that grow into normal plants.
- Flowers on variegated branches yield offspring of three kinds- green, white and variegated in variable proportions.

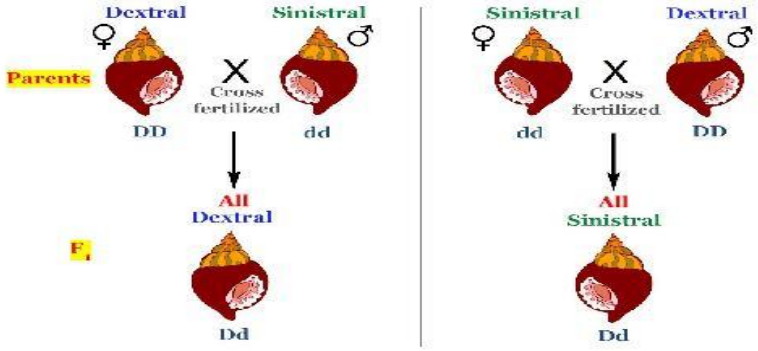
- Flowers from branches wholly white produce seeds that grow into white plants that is without chlorophyll.
- But in every case the source of pollen has no influence on the offspring.
- In other words, the phenotype of the progeny always resembled the female parent and the male made no contribution at all to the character. So cytoplasm of the egg influences the type of leaf in Mirabilis.
- The explanation for this unusual pattern of inheritance is that the genes concerned are located in the *plastids* within the cytoplasm, not in the nucleus and are therefore transmitted only through the female parent.
- Plastids are of two types, namely green *chloroplasts* and colourless *leucoplasts.*
- *Green* branches contain Green plastids in their leaves, Variegated branches contain Green plastids and Colourless plastids and Colourless branches are due to the presence of Colourless plastids.



SNAIL SHELL COILING

- The classic phenotype which exhibits maternal effects is coiling direction of snail shells.
- Shell coiling in *Limnaea peregra*, a fresh water snail , is of two types, Dextral (clockwise) and Sinistral (anticlockwise).
- The dextral shell is dominant and is controlled by dominant gene D.
- The sinistral shell is recessive and is controlled by recessive gene d.
- The following crosses were made between pure line snails.
- When dextral female (DD) was crossed with sinistral male (dd), all the offsprings of F1 generation (Dd) have dextral coiling.
- If sinistral female (dd) is crossed with dextral male (DD), the offspring have Dd genotype but coiling is sinistral.

- In the above two crosses, the F1 snails have the same genotypes.
- The F1 phenotype is not the same for both crosses.

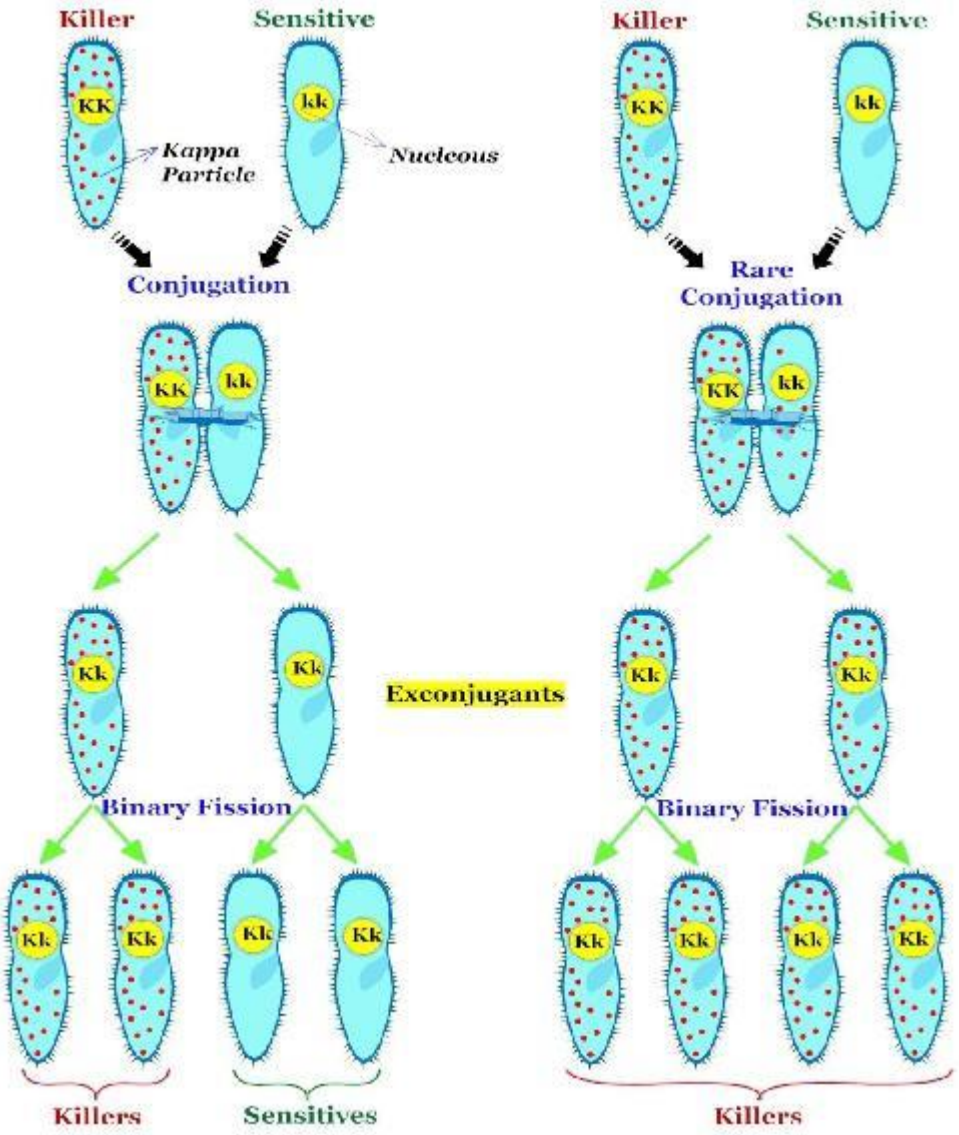


- The coiling phenotype that is seen in the offspring is controlled by the genotype of the mother.
- In the first cross, the offspring has dextral shell because the mother's genotype is DD.
- In the second cross, the offspring has sinistral shell because the mother's genotype is dd.
- Since, zygote receives whole of its cytoplasm from the egg, the direction of shell coiling in the offspring is governed by cytoplasm of the mother.

KAPPA PARTICLES IN PARAMECIUM

- T. M. Sonneborn described the inheritance of some cytoplasmic particles known as kappa and their relation to nuclear gene in the common cillate protozoan, *Paramecium aurelia.*
- There are two strains of Paramecium. They are killer and sensitive.
- Killer strain produces a toxic substance called paramecin that is lethal to other individuals called "sensitives" .
- The production of paramecin in killer type is controlled by certain cytoplasmic particles known as kappa particles. The sensitive strains lack these particles.

- The kappa particles are transmitted through the cytoplasm.
- The existence, production and maintenance of kappa particles
- are controlled by a dominant gene 'K' present in the nucleus. However, 'K' cannot initiate the production of kappa in the total absence of kappa in the cytoplasm.
- When a Paramecium of killer strain is having the genotype "KK" or (K+) conjugates with the Paramecium of non-killer strain having the genotype "kk", the exconjugants are all heterozygous for "Kk" genes.



- The development of a particular type depends upon the duration of cytoplasmic exchange
- oIf conjugation is normal, i.e., lasts only for a short time, and no exchange of cytoplasm takes place between the two, both killers and non-killers
- (sensitive) are produced.
- oHowever in rare or prolonged conjugation (i.e., lasting for long time) the cytoplasmic bridge between the two conjugants is larger. In such cases, in addition to the nuclear material, the cytoplasmic materials are also exchanged.
- oDuring this cytoplasmic exchange, the kappa particles present in the cytoplasm of the killer type enter the non-killer type and convert it into a killer type. So all the offspring produced by the exconjugants are killer type.
- This shows that a Paramecium becomes a killer when it receives kappa particles and it becomes a sensitive when it does not receive kappa particles.

• **Uniparental inheritance** occurs in extranuclear genes when only one parent contributes organellar DNA to the offspring. A classic example of uniparental gene transmission is the maternal inheritance of human mitochondria. The mother's mitochondria are transmitted to the offspring at fertilization via the egg. The father's mitochondrial genes are not transmitted to the offspring via the sperm. Very rare cases which require further investigation have been reported of paternal mitochondrial inheritance in humans, in which the father's mitochondrial genome is found in offspring Chloroplast genes can also inherit uniparentally during sexual reproduction. They are historically thought to inherit maternally, but paternal inheritance in many species is increasingly being identified. The mechanisms of uniparental inheritance from species to species differ greatly and are quite complicated. For instance, chloroplasts have been found to exhibit maternal, paternal and biparental modes even within the same species.

• **Biparental inheritance** occurs in extranuclear genes when both parents contribute organellar DNA to the offspring. It may be less common than uniparental extranuclear inheritance, and usually occurs in a permissible species only a fraction of the time. An example of biparental mitochondrial inheritance is in the yeast, *Saccharomyces cerevisiae*.

Hardy-Weinberg Principle

The **Hardy-Weinberg Principle** (also **Hardy Weinberg Equilibrium**) states that the allele frequency (and genotype frequency) of a population remains constant over generations, unless a specific factor or combination of factors disrupts this equilibrium. Such factors might include non-random mating, mutation, natural selection, genetic bottlenecks leading to increased genetic drift, the immigration or emigration of individuals (gene flow) or meiotic drive. The Hardy-Weinberg Equilibrium does not actually exist in nature because one or more of these factors is *always* in play.

The concept of an equilibrium exists instead as a baseline against which to measure genetic change between generations.
According to the Hardy-Weinberg principle, then, changes in allele frequency (and thus evolution) would be theoretically impossible if the following conditions were met:
1.There was no mutation
2. There were no selective pressures
3. The population size was infinite (this would bring the rate of genetic drift infinitely close to zero)
4. All members of the population were breeding
5. All breeding was random
6. All individuals produced the same amount of offspring
7. There was no migration of individuals into, or out of, the population (i.e. the rate of gene flow was zero)

Representing the equilibrium mathematically:
When considering a locus that has two alleles, **A** and **a:**
The frequency of **A** (the dominant allele) is denoted ***p***
The frequency of **a** (the recessive allele) is denoted **q**
And ***p*** **+** ***q*** **= 1,** since every locus in the population must carry either allele.

If the population is in Hardy-Weinberg equilibrium, then the alleles are distributed evenly among heterozygotes and homozygotes:

Dominant homozygotes (genotype **AA**) are denoted ***p*^2** because the probability of inheriting two dominant alleles is **p*p**

Recessive homozygotes (genotype **aa**) are denoted ***q*^2** because the probability of inheriting two recessive allelesis **q*q**

Heterozygotes (genotype **Aa**) are denoted **2*pq*** because the probability of inheriting both alleles is **(p*q)+(q*p)** And ***p*^2 + 2*pq* + *q*^2 = 1**, since every individual in the population must be one of these genotypes This equation can be used, for example, to predict the frequency of carriers of a disease in a population. Consider the autosomal recessive disease, phenylketonuria. If the frequency of the recessive (in this case, harmful) allele is 1% (***q* = 0.01**), then the number of people who suffer (i.e. who are homozygous recessive) is ***q*^2 = 0.0001** or 0.01% of the population.

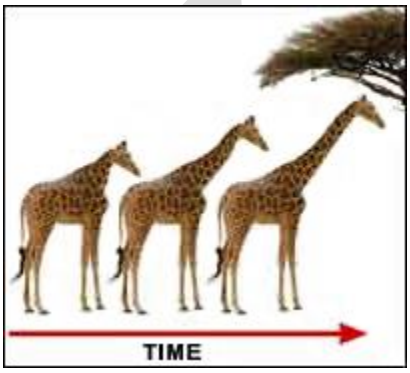
The number of carriers - or heterozygotes - is **2*pq* = 2 x 0.99 x 0.01 = 0.198**, or 1.98% of the population. That means that carriers of the disease exist in the population at a frequency of almost 200 times more than actual sufferers. This can help us to identify the likelihood of one carrier mating with another, and potentially producing an offspring who suffers from the condition. One exception to the Hardy-Weinberg principle is a phenomenon called *recurrent mutation*. When looking at the rate of mutation in a population, forward mutations (i.e. those that cause functional genes to become non-functional) are more common than reverse mutations (i.e. those that cause non-functional genes to restore functionality). This is simply because a forward mutation can involve any number of base changes, while a reverse mutation requires the specific reversal of the change that initially made the gene non-functional. Hence, forward mutations occur at far greater rate than reverse mutations, and will proliferate in a population when they pose no selective disadvantage. This is evident in the proliferation of the O allele (which does not code for a glycosyltrasnferase enzyme) in the ABO blood-grouping system, at the expense of the A and B alleles.

Thus if q to determines the frequency of the non-functional allele, and μ determines forward mutation rate while v determines reverse mutation rate, where μ is taken to be 10 times the value of v:q(equilibrium) = $\mu / (\mu + v)$

NATURAL SELECTION

History of Evolution:

- Plato & Aristotle believed species were fixed & could be arranged according to their complexity
- In the mid eighteenth century, Carolus Linnaeus developed a system of classification that called binomial nomenclature
- George Cuvier, in the eighteenth century, explained changes in the fossil record by proposing that a whole series of catastrophes (extinctions) and re-populations from other regions had occurred giving the appearance of change over time
- Prior to Darwin, it was thought that the world was young & species did not change
- Lamarck (1744-1829) was first to state that descent with modification occurs and that organisms become adapted to their environments
- Inheritance of acquired characteristics was the Lamarckian belief that organisms become adapted to their environment during their lifetime and pass on these adaptations to their offspring
- Lamarck believed that the long necks of giraffes evolved as generations of giraffes reached for ever higher leaves; known as the Law of Use & Disuse



- Because it is supported by so many lines of evidence, evolution is no longer considered a hypothesis
- Evolution is one of the great unifying theories of biology

Darwin's Background & Voyage:

- His nature was too sensitive to become a doctor like his father so he studied divinity
- He attended biology and geology lectures and was tutored by the Reverend John Henslow who arranged his trip on the HMS Beagle
- In 1831, at the age of 22, Charles Darwin accepted a naturalist position aboard the ship HMS Beagle & began a five-year voyage around the world

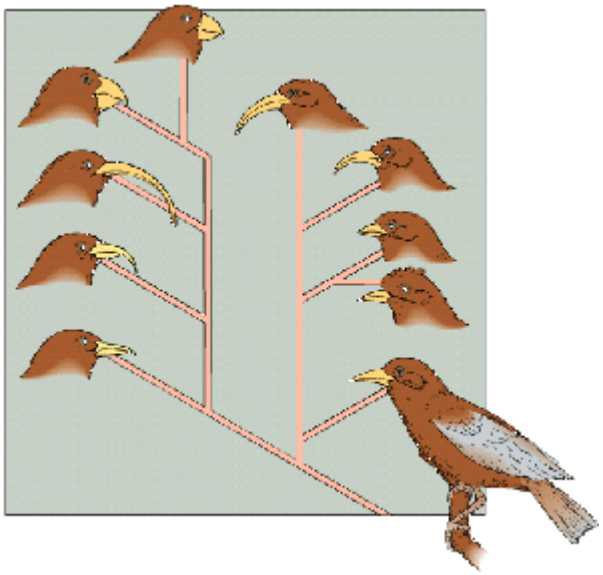
- He read Principles of Geology by Charles Lyell that stated that the observed massive geological changes were caused by slow, continuous processes (erosion, uplifting…)
- Darwin carried this book with him on his voyage as he witnessed Argentina coast earthquakes raising the earth several feet, & marine shells occurring far inland and at great heights in the Andes
- Darwin's many observations led him to the idea that species slowly change over time
- Darwin's comparison of the animals of South America and the Galapagos Islands caused him to conclude that adaptation to the environment can cause diversification, including origin of new species

Examples: Patagonian hares replaced rabbits in the South American grasslands

The Galapagos Islands:Volcanic islands off the South American coast

- Island species varied from the mainland species, and from island-to-island
- Each island had either long or short necked tortoises depending on the island's vegetation

- Finches on the Galapagos Islands resembled a mainland finch, but there were more types
  - Bill shapes are adaptations to different means of gathering

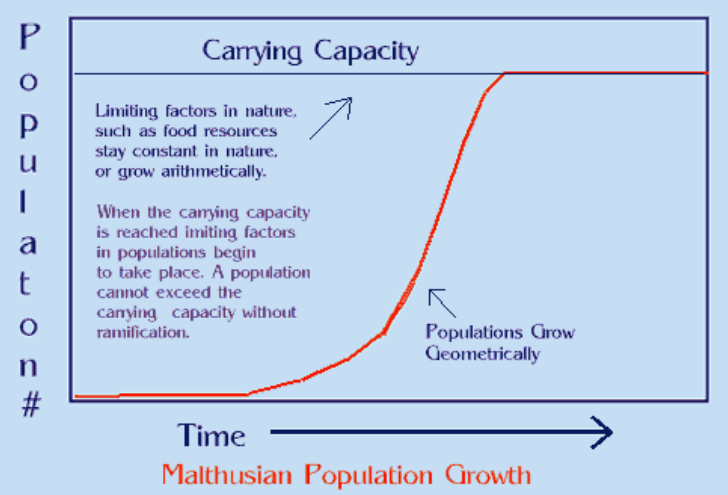


food.

- Galapagos finch species varied by nesting site, beak size, and eating habits

Darwin's Theory of Evolution:

- An adaptation is a trait that helps an organism be more suited to its environment
- Darwin decided adaptations develop over time
- Natural selection was proposed by both Alfred Russell Wallace and Darwin as a driving mechanism of evolution
- Darwin and Wallace both read an essay by Thomas Malthus that proposed that human populations outgrow resources so there is a constant struggle for existence



- Fitness is a measure of an organism's reproductive success
- Organisms most fit to reproduce are selected by environment which results in adaptation of the population
- Natural selection is also called "survival of the fittest"
- Conditions for natural selection include:
  a. Variations exist among members of a population
  b. Many more individuals are produced each generation than will survive
  c. Some individuals are better adapted so they survive & reproduce
  d. Members of a population compete for food, space, mates…
- Variations that make adaptation possible are those that are passed on generation to generation
- Extinction occurs when previous adaptations are no longer suitable to a changed environment

On the Origin of Species by Darwin:

- After the HMS Beagle returned to England in 1836, Darwin waited over 20 years to publish
- Darwin was forced to publish Origin of Species after reading a similar hypothesis by Alfred Russell Wallace

- Both men concluded that life forms arose by descent from a common ancestor, and that natural selection is the mechanism by which species change and new species arise

Fossil Evidence:

- Fossils are relics or impressions of ancient organisms
- Most fossils are found in layers (strata) of sedimentary rock

- The fossil record traces history of life and allows us to study history of particular organisms
- Through radioactive dating, geologists estimate the age of the earth at about 4.6 billion years

- Fossils are at least 10,000 years old and include skeletons, shells, seeds, insects trapped in amber, imprints of organisms, organisms frozen in ice (wooly mammoth), or trapped in tar pits (saber-toothed tiger)
- Transitional forms reveal links between groups (Example: Therapsids were mammal-like reptiles and Pterosaurs were bird like reptiles)

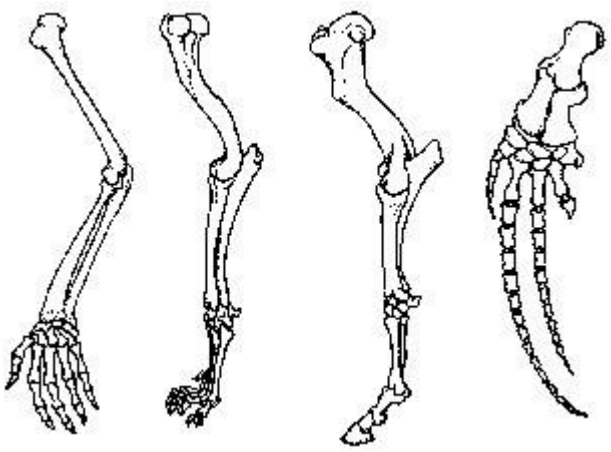
PTEROSAURS

Biogeographical Evidence:

- Biogeography is the study of the geographic distribution of life forms on earth
- Physical factors, such as the location of continents, determine where a population can spread
- Example: Placental mammals arose after Australia separated from the other continents, so only marsupials diversified in Australia

Anatomical Evidence:

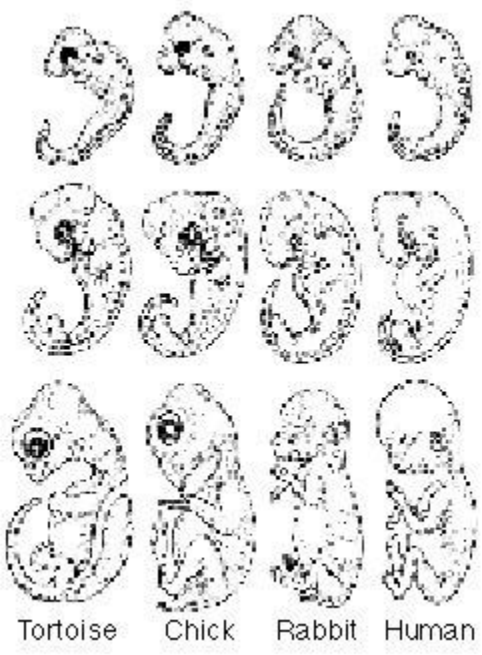
- Organisms have anatomical similarities when they are closely related because of common descent
- Homologous structures in different organisms are inherited from a common ancestor have have similar structures
- Example : Vertebrate forelimbs contain the same sets of bones organized in similar ways, despite their dissimilar functions



- Analogous structures are inherited from different ancestors and have come to resemble each other because they serve a similar function
- Example: Bird wing & bat wing are both for flight but they are structurally different
- Vestigial Structures are remains of a structure that is no longer functional but show common ancestry
- Example: Humans have a tailbone but no tail

Embryological Evidence:

- During development, all vertebrates have a post-anal tail and paired pharyngeal pouches
- Organisms that show similarities in their embryonic development may have a common ancestry



Biochemical Evidence:

- Almost all living organisms use the same basic biochemical molecules, e.g., DNA, ATP, enzymes …
- Similarities in amino acid sequences, DNA codes, etc. can be explained by descent from a common ancestor

Examples of Evolution in Modern Times:

- Peppered moth — light colored vs. dark colored (industrialization influence) Manchester, England
- Insect resistance to insecticides
- Bacterial resistance to antibiotics