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15. Mutation

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Abstract:

Mutation, a crucial component of genetic variation and evolution, pertains to enduring, inheritable alterations in an organism's DNA sequence. Mutations, first detected by Wright in 1791 and later defined by de Vries in 1900, are fundamental to the field of evolutionary biology as they supply the necessary material for natural selection.

Mutations are spontaneous changes in genetic material that can be caused by mutagens. They can vary in their frequency, happening repeatedly, and can have a wide variety of effects, from changes in appearance to being deadly.

These effects can include variations in metabolic processes and replacements of amino acids. Significant historical contributions include Morgan's methodical genetic mutation investigations in Drosophila in 1910 and Muller's Nobel Prize-winning revelation in 1927 that x-rays can cause mutations.

Contemporary applications utilize induced mutations to boost crops, resulting in more than 2000 varieties with improved features like higher yield and resistance to diseases. Although detrimental mutations are prevalent, induced mutation breeding continues to be a crucial method for improving agricultural output. However, it does come with hurdles in terms of identifying mutations and requiring significant resources for screening.

Keywords:

Mutation, Evolution, Variation, Heredity, Pleiotropy

15.1 Introduction:

Mutation, initially observed by Wright in 1791 and further investigated by Hugo de Vries in 1900 and Morgan in 1910, denotes an abrupt and heritable modification in an organism's genetic material, primarily characterized by modifications in nucleotide sequences.

These infrequent, enduring genetic mutations, crucial for the progression and variety of life, function as the main origin of genetic diversity in populations. Mutations can arise from errors in DNA replication or external influences such as radiation.

They play a vital role in supplying the necessary material for natural selection, hence propelling species evolution. De Vries introduced the term "mutation," which played a crucial role in advancing our comprehension of genetic alterations in biological evolution.

A. History

- **1791:** Seth Wright, an English farmer, records the initial documented instance of a mutation after spotting a male lamb with abbreviated limbs.
- **1900:** Hugo de Vries coined the term "mutation" when investigating abrupt heritable alterations in Oenothera. However, subsequent studies revealed that these changes were primarily related to chromosomes rather than gene mutations.
- **1910:** T.H. Morgan initiates a methodical investigation of mutations by analyzing the genetics of a white-eyed mutant in the fruit fly Drosophila.
- **1927:** H.J. Muller made the significant discovery that X-rays have the ability to cause mutations in Drosophila, which ultimately earned him the Nobel Prize in Physiology and Medicine in 1946.
- **1929:** L.J. Stadler conducted an experiment where he showcased the carcinogenic properties of X-rays on barley.
- **1946:** Auerbach and Robson made a significant contribution to the field of genetic mutation studies by uncovering the mutagenic properties of mustard gas and other substances.

15.2 Characteristics of Mutations:

Key characteristics of mutations:

- **Permanence and heritability**: Mutations are permanent, heritable changes in an individual's phenotype, usually due to alterations in the DNA sequence.
- **Mutation frequency**: Spontaneous mutations are rare but can be increased with mutagens. The mutation frequency is calculated as M / (M+N), where M is the number of mutants and N is the number of normal individuals.
- **Variation in mutation rates**: Mutation rates vary among genes. Mutable genes have higher mutation rates, while mutator genes increase and antimutator genes decrease mutation rates of other genes.
- **Direction of mutation**: Mutations often occur from dominant to recessive alleles, but reverse mutations are also possible. Some mutations are inherently dominant.
- **Effects on organisms**: Most mutations are harmful, with only a small fraction being beneficial for crop improvement. They often have pleiotropic effects, influencing multiple traits.
- **Mutation sites**: The smallest unit of mutation is the muton, and genes have varying numbers of mutational sites, including highly mutable 'hot spots'.
- **Randomness**: Mutations occur randomly in any gene and any cell, at any developmental stage.
- **Recurrent nature**: The same type of mutation can recur in different individuals of the same species.
- **Similarity of Spontaneous and Induced Mutations**: There's no fundamental difference in the nature of spontaneous and induced mutations.
- **Role of mutagens**: Physical and chemical agents known as mutagens significantly increase mutation frequency.
- **Variability in induced mutation rates**: The rate of induced mutations varies among genes and is influenced by environmental conditions.
- **Pleiotropy of mutant alleles**: Most mutant alleles affect multiple traits.
- **Forward and reverse mutations**: Mutations occur in both forward and reverse directions, though forward mutations are more common.
- **Irreversible mutations**: Some mutations, often resulting from deletions, do not revert to the original state.

15.3 Classification of Mutations:

A. Direction of mutation:

- **Forward mutation**: Transition from the wild type allele to a mutant allele.
- **Reverse mutation**: Reversion from a mutant allele to the wild type.

B. Cause of mutation:

- **Spontaneous mutations**: Occur naturally without a discernible cause.
- **Induced mutations**: Result from exposure to mutagens.

C. Dominance relationship:

- **Dominant mutation**: mutant allele is dominant over wild type.
- **Recessive mutation**: mutant allele is recessive to wild type.
- **Codominant mutation**: both alleles are equally expressed.
- **Incomplete dominance**: partial dominance of the mutant allele.

D. Tissue of origin:

- **Somatic mutation**: occurs in somatic cells.
- **Germinal mutation**: Occurs in reproductive cells.

E. Effect on survival:

- Categories include lethal, sublethal, subvital, vital, and supervital mutations.

F. Environmental influence on lethal action:

- **Conditional lethals**: lethal under specific conditions.
- **Auxotrophic mutants**: require specific biochemicals.
- **Temperature-sensitive mutants**: affected by temperature extremes.
- **Suppressor-sensitive mutants**: Counteracted by another gene.

G. Type of trait affected:

- **Visible mutation**: alters physical traits.
- **Biochemical mutation**: affects biochemical production.

H. Intensity of character expression:

- **Amorphic mutations**: almost complete loss of function.
- **Hypomorphic mutations**: partial loss of function.
- **Isoallelic mutations**: no effect on trait intensity.
- **Hypermorphic mutations**: Increased expression over wild type.

I. Effect on the function of protein product:

- **Null mutation**: total elimination of gene function.
- **Loss of function mutation**: reduced gene function.
- **Gain of function mutation**: altered gene expression or function.

J. Quantum of morphological effect:

- **Macromutations**: large, distinct morphological changes.
- **Micromutations**: minor changes, often confused with environmental effects.

K. Effect on the expression of neighbouring genes:

- **Polar mutations**: suppress downstream gene expression.
- **Nonpolar mutations**: affect only the mutated gene.

L. Cytological basis:

- **Chromosomal mutations**: changes in chromosome number or structure.
- **Gene mutations**: alterations in dna base sequences.
- **Cytoplasmic mutations**: changes in mitochondrial or chloroplast dna.

M. Molecular basis:

- Subdivided into base substitution, deletion, addition, transposition, and trinucleotide expansion mutations.

N. Type of amino acid replacement:

- **Missense mutation**: replacement of one amino acid.
- **Nonsense mutation**: generates a stop codon, terminating polypeptide growth.
- **Frame-shift mutation**: alters the reading frame of the gene.

15.4 The Spectrum of Genetic Mutants: Classifications and Characteristics:

Genetic mutations produce morphological, lethal conditional, and biochemical mutants. A brief summary of these classes:

- **Morphological mutants:** changes in form, size, shape, and colour. Short-legged sheep, dwarf peas, curly-winged drosophila, and neurospora albino spores are examples.
- **Lethal mutants:** these mutations kill the organism. Some carriers may survive a mutant allele, which can be entirely lethal or semi-lethal/sub-vital.
- **Conditional lethal mutants:** these mutants only exhibit mutant traits under constrained environmental conditions. Under permissive situations, they are normal. This facilitates their growth and analysis under permissive and restrictive conditions.
- **Biochemical mutants:** these mutants lose cellular biochemical function yet can operate normally with particular nutrients. In contrast to the wild type, adenine auxotrophs need exogenous adenine to thrive.

15.4.1 Spontaneous Mutations:

Spontaneous mutations naturally occur at a relatively low rate, ranging from 10^-8 to 10^-10 per nucleotide per generation in bacteria and viruses, and from 10^-7 to 10^-9 in eukaryotes. For prokaryotes and eukaryotes, the mutation rates range from around 10^-5 to 10^-7 and 10^-4 to 10^-6 per gene each generation, respectively. Reverse mutations in prokaryotes are less frequent, typically occurring at a rate that is around one-tenth of forward mutations.

Although eukaryotes often exhibit higher rates of spontaneous mutations, certain genes, such as the waxy locus in maize, are known for their exceptional stability.

One of the main reasons for spontaneous mutations in E. coli is the alteration of DNA bases after synthesis, particularly the methylation of cytosine at position 5. This modification leads to transitions from G.C to A.T, which is a major contributor to mutation hotspots. Transposable elements, present in both prokaryotes and eukaryotes, have the ability to relocate within the genome, which might potentially lead to mutations.

The precise influence of environmental elements, such as UV light and various natural or manufactured substances, in causing spontaneous mutations remains uncertain, but they are believed to be potential contributors.

15.4.2 Induced Mutations:

Mutations that are created by exposure to certain chemical or physical agents, known as mutagens, are referred to as induced mutations. This process is called mutagenesis. This technology, particularly employed in mutation breeding for the improvement of crops, has the potential to greatly increase mutation rates. Muller's observation of a 150-fold rise in mutation rate in Drosophila exposed to X-rays serves as an illustrative case. The extensive utilization of insecticides and antibiotics has resulted in the rise of resilient types of insects and bacteria, showcasing the tangible consequences of induced mutations in both agricultural and medical domains. This phenomenon highlights the ability of organisms to adapt to environmental changes caused by human activities.

15.4.3 Mutagens:

Mutagens refer to physical or chemical agents which greatly enhance the frequency of mutations. Various radiations and chemicals are used as mutagens. Radiations come under physical mutagens. A brief description of various physical and chemical mutagens is presented below:

Sr. No.	**Class**	**Mutagen**
Physical mutagens		
1.	Ionizing radiation	
	a) particulate radiations	• Alpha rays • Beta rays • Fast neutrons • Thermal neutrons
	b) non particulate radiations	• X- rays • Gamma rays
	Non- ionizing radiations	• Ultraviolet rays

Sr. No.	Class	Mutagen
Chemical mutagens		
1.	Alkylating agents	• Mustard gas or sulphur gas • Nitrogen gas • Ehtylmethane sulphonate • Methylmethane sulphonate • Ethylethane sulphonate • N-Methyl-N-nitro-Nnitrosogunanidine
2.	Base analogues	• 5-Bromouracil • 2-Aminopurine
3.	Acridine dyes	• Acriflavin • Proflavin • Acridine orange • Ethidium bromide
4.	Deamination agents	• Nitrous acid
5.	Other chemical mutagens	• Hydroxylamine • Sodium azide • DNA sequences

15.4.4 Physical Mutagens:

Physical mutagens consist of a range of radiations, each possessing unique characteristics and mutagenic impacts:

X-Rays: X-Rays were first discovered by Roentgen in 1895. They have a wavelength ranging from 10^-11 to 10^-7 meters. They have the ability to induce several types of changes in nucleotides, such as addition, deletion, and inversion, by methods such the addition of oxygen to deoxyribose.

Muller first utilized X-rays in 1927 to examine Drosophila, and then, in 1928, Stadler employed them to study plants. Their main mutagenic effect is attributed to the generation of free radicals and ions.

Gamma rays: Gamma rays are similar to X-rays in both physical and biological aspects, although they have shorter wavelengths and more penetrating power. They are generated by the process of radioactive decay of metals such as Cobalt-60.

These particles induce chromosomal and gene changes by expelling electrons. They are currently commonly employed in genetically modifying crop plants.

Alpha particles: Alpha particles are composed of two protons and two neutrons. They have a high ionization density but limited penetrating capability due to their positive charge. They cause chromosomal alterations by ionizing and exciting.

Beta particles: Beta particles are characterized by their low ionization density and higher penetration capability compared to alpha particles. Beta particles, which are produced from the decay of elements such as 3H and 32P, cause chromosomal and gene changes by means of ionization and excitation.

Neutrons (Fast and Thermal): Neutrons possess a high density of ionization and are capable of deeply penetrating materials. Fast neutrons, generated within nuclear reactors, can be decelerated to thermal neutrons by utilizing substances such as graphite. Both types of radiation induce chromosomal fragmentation and genetic alterations, and are employed in mutant breeding, particularly in crops that reproduce asexually.

Ultraviolet photons: Non-ionizing ultraviolet photons, emitted from sources such as mercury vapour lamps, have the ability to penetrate only a few layers of cells. They are mostly employed for the purpose of subjecting microbes, plant pollen, and Drosophila eggs to irradiation. UV radiation induces chromosomal fragmentation and disrupts the synthesis of DNA and RNA by generating pyrimidine dimers.

15.4.5 Chemical Mutagens:

Chemical mutagens are essential in causing genetic changes, with each mutagen having distinct methods and consequences:

Alkylating Agents: Alkylating compounds, such as ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), and ethylene imines, are highly strong mutagens that cause mutations by attaching alkyl groups (ethyl or methyl) to DNA. Alkylation modifies hydrogen bonding, resulting in changes to base pair transitions and transversions. These substances are known for producing effects that are similar to ionizing radiations, and are hence referred to as radiomimetic chemicals. They have the ability to induce substantial modifications in the fundamental composition of DNA, resulting in transversions by causing changes in the size of purines or pyrimidines.

Base Analogues: Base analogues, such as 5-bromo uracil (5BU) and 2-amino purine (2AP), bear a strong resemblance to DNA bases and can be integrated into DNA during the replication process. This integration can result in mutations due to erroneous base pairing. Bromine in 5BU enables a tautomeric shift, leading to erroneous pairing and triggering transitions or transversions. These analogues function by imitating the typical DNA bases but modifying the process of pairing during replication.

Acridine Dyes: Acridine dyes, such as proflavin and acriflavin, have a significant capacity to cause mutations. They interpose themselves amidst DNA base pairs, inducing the addition or removal of base pairs during DNA replication, resulting in frameshift mutations. These colours are frequently employed in mutation research involving bacteriophages, bacteria, and higher species.

Other mutagens: Additional mutagens of significance include nitrous acid and hydroxylamine. Nitrous acid alters the amino groups in cytosine and adenine, resulting in transversions. On the other hand, hydroxylamine is more selective, usually causing guanine-cytosine (GC) to adenine-thymine (AT) conversions. Unlike base analogues, these compounds are categorized as DNA modifiers since they directly interact with the structure of DNA.

15.4.6 Molecular Basis of Mutation:

The primary cause of mutations in genetic traits is modifications in the DNA base sequences, which then lead to changes in the amino acid sequence of proteins.

These modifications can arise through four primary mechanisms:

Base substitution: Base substitution refers to the substitution of one DNA base with another. There are two categories: transitions, which include the replacement of a purine with another purine or a pyrimidine with another pyrimidine, and transversions, which involve the replacement of a purine with a pyrimidine or vice versa. Transitions occur more often because DNA polymerase has lower mistake rates and there is a larger probability of repair. Base substitutions generally have a specific impact on a single amino acid inside a protein. Although typically less damaging, they can have detrimental consequences if they cause nonsense codons, resulting in incomplete and frequently nonfunctional polypeptides.

Base addition and deletion: Base addition and deletion in DNA, often known as insertions or deletions, result in genetic alterations. When the number of bases changed is divisible by three, it can result in the addition or removal of amino acids in a protein without significant consequences. Nevertheless, in the event that the number is not divisible by three, it causes a modification in the reading frame, commonly known as frame-shift mutations, which typically lead to the production of dysfunctional proteins. Frame-shift mutations are frequently more detrimental than base substitutions.

Transposition: Transposition refers to the movement of DNA sequences, which are also called transposable elements, within the genome. Their incorporation into a gene frequently renders it non-functional, resulting in mutant phenotypes. Transpositions have been identified as the cause of numerous classical mutations in species such as Drosophila, maize, and E. coli.

Trinucleotide repeat expansion: Trinucleotide repeat expansion refers to the augmentation of trinucleotide repeats, which are sequences of three nucleotides, within genes. This phenomenon is linked to several genetic illnesses.

15.5 Methodology of Mutation Detection Techniques:

Morphological mutation detection:

- **Observation:** Examine physical traits of organisms or offspring from crosses between normal and irradiated parents.

- **Identification:** Detect mutations based on visible changes in size, color, shape, or other phenotypic attributes.

Molecular mutation detection:

- **DNA Analysis:** Sequence DNA to pinpoint specific nucleotide changes.
- **Detection:** Identify mutations through differences in DNA sequences compared to a reference genome.

Biochemical mutation identification:

- **Enzyme Assays:** Conduct tests to evaluate enzyme activities and metabolic processes.
- **Comparison:** Compare the biochemical activity in normal and mutated specimens.

Prokaryotic mutation detection in bacteria:

- **Cultivation:** Grow bacterial cultures in a nutrient-rich, non-selective medium.
- **Transfer and Growth:** Move cultures to a selective medium where only mutant bacteria can survive and proliferate.
- **Quantification:** Estimate mutation frequency by comparing growth on both media.

Auxotrophic mutation detection via replica-plating:

- **Replica-plating:** Grow bacterial colonies on a master plate, then transfer impressions to a minimal medium plate.
- **Growth observation:** Identify auxotrophic mutants by their inability to grow on minimal medium.

Ames test for histidine-dependent mutants:

- **Culturing mutants:** Grow histidine-dependent mutants on a medium lacking histidine.
- **Chemical testing:** Expose mutants to test chemicals, with and without liver enzymes.
- **Colony counting:** Measure mutation frequency by counting colonies that form in the presence of test chemicals.

15.6 Methodology of Mutation Detection in Drosophila:

CIB Method:

- **Crossing:** Conduct a cross between CIB females (with a paracentric inversion in X-chromosome) and mutagen-treated males.
- **F1 Generation Analysis:** In the F1 generation, males with a normal X-chromosome survive, while those carrying the CIB chromosome do not. Among females, half have the CIB chromosome and half have a normal chromosome.

- **F2 Generation Cross:** Cross F1 females with the CIB chromosome and males with a normal chromosome. The CIB female has one CIB chromosome and one mutagen-treated chromosome.
- **Mutation Detection:** In F2, absence of male progeny indicates the induction of sex-linked recessive lethal mutations in the mutagen-treated Drosophila male.

Muller 5 Method:

- **Initial cross:** Mate a homozygous bar apricot female with a mutagen-treated male.
- **F1 progeny analysis:** The F1 generation yields heterozygous bar females and bar apricot (Muller) males.
- **F1 interbreeding:** Mate the F1 progeny with each other. This produces various combinations of females and males.
- **Mutation identification:** The absence of normal males in the F2 progeny indicates the presence of lethal mutations.

Attached X-Method:

- **Crossing:** Use females with attached X-chromosomes (XXY) and cross them with mutagen-treated males.
- **Progeny Analysis:** The cross produces super females (XX-X), attached females (XXY), mutant males (XY), and YY (which usually die).
- **Mutation Detection:** Surviving males, with an X-chromosome from the mutagen-treated male and a Y chromosome from the attached X-female, are analyzed. The expression of recessive mutations on the X-chromosome can be easily observed.

Curly Lobe-Plum Method:

- **Initial cross:** Mate curly lobe plum (CYL/Pm) females with mutagen-treated males.
- **F1 progeny analysis:** The first generation produces 50% curly lobe and 50% plum offspring.
- **F2 generation cross:** Cross F1 curly lobe females with curly lobe plum males.
- **Further crosses and analysis:** In subsequent generations, examine the progeny for the presence of homozygous curly lobe, which die due to the lethal effect, and other combinations. Mutation is indicated by the presence of progeny with autosomal recessive mutations.

Plant mutation detection:

- **Visible mutations:** Grow plants from mutagen-treated seeds and observe for phenotypic changes.
- **Invisible mutations:** Compare yield or protein content between treated and untreated plant varieties.

Nutritional deficiency method in micro-organisms:

- **Growth in Minimal Medium:** Culture mutated strains on minimal media.

- **Supplementation:** Add specific nutrients to identify deficiencies caused by mutations.

Directed mutagenesis techniques:

- **In Vitro manipulation:** Use methods like restriction enzymes, transposons, and synthetic oligonucleotides to induce specific DNA changes.
- **Result analysis:** Analyze the altered DNA to confirm targeted mutations.

15.7 Applications in crop improvement

- **Development of improved varieties**: over 2000 varieties enhanced for yield, quality, earliness, dwarfness, and disease resistance. examples include wheat (np 836, sarbati sonora), barley (rdb 1), and sugarcane (co 8152, 8153).
- **Trait-specific improvements**: specific improvements like earliness in castor, disease resistance in wheat, and toxin reduction in rapeseed and mustard.
- **Induction of male sterility**: facilitates efficient hybrid seed production, with examples in durum wheat, barley, and cotton.
- **Production of haploids**: accelerates the development of inbred lines for hybrids, particularly in crops like rice.
- **Creation of genetic variability**: broadens the range of traits in crops such as barley, wheat, sugarcane, and potato.
- **Overcoming self-incompatibility**: achieved in species like prunus ovium, aiding in the production of self-fertile plants.
- **Quality enhancements**: nutritional improvements like increased protein in wheat and rice, and higher oil content in mustard.
- **Genetic research and breeding**: provide valuable insights into gene functions and interactions, supporting breeding programs.

15.8 Limitations:

- **Predominance of deleterious mutations**: Most induced mutations are harmful, overshadowing beneficial ones.
- **Identification challenges**: Difficult to pinpoint useful micro-mutations among a vast number of mutations.
- **Extensive screening required**: Due to low frequency of beneficial mutations, large-scale screening of plant populations is necessary.
- **Resource intensive**: The process of inducing and identifying beneficial mutations demands significant resources and time.

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