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Stream	**BIO-GROUP**
Faculty	**SCIENCE**
Department/ Subject	**ZOOLOGY**
Course	ZOOL202- Systematics, Biodiversity & Evolution
Course Duration	IISemester (CBCS)
Sub Topic	**Unit-I:Concepts of different conventional and newer aspects**
Content	The Science of Classification, Binomial System of nomenclature Species, Modern trends in Taxonomy.
Search KeyWords	Chemotaxonomy, cytotaxonomy, ethotaxonomy, molecular taxonomy, DNA fingerprinting, Molecular markers, RFLP, RAPD, & numerical taxonomy.

Biosystematics

Concepts of different conventional and newer aspects-

The Science of Classification/Taxonomy

This study involves naming of organisms (nomenclature) and systematic placing of them into groups (taxa) on the basis of certain relationship between organisms. Though many Greek scholars have studied living plants and animals, the work of Aristotle (384-322 B.C.) stands unique, because he characterized animals according to their actions, way of living, body parts and habitats therefore he is called the "Father of Biological Taxonomy". A more rational approach to the scientific method of classification, particularly on plants was carried out by John Ray (1627-1705). The most remarkable person to give an almost perfect 2-kingdom classification of plants and animals was the Swedish Naturalist, Carlous Linnaeus (1709-1778), rightly called the Father of Taxonomy for his outstanding contribution to systematics. He was the first to introduce the Binomial Nomenclature System, where every plant and animal will have two scientific names, the first word in the genus (where the first letter will be written in capital letter) and second word is the species (all words written in small letters. Example, *Pavo cristatus* (Peacock). He published his scheme of classification in the book entitled *Systema Naturae* in 1753. He strongly believed in the immutability or the fixation of the species.

Binomial system of Nomenclature

The binomial system classifies organisms into groups at various hierarchic levels, on the basis of easily observable and shared morphological features like shape, number and position of limbs etc. in a descending order of group size. As the word binomial suggests, the name of a species is made up of two parts: one indicating the genus and indicating the species. Binomial nomenclature means "two part name" or "system of two part names". The person who popularized this system for use was Swedish Botanist and physician Carlous Linnaeus (1707-1778) who tried to name all things in the natural world and gave every species that he knew a two-part name. This kind of naming had been used before Linnaeus about everybody did. In modern usage, the first letter of the first part of the name, the genus, is always capitalized in writing, while that of the second part is not, even when derived from a proper noun such as the

name of a person or place similarly both parts are italicized when a binomial name occurs in normal text thus the binomial name of the human is *Homo sapiens* in zoology. "*Patella vulgata* Linnaeus, 1758". The name "Linnaeus" tells the reader who it was that first published a description and name for this species of sea snail; 1758 is the date of the publication in which the original description can be found (in this case the 10th edition of the book Systema Naturae). "*Passer domisticus* (Linnaeus, 1758)" The original name given by Linnaeus was *Tringilla domestica*; the parentheses indicated that the species is now considered to belong in a different genus. The ICZN does not require that the name of the person who changed the genus be given, nor the date on which the change was made although nomenclature catalogs usually include such information.

Relationship to classification and taxonomy

Nomenclature (including binomial nomenclature) is not the same as classification, although the two are related. Classification is the ordering of items into groups based on similarities and/or differences; in biological classification species are one of the binds of item to be classified. In principle, the names given to species could be completely independent of their classification. This is not the case for binomial names, since the first part of a binomial is the name of the genus into which the species is placed. Above the rank of genus, binomial nomenclature and classification are partly independent; for example, a species retains its binomial name if it better fits a different genus in the same or different family, or it is split from its old genus and placed in a newly created genus. The independence is only partial since the names of families and other higher taxa are usually based on genera. Taxonomy includes both nomenclature and classification. Its first stage (sometimes called alpha taxonomy) is concerned with finding, describing and naming species of living or fossil organisms. Binomial nomenclature is thus an important part of taxonomy as it is the system by which species are named. Taxonomists are also concerned with classification, including its principles, procedures and rules.

Species

In biology, a species (abbreviated sp., with the plural form species abbreviated spp.) is one of the basic units of biological classification and a taxonomic rank. The scientific system of naming 'kinds' of plants and animals revolves around the species level. The term 'species' is Latin for 'kinds'. Since ancient time; philosophers and naturalists realized the necessity for a basic unit by which biodiversity on this planet may be described and estimated. But the development of a scientific theory of classification is relatively recent phenomenon. Simpson and Mayr have elaborated on the historical developments of taxonomy and its concepts early Greek Philosophers and Naturalist like Hippocrate, Plato and Aristotle also paid attention to biological classification Hippocrates (460-377 B.C.) described types of animals, but there is no indication of useful classification in his work. Plato (427-347 B.C.) was, in the words of Mayr, 'the great antihero of evolution as he believed in essentialism which is also referred to as the theory of forms. Aristotle (384-322 B.C.) was the father of biological classification. As far as evolution is concerned, he gave the idea of ladder of lip a series in which organisms could be arranged in the order of increasing complexity. He studied morphology of animals and also paid attention to embryology, habits and ecology. He emphasized that all the attributes of animals such as living actions habits and bodily parts may be taken into consideration in classification. His idea was also a kind of typological or essentialism as far as species is concerned. Linnaeus (1707-1778), a great taxonomist and sometimes called the 'father of taxonomy', adhered to downward classification. His thinking was that of an essentialist for whom species reflects the

existence of fixed, unchangeable type (essence). He proposed binomial nomenclature. The typological definition of species based on the concept of Linnaeus is called essentialist species concept. Occam and his followers suggested that nature produces individuals and nothing more, and species has no actual existence in nature; it is only a mental concept. It is the basis of nominalistic species concept which was popular in France in the eighteenth century. A particular species concept is associated with a definition and definitions differ in different concept of species. It may be mentioned here that nearly all of the older definitions of the species, including those of Buffon, Lamark and Cuvier refer to the morphological similarities of individuals of the same species.

An entirely new species concept has begun to emerge in the seventeenth century. Ray believed in the morphological definition of species and his species characterization also contained the germ of biological species concept, which considers the reproductive relationship to be a principle species criterion. As early as 1760, Koelreuter mentioned that all the individuals which are able to interbreed and produce fertile progeny belong to the same species. Hundred years before Darwin, Buffon in his Historie Naturelle describes everything known in the natural world and believed in organic change but did not provide any mechanism to explain the evolutionary change. Although initially he believed in morphological species concept, Buffon prepared the way for biological species concept using sterility barrier (instead of morphological similarities) as species criterion later on, the biological species concept was developed due to contribution of Merrem, Voigt, Walsh and many other naturalist and taxonomist of the Nineteenth century.

The biological species concept was clearly formulated by Jordan, Dobzhansky and Mayr. According to Mayr a species is a group of potentially or actually interbreeding natural population which are reproductively isolated from other such groups. However, Dobzhansky, being an evolutionary geneticist defined species as a reproductive community of sexually and cross-fertilizing individuals which share a common gene pool. The biological species concept is the most widely accept, but it has certain difficulties in its application. Since biological species concept is applicable to non –dimensional situation, Simpson, faced with the problems of studying the evolutionary species concept in which a species is alineage (an ancestral-descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies. Darwin explains the mechanism of evolution in his book Origin of species and his theory has two components:

i) descent with modification- all species living and extinct have descended from one or a few original form of pre-existing species, and
ii) natural selection as casual agent of evolutionary change.

Darwin also recognized that species not only evolve but also divide. Darwin unquestionably had adopted a biological species concept ion the 1830s even though later he gave it up. He did not define species but appear to have a morphological concept of species which was central to his theory of natural selection. According to Darwin, the term species is arbitarly used for sake of convenience to a set of individuals closely resembling each other and it does not differ from the term ‘variety’ which is given to less distinct and more fluctuating forms. Probably Darwin believed that the concept of species is unnecessary because gradual evolutionary changes can account for the diversity of life. In his article a number of species concepts, including those which have been rejected and are also of historical significance, have been described. Further, various modes of speciation have also been discussed with suitable examples.

Concept of Species

Species concepts originate in taxonomy in which species is the basic unit of classification according to the international commission of Zoological nomenclature. Survey of taxonomic literature shows that there are a large number of species concepts which have been suggested by naturalists, taxonomists and evolutionary biologists from time to time. There are more than 20 species concept which are listed below:

Agamospecies: Asexual lineages, uni-parental organisms (parthenogens and apomicts) that cluster together in term of their genome, may be secondarily uni-parental from bi-parental ancestors.

Biological Species: Mendelian population of sexually reproducing organisms, interbreeding natural populations isolated from other such groups, depending upon reproductive isolating mechanisms.

Cladistic Species: Set of organisms between speciation events or between speciation and extinction events, or a segment of a phylogenetic lineage between modes.

Cohesion Species: Evolutionary lineage bounded by cohesion mechanisms that causes reproductive communities, particularly genetic exchange and ecological interchangeability.

Composite Species: All organisms belonging to an internodon and their descendents until a subsequent internodon (internodon is a set of organisms whose parent-child relations are not split.

Ecological Species: A lineage which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range.

Evolutionary Species: A lineage (ancestral- descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies.

Evolutionary significant unit: A population (or group of population) that is substantially reproductively isolated from other conspecific population units and represents as important component in the evolutionary legacy of the species.

Geological concordance: Population subdivisions concordantly identified by multiple independent genetic units constitute the population units worthy of recognition as phylogenetic taxa.

Genetic Species: Group of organisms that may inherit characters from each other, common gene pool, reproductive community that forms a genetic unit.

Genotypic cluster definition: Clusters of monotypic or polytypic biological entities, identified using morphology or genetics, forming groups that have few or no intermediates when in contact.

Hennigian species: A tokogenetic community that arises when a stem species is dissolved into two new species and ends when it goes extinct or speciates.

Internodal species: Organisms are conspecific in virtue of their common membership of a part of a genealogical network between two permanent splitting events or a splitting event and extinction.

Morphological species: Similar to typological species concept of Linnaeus; species are the smallest groups that are consistently and persistently distinct and distinguishable by ordinary means.

Nominalistic species: Only individuals exist and nothing more. Species have no actual existence in nature.

Non-dimensional Species: Species delimitation in a non-dimensional system (a system without the dimensions of space and time).

Nothospecies: Species formed from the hybridization of two distinct parental species, often by polyploidy.

Phenetic species: A cluster of characters that statistically co-vary, a family resemblance concept in which possession of most characters is required for inclusion in a species, but not all. A class of organisms that share most of a set of characters.

Phylogenetic species: A species is the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent.

Recognition species: A species is that most inclusive population of individuals, biparental organisms which share a common fertilization system.

Reproductive competition species: The most extensive units in the natural economy such that reproductive competition occurs among their parts.

Successional species: Arbitrary anagenetic stages in morphological forms, mainly in the palaeontological records.

Taxonomic species: Specimens considered by a taxonomist to be a member of a kind on the evidence or on the assumption that they are as alike as their offspring of hereditary relatives within a few generations. Specifications of various species concepts mentioned above have been taken from Wilkins. Mayr and Ashlock have stated that 'The taxonomic literature reports innumerable species concepts, but they fall into four groups. The first two have mainly historical significance but are still upheld by a few contemporary authors'. These groups are: (i) typological species concept, (ii) nominalistic species concept, (iii) biological species concept and (iv) evolutionary species concept.

Typological species concept

This concept was proposed by Linnaeus and his followers and before that Plato and Aristotle also believed in this. The term 'Eidos' coined by Plato is also related to this. In nature, there are limited numbers of types or universals and members of a species form a class. It is also referred to as essentialism and the definition of species based on this concept is also called essentialist species concept. It is based on the degree of morphological differences used by the taxonomists. Under this concept, each species is entirely constant through time and thus the concept does not allow any change in a particular species. Since it is known that there are individual variations within the species and different species may be morphologically identical as in the case of sibling species (morphologically indistinguishable but reproductively isolated), the essentialist species concept has been rejected.

Nominalistic species concept

Occam and his followers did not believe in the existence of universals or types and for them only individuals existed and species had no real existence. This species concept was popular in France in the 18th century. According to Bessey, nature produces only individuals and nothing more. Species is merely a mental concept. But it is known that species are not human constructs. So this species concept has also been rejected.

Biological species concept

Jordan , Dobzhansky and Mayr clearly formulated the biological species concept. Mayr defined species as a group of potentially or actually interbreeding natural populations which are reproductively isolated from other such groups. Dobzhansky , being an evolutionary geneticist, added the term gene pool, and defined species as a reproductive community of sexually and cross-fertilizing individuals which share in a common gene pool. The members of a species form a reproductive community, an ecological unit and a genetic unit. These three properties (reproductive community, ecological and genetic units) show that species cannot be defined by

the typological or nominalstic concepts. The biological species concept is the most widely accepted, but there are three main difficulties in its application: insufficient information, uniparental reproduction and evolutionary intermediacy. With regard to insufficient information in a particular species, there are individual variations due to sexual dimorphism, age differences, polymorphism and other types of morphological changes, but these difficulties may be overcome through a study of life histories and analysis of natural populations. In the biological species concept, interbreeding among the individuals of the same species and reproductive isolation from other species are the principal criteria. During the process of sexual reproduction, recombination of genetic materials takes place between parental individuals which leads to new combinations of genes in the progeny. However, there are many examples which do not come under this category such as hermaphroditism, automixis, parthenogenesis, gynogenesis and vegetative reproduction which show uniparental reproduction. There are numerous examples of such uniparental reproduction in invertebrates and vertebrates. Mayr has given a new terminology to such uniparental lineages, i.e. paraspecies, but Grant has designated such cases as agamospecies. Any terminology may be given to such cases, but they may not be considered as subdivision of biological species because they are quite different from biological species. There is difficulty in the application of biological species concept in those situations in which speciation is incomplete (evolutionary intermediacy).

The species as a reproductive community exists in nondimensional situation of a deme. As soon as it extends in dimension of space and time, the stage is set for incipient speciation. The populations may be found in the process of becoming new species which have not yet acquired the characters of entirely new species. It is difficult to assign any stage to such populations, particularly when morphological distinctness is not correlated with the acquisition of reproductive isolation. Further, there may be acquisition of reproductive isolation without the development of equivalent morphological change. Numerous difficulties may be faced by the taxonomists for such cases of evolutionary intermediacy. There are several examples of such situations which are consequences of the gradual nature of the speciation process. It is difficult to assign species status to a given population in these cases of evolutionary intermediacy. This temporal inextensibility of biological species concept makes it non-evolutionary because of its non-dimensional character. Mayr has explained this limitation by stating that ‘the species concept has its full meaning only where populations belonging to different species come into contact. This takes place in local situations without the dimension of space (geography) and time. The function of the species concept is to determine the status of co-existing individuals and populations.’

Evolutionary species concept

Because of non-dimensional character of the biological species concept, some palaeontologists are not satisfied with biological species definition. Their argument is that the species definition must involve evolutionary criteria. Simpson1 proposed the evolutionary species concept and defined the species as a lineage (an ancestral–descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies. Mayr has criticized the evolutionary species definition saying that it is the definition of a phyletic lineage, but not of the species. It is also applicable to incipient species or isolated populations. Further, it ignores the core of the species problem and tries to delimit species taxa in the time dimension. Wiley attempted to make certain improvement in evolutionary species concept by suggesting that no presumed separate, single, evolutionary lineage may be subdivided into a series of ancestral and descendent species. But this definition is of species taxon and not of

species category. Thus Mayr did not accept the evolutionary species concept and he strongly advocated for the biological species concept in spite of certain difficulties in its application.

Modern Trends in Taxonomy

Taxonomy in Science and method of naming organisms is a fundamental basis for all biological Science and its application. The principal task of taxonomy is to describe, establish and give an account of the order that is an inherent property of biological diversity. The order of names provided by taxonomy is arranged as a hiearchial classification, which is considered to potray the hiearchy of species and more inclusive taxa as a result of the continuous chain of species splitting in the evolutionary history of life on earth. Generalizations on organisms as a basic principle in biology are only possible if the infinite number of items in Science is classified statements about the overwhelming diversity of nature would be impossible without methods for bringing order to this diversity .The world's biota is a vast library of information concerning any aspect of life and taxonomy is the cataloguing system that everybody must use to access its information .All kinds of biological Science and application link their specific data to species names and use these names for effective communication.

As Longino (1993) has paraphrased "taxonomy is the raw material from which hypothesis of phylogeny are derived". All kinds of comparative biology rely on sound phylogenetic hypotheses immediately depends on the reliability of the underlying taxonomic data. Moreover society has an increasing need for reliable taxonomic information in oder to allow to manage and understood the world's biodiversity .Until recently , taxonomy was confronted with what Godfray called a new bioinformatics crisis evidenced " by a lack of prestige and resources that is crippling the continuing cataloguing of biodiversity ". Current biological taxonomy quite successfully adopt methods ,data structure and other demands of techniques and theories invented by new entrant to the biological Science as the fields of molecular biology .However ,all other useful sources of information are simultaneously gathered in modern taxonomy and this multicharacter integrative approach has been called integrative taxonomy . It allows taxonomists to create new common visions to meet changing demands of a changinh global view on global diversity and threats to it.

Cytotaxonomy

It is the branch of biology dealing with the relationship and classification of organism using comparative studies of chromosomes. The structure, number and behavior of chromosomes is of great value in taxonomy, with chromosome number being the most widely used and quoted character. Chromosome numbers are usually determined at mitosis and quoted as the diploid number(2n), unless dealing with a polyploidy series in which case the base number of chromosomes in the genome of the original haploid quoted.

Another useful taxonomic character is the position of the centromere. Meiotic behavior may show the heterozygosity of inversions. This may be constant for a taxon, offering further taxonomic evidence. The cytotaxonomy is more significant over physiological taxonomy because cytotaxonomy is dealing with the comparative study of chromosome and with this method minute variation among the individuals among the individuals can be detected. DNA are present in the chromosome and the variation in DNA are responsible for the variation among the individuals, species, genus and so on. The difference in physiological variation are too less among the individuals of same species and other higher taxa.

Bioacoustic tools: Sonotaxonomy

Field identification methods involving acoustic sampling for taxa such as birds, frogs, and crickets, and visual sampling based on diagnostic morphological characters are rapid and

inexpensive, and they can be developed to be accurate (Riede, 1993). Morphological characteristics often are found insufficient for the identification of cryptic species. Several cryptic species of anurans display a high level of morphological similarities that often make them virtually impossible to distinguish on the basis of morphological parameters. The taxonomic status of some very poorly known groups of frogs of the family Dicroglossidae from the central Aravalli ranges of Western India and the family Microhylidae from the southern parts of India, is assessed by means of acoustic and statistical analyses of differences in temporal parameters of advertisement calls, such as the number of pulses and the call duration, as well as a spectral parameter, dominant frequency, harmonics, peak frequency, amplitude, and power, etc. As these species usually are misidentified or ignored because of their taxonomic complexity in both ecologically diversified regions, we have found bioacoustical diagnosis for each species in order to facilitate identification in the field. Differences in acoustic parameters support the specific status of Sphaerotheca breviceps, S. rolandae, Microhyla ornata, and M. rubra. Populations from these distinct biodiversity regions can be recognized by distinctive advertisement calls, usually corresponding to a recognized species. The individuals of family Dicroglossidae (S. breviceps and S. rolandae) and Microhylidae (M. ornata and M. rubra), being sympatric species, show great similarities in their morphological characteristics as well as eco-biological needs, but their advertisement call characteristics analyzed using sound analysis softwares, viz., Raven, Avisoft, and Sound Ruler, are very different and species-specific, and they are very useful, particularly in field identification and monitoring (Sharma, 2005). Furthermore, identification and monitoring of species using bioacoustics tools is a humane approach that avoids unnecessary killing of animals. 4.1 Sound analysis system. The call analysis system includes the following steps: Transmitter > Medium (air) > Receiver. The transmitter, e.g., a male frog, emits the sound, which is transmitted through the medium, usually the air, as longitudinal pressure waves. The receiver processes the sound and presents the waves as visual spectrograms that are used for the identification of species. The system of call analysis includes: 1. Recording 2. Storage and conversion into a proper format 3. Generation of a spectrogram 4. Analysis of spectral pattern and development of classifiers Investigation of animal sounds includes signal recording with electronic recording equipment. Due to a wide range of signal properties and media they propagate, specialized equipment is used instead of the usual microphones.

Video cameras are used for the confirmation of the call of a particular species. A sound bank is prepared to store calls in a format applicable to the software. Specific computer programming is designed for the storage and analysis of recorded data, and specialized sound analyses are used for describing and storing signals according to their intensity, frequency, duration, and other parameters. Before the analysis of an unknown call the software is calibrated with the help of audio-frequency generators. All the sound signals are analyzed on the same frequency and timescale to ensure that the recorded sound belongs to a different species or the same species. Data collection involves two main components: sampling followed by processing. Sampling depends on rate and resolution. Rate should be greater than twice the highest frequency to be sampled. Resolution depends on processor intake, i.e., 8-bit or 16-bit. The resolution of 8-bit code is 256 combination steps and that of 16-bit code is 65,536 combination steps. Most graphical display devices present sound as a time domain feature. The time domain display of sound waves has limitations in analysis. Frequency domain representatives of spectrum are an improved display system achieved by Fourier transform that provides better opportunities for sound analysis. The Fourier transform is a mathematical function that converts the time domain forms of a signal (produced by most measuring and graphical display devices)

to a frequency domain representation or spectrum. The input to the DFT is a sequence of digitized amplitude values (xo. x1. x2. …. xN- 1) at N discrete points in time. The output is a sequence of amplitude values ((Ao. A1. A2. … AcNl2)-1) at N/2 discrete frequencies. The highest frequency, (Nl2)-1, is equal to half the sampling rate (=1/ (2T)), where T is the sampling period. The output could be plotted as a magnitude spectrum. Frequency composition of a signal changes over time and can be plotted as a sound spectrogram using spectrum generation and analysis software (Avisoft SAS PRO, Raven 1.4, Sound Ruler). The spectrograms produced by sound plot have frequency on the vertical axis versus time on the horizontal; the amplitude of a given frequency component at a given time is represented by color combinations as per the parameters of sub-menus of the software. 4.2 Microscale analysis of calls Furthermore, microanalysis of a spectrogram could be achieved by slicing the spectrogram (Raven 1.4). A spectrogram slice view is a plot of relative intensity versus frequency at a particular point in time within a signal. A spectrogram slice represents a vertical cross section through a spectrogram at a single time, but rotated 90° so that the frequency axis is horizontal. In fact, a spectrogram is built of a series of spectrogram slices stacked side by side (with their frequency axis running vertically). Whereas a spectrogram view shows a series of slices at successive points in time and represents power at each frequency by a color (by default, grayscale) value, a spectrogram slice view shows only one slice and represents power at each frequency on a line graph. Using a sound ruler, pulse rate, call rate, dominant frequency, fundamental frequency, etc., are recorded. Classifiers and filters are used to sort particular elements or symbols of a call. Generally, sound spectra are discrete in most species, and such diagnostic characteristics of the spectral pattern can be used for the identification of a species. Sound-based identification, classification of call, and spectral parameters can be developed as a strong tool in taxonomy as sono-taxonomy. Sonotaxonomy may be used independently for the identification and categorization of various taxa or as a supporting tool to the conventional taxonomy.

Chemotaxonomic Classification:

Nature which consists of so many variabilities of living components of the environment possesses useful, harmful and inactive chemical constituents. The classification based on these chemical constituents is known as chemotaxonomy. All the living components of the environment produce secondary metabolites that are derived from primary metabolites. The chemical structure of the secondary metabolites is often specific and restricted to taxonomically related organisms. The classification of plants on the basis of specific class of secondary metabolites and their biosynthetic pathways constitutes chemotaxonomy. Its study is helpful to taxonomist, phytochemists and pharmacologists to solve selected taxonomical problems. The phenolics, alkaloids, terpenoids and non-protein amino acids, are the four important and widely exploited groups of compounds utilized for chemotaxonomic classification. These groups of compounds exhibit a wide variation in chemical diversity, distribution and function. The system of chemotaxonomic classification relies on the chemical similarity of taxon. Three broad categories of compounds are used in chemotaxonomy: primary metabolites; secondary metabolites and semantices.

Ethnobotany as an ethnoscientific discipline

This approach also consists of a union of ethnobotany with anthropology, but there is a difference. The aforementioned approaches study the relationships between people and plants without necessarily considering the minds of the people themselves about their culture. A study ruled by previous approaches, for example, could select useful plants within a human group and identify and classify these plants from a scientific point of view. However, a study using the

ethnoscientific approach could verify the way the people of a culture themselves identifies and classifies the plant resources of the environment. Here, ethnobotany relates to the ethnosciences and can be described as a line of research that studies the understanding of people about their own culture. Thus, ethnobotanical studies from this approach seek to understand how people name and classify the plants in the environment from their own classificatory logic. This type of ethnobotanical study became known as studies of folk classification, ethnotaxonomy, or even folk taxonomy.

Ethnobotany as an integrative or synthesis science: According to the three previous approaches, ethnobotany studies the relationship between people and plants. However, they differ in the sense that the research mainly focuses on one of these two components of the relationship (people or plants). In the first approach (of ethnobotany as a field of botany), for example, the focus of research is the useful plant; in the second approach (the meeting of ethnobotany with ethnography), the focus is on culture, that is, on the cultural aspects that can be described from the useful plants; in the third approach (the meeting of ethnobotany with ethnoscience), the focus is to understand the way the people belonging to a particular culture apprehend the plants they use.

Molecular Taxonomy

Molecular Taxonomy is the classification of organisms on the basis of the distribution and composition of chemical substances in them. Molecular techniques in the field of biology have helped to establish genetic relationship between the members of different taxonomic categories. DNA and protein sequencing, immunological methods, DNA-DNA or DNA-RNA hybridization methods are more informative in the study of different species. The data obtained from such studies are used to construct phylogenetic trees. Fitch and Margoliash, (1967) made first phylogenetic tree based on molecular data .This tree was so close to the already established phylogenetic trees of the vertebrates that the taxonomists realized significance of molecular data and this made them understand that other traditional methods are although important but molecular evidences could be final or confirmatory evidences.

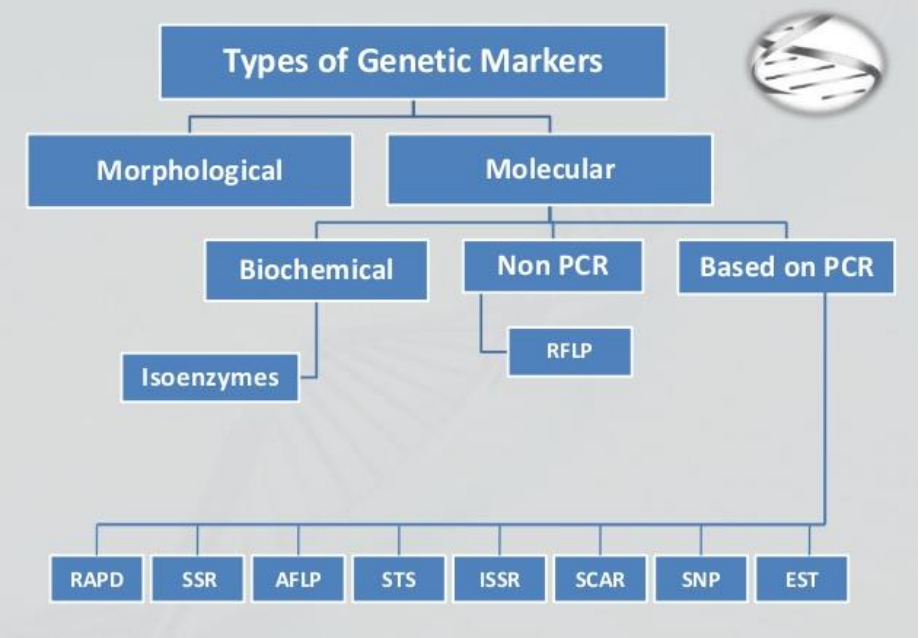
Protein based markers

Iso -enzyme s and allozymes were first discovered by R L Hunter and Clement Markert in 1957.

Both these two variables are now used as interchangeable eg. Lactate dehydrogenase (LDH) chr.-12 and chr-15, malate dehydrogenase, glucose phosphate and glucokinase etc.

Alloenzymes are common biological enzymes that exhibit high levels of functional evolutionary conservation throughout specific phyla and kingdoms. They are used by phylo-geneticists as molecular markers to gauge evolutionary histories and relationships between different species.

A Allozyme electrophoresis is a method which can identify genetic variation at the level of enzymes that are directly encoded by DNA protein variants and they will differ slightly in electric charge. Allozyme provides us a data of single locus genetic variations which can answer many questions.



Steps in Allozyme analysis

1.Extract allozyme from tissues using standard specific protocol.

2.Then the variation is detected through electrophoresis in an acrylamide or in cellulose acetate gel.

3.Individuals that are homozygous show a single band where as heterozygous individuals show two bands.

4. it is a codominant Mendelian character

Steps in Allozyme analysis

Extract allozyme from tissues using standard specific protocol.

Then the variation is detected through electrophoresis in an acrylamide, starch gel or in cellulose acetate gel.

The protein bands obtained are observed carefully. Individuals that are homozygous show a single band where as heterozygous individuals show two bands.40-50 individuals can be analyzed per gel.

It is a co-dominant Mendelian character.

Molecular markers are versatile tools in various fields other than taxonomy like physiology, embryology, genetic fingerprinting etc.

Molecular phylo-genetics and systematics have been found to be greatly promising in recent years, due to the development of new and diverse method.

Molecular taxonomic approaches permit an exact and rapid method of distinguishing specimens based on their interspecific variations. These methods allow estimation of the genetic variability of the biota carrying to a super-estimation on the global biodiversity besides the relationships among

Isozyme

Isozymes are protein markers.

Isozyme staining is a kind of activity staining – a molecular marker technique based on the principle that allelic variation exists among many proteins which perform same enzymatic function but the electrophoretic mobility of the proteins may differ (depending on their respective molecular weights); therefore they migrate to different extents in a starch or polyacrylamide gel. In this technique, a crude protein extract is made from some tissue sources and separated by electrophoresis in a solution containing reagents required for the activity of the enzyme being monitored. The solution contains a dye that the enzyme can catalyse into a colour reagent that stains the protein. The allelic variants of the protein can be visualized in the gel. Isozyme markers are much used in molecular taxonomy as the technique is easy to

perform, is cost-effective, less time-consuming, and gives a vivid representation of gel electrophoresis band patterns, as far as studies on taxonomic diversity.

Mitochondrial DNA marker

Mitochondrial DNA is non nuclear, remain present within mitochondria.

Mt DNA is maternally inherited with haploid genome

The entire genome undergoes transcription as one single unit. They are not subjected to any recombination and hence they are homologous marker .

They are selectively neutral, occurring in multiple copies in each cell.

Mt DNA is physically separated from the rest of the cells DNA and so it is relatively easier to isolate from any tissues or blood samples.

Due to maternal inheritance of Mt. DNA, the effective population size is smaller than nuclear DNA and so Mt DNA variation is more sensitive to population bottle neck and hybridization.

The difference in the nucleotide sequence of the DNA molecule in mitochondria can be determined directly or indirectly by several methods like-RFLP.

The newly emerged sequencing technologies have enabled direct sequencing of Mt DNA and several sets of universal primer have been developed from conserved sequence region. Slow evolved gene regions are constantly being used for interspecies comparison while fast evolving gene region are used for population comparison eg. D-loops

The only non coding region of Mt DNA is D-loop region which is fast evolving gene region and hence mostly used for population comparison. Besides the Cyt.b and ND-1and ND-5/6gene regions are also being used.

Mit cytochrome c oxidase I gene (CO I gene) has been identified as universal barcode for species level identification due to its conserved nature across a wide range of taxa.

Signature sequence and its importance in tracing phylogeny among prokaryotes

The rRNA molecule has been described as ultimate „Molecular chronometer" enabling classification across all major taxonomic Classes.

Why rRNA molecule is considered as molecular chronometer or tool for molecular taxonomy?

Ubiquitous occurrence in all living beings 2. Functionally constant i.e. conserved over time (evolution of 16SrRNA is very slow). 3. Relatively smaller in size (50 helical stalk). 4. Having carrier regions which mutate at different rates. 5. Can easily be multiplied/amplified/sequenced and compared.

Why bacterial 16SrRNA used as a molecular clock in prokaryotes ?

The bacterial 16S rRNA has a length of 1650 nucleotides, found on smaller ribosomal subunits (30S) of prokaryotic ribosomes. Since both mitochondria and chloroplast have their own ribosomes. So it is found in all three kingdoms.

The sequence of 16S rRNA has been divided into distinct areas according to their degree of variability among different taxonomic groups.

Actually the different areas of on rRNA molecule have a different mutation rate. Highly conserved region therefore be used to group bacteria into higher taxonomic order, where as more variable regions allow classification at lower taxonomic level such as genes or species level.

In recent times two organisms that differ only by a few bases have diverged more recently in evolutionary time than organism that differs by more bases.

Random Amplified Polymorphic DNA (RAPD)

Random amplified Polymorphic DNA(RAPD) markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence.

RAPD analysis is a PCR based molecular marker techni1que. Single short oligonucleotide primer is arbitrarily selected to amplify a set of DNA segments distributed randomly through out the genome (anonymous loci).

RAPD uses random primer to generate multiple PCR products resulting in a fingerprint for a particular species. It is very fast, cheap and show very high amount of polymorphism and the marker does not require the prior

Amplified fragment length polymorphisms (AFLPs) provide an effective means of genotyping, particularly when little is known about the genome or genetics of an organism. It involves ligation of adaptors to digested DNA followed by PCR amplification using primer that are primarily adaptor and partially gene specific.

They may combine the benefit of both RAPD and RFLP. The total genomic DNA is digested using two restriction enzymes.

Restriction enzymes cut the DNA and double stranded nucleotide adaptors are ligated to the ends of the fragments to serve as primer biding site for PCR amplification.

Specific DNA markers in Molecular taxonomy: Polymerase chain reaction (PCR)

Fragments are then amplified using PCR and the presence or absence of their varying lengths can then be visualized on polyacrylamide gel or capillary-based platform. Thus genetic polymorphism is studied.

Polymerase chain reaction (PCR) is a method used widely in molecular biology to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

PCR was invented in 1983 by the American biochemist Kary Mullis. It is fundamental to much of genetic testing including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series or cycles of temperature changes.

Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis**; construction of DNA-based phylogenies**, or functional analysis of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA;] analysis of genetic fingerprints for DNA profiling (for example, in forensic science and parentage testing); and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

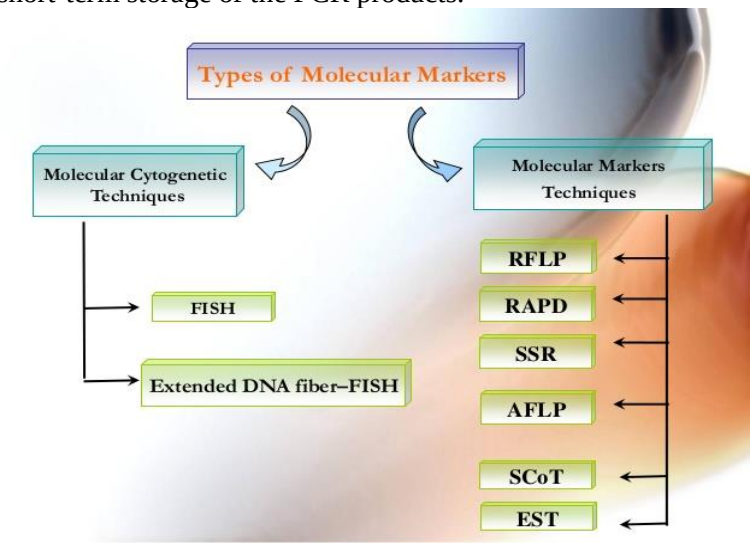
A basic PCR set-up requires several components and reagents, including:a DNA template that contains the DNA target region to amplify, a DNA polymerase(heat-resistant Taq polymerase),two DNA primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target, deoxynucleoside triphosphates, or dNTPs (sometimes called "deoxynucleotide triphosphates"; buffer solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase and bivalent cations, typically magnesium (Mg) or manganese (Mn) ions; Mg2+ each cycle commonly consisting of two or three discrete temperature steps. The individual steps common to most PCR methods are as follows:

Initialization: This step is only required for DNA polymerases that require heat activation by hot-start PCR. It consists of heating the reaction chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if extremely thermostable polymerases are used, which is then held for 1–10 minutes.

Denaturation*:* This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

Annealing: In the next step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand. The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies. The formula used to calculate the number of DNA copies formed after a given number of cycles is 2n, where n is the number of cycles. Thus, a reaction set for 30 cycles results in 230, or 1,073,741,824, copies of the original double-stranded DNA target region.

***Final elongation**:* This single step is optional, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.
Final hold: The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products.



DNA Fingerprinting Techniques

Restriction fragment length polymorphism (RFLP): This technique is based upon the polymorphic nature of restriction enzyme digestion sites within a defined genetic region. RFLP involves restriction digestion of the chromosomal DNA followed by southern blotting for detection of specific genetic loci. Whole chromosomal DNA is restriction digested, fragments are then separated on agarose-gel and transferred on to the nitrocellular / nylon membrane. The DNA fragments immobilized on the membrane are then hybridized with one or more labeled probes. Only the genomic DNA fragments, which will have specificity for the probe, will be detected, simplifying the restriction endonuclease analysis greatly. This differentiates the strains as the position and length of target sequences will vary in the fragments of different isolates. RFLP has been used to subtype *Brucella* sp. (Grimont *et al.*, 1992), *Legionella pneumophila* (Tram *et al.*, l990), *Pseudomonas* (Loutit *et al.*, l99l), *Mycobacterium tuberculosis* (van Embden *et al.*, 1993). Ribotyping is also a form of RFLP typing in which the probe's target is in a multicopy operon i.e. the *rrn* operon. Ribotyping is a particular form of RFLP- based typing in which probe's target sequence is the multicopy *rrn* operon. *rrn* operons are highly conserved and most bacteria have multiple copies of these, permitting both inter- and intraspecies discrimination. Also intergenic spacer regions (the region present between 16S and 23S regions) are variable in length among different copies of the *rrn* operon and thus this property can also be exploited for typing. Use of multiple restriction enzymes provides additional discriminatory capacity to this technique. Strains differentiated by ribotyping are referred as 'ribotypes'. Jorden and Leaves (1997) observed that the traditional ribotyping was most discriminatory to detect variations between strains of *H. influenzae*. Ribotyping is used for typing and subtyping of several microbes like *L. pneumophila* (van Belkum *et al.*, 1996), *Yersinia ruckeri* (Garcia *et al.*, 1998), *V. parahaemolyticus* (Marshall *et al.*, 1999) for epidemiological studies. Ribotyping offers a good typing method and is highly useful for taxonomical and long term epidemiological studies. Ribotyping and other RFLP techniques generate interpretable banding patterns that are highly reproducible. However, this technique is time-consuming and requires considerable technical expertise. Recently an automated typing system, the Riboprinter (Qualicon. Welmington, DE, USA) has been designed for characterizing the bacteria to the strain level in 8 hours. However, 'riboprinter' has low discriminatory ability and low cost-effectively as compared to the traditional ribotyping hindering its usage in most laboratories

Random amplified polymorphic DNA (RAPD) : This technique is based on arbitrary amplification of polymorphic DNA sequences. Amplification is carried out using single or multiple, non-specific primers whose sequences are random and not designed to be complementary to any particular site in the chromosome. These primers bind at various 'best-fit' sequences on the denatured DNA under low stringency conditions and extend efficiently to give short amplicons. In subsequent cycling, conditions are made more stringent so that primers continue to bind to best-fit sequences and generate products of fixed lengths. Their (products) electrophoresis and staining produces the fingerprint. RAPD has the main advantage that no prior sequence information is required. Moreover, the entire genomic sequence is explored for comparison. Since the primers are not directed against any particular genetic locus, several priming events can result from variations in experimental conditions, making rigorous standardization of the method essential. The major disadvantage of this method is lack of inter-laboratory reproducibility. A little change in protocol, primers, polymerase or DNA extraction may give different results. RAPD has been used for typing of a number of bacteria using 10-mer primers (oligonucleotides consisting of 10 nucleotides). It was carried out for *Campylobacter coli* and *C. jejuni* (Madden *et al.*, 1996), *Listeria monocytogenes* (Czajka *et al.*, 1993), *Staphylococcus haemolyticus* (Young *et al.*, 1994), *Vibrio vulnificus* (Warner and Oliver, 1999). In *V. vulnificus* typing, discriminatory power of RAPD was highlighted; a difference in band patterns was obtained between encapsulated and non-encapsulated isogenic morphotypes. Another version of RAPD is AP-PCR i.e. arbitrarily primed PCR in which PCR is carried out with arbitrary primers. Here, PCR is carried out using >20-mer primers instead of 10-mer (RAPD). Other details of the methodology remain similar (Welsh and McClelland, 1990; Williams *et al.*, 1990, Welsh and McClelland, 1993). Recently, to promote reliability and reproducibility of arbitrarily primed PCR, various procedures have been recommended by Tyler *et al.* (1997).

***Repetitive sequence-based PCR (rep-PCR*):** rep-PCR genomic fingerprinting is a DNA amplification-based technique involving repetitive DNA elements present within bacterial genome (Versalovic *et al.*, 1991). Repetitive DNA sequences are universally present in eubacteria and have been applied to fingerprinting of bacterial genomes. Three main types of repetitive sequence used for typing purposes are repetitive extragenic palindromic sequences (REP elements), enterobacterial repetitive intergenic consensus (ERIC) sequence and BOX elements. All these motifs are genetically stable and differ only in their number and chromosomal locations between species, permitting differentiation of bacterial isolates to species, subspecies and strain levels. REP elements are 33-40 bp sequences consisting of a conserved palindromic stem, 5-bp variable loop and six degenerate positions (Stern *et al.*, 1984). ERIC sequences are 124-127 bp elements containing a highly conserved central inverted repeat, located in extragenic regions of various enterobacteria in approximately 30- 150 copies (Hulton *et al.*, 1991; Sharples *et al.*, 1990). They were first identified in *Salmonella typhi* and *E. coli* and constitute approximately 1% of the bacterial genome. Although REP and ERIC sequences are most widely used targets in gram negative bacteria, however gram-positive equivalents of these elements are BOX sequences. BOX elements are intergenic sequences of 154 bp each present in approximately 25 copies. These sequences appear to be located in distinct intergenic positions around the genome. The repetitive elements may be present in both the orientations and oligonucleotide primers have been designed to prime DNA synthesis outward from the inverted repeats in REP and ERIC and from the Box A subunit of BOX. Rep-PCR genomic fingerprinting is becoming a method of choice for bacterial typing. Rep-PCR has been applied to differentiate strains of *L. monocytogenes* (Jersek *et al.*, 1999), *V. parahaemolyticus* (Marshall *et al.*, 1999), *Acinetobacter baumanii* (Reboli *et al.*, 1994), *Burkholderia cepacia* (Hamill *et al.*, 1995), *Citrobacter diversus* (Woods *et al.*, 1992), and *Rhizobium meliloti* (De Bruijn, 1992). ERICPCR is used for typing *Haemophilus somnus* (Appuhamy *et al.*, 1997), viridans Streptococci (Alam *et al.*, 1999) and *Vibrio parahaemolyticus* (Marshall *et al.*, 1999). Rep- PCR was used to study the role of genetic rearrangement in adaptive evolution. One distinct advantage of rep-PCR is its broader species applicability. Moreover this technique has a good discriminatory power which can be further increased by the use of multiple sets of primers.

Numerical taxonomy

Numerical taxonomy or taximetrics, nowadays frequently and perhaps more appropriately referred to as phenetics, refers to the application of various mathematical procedures to numerically encoded character state data for organisms under study. Thus, it is the analysis of various types of taxonomic data by mathematical or computerized methods and numerical evaluation of the similarities or affinities between taxonomic units, which are then arranged into taxa on the basis of their affinities. So, Numerical taxonomy is a classification system in biological systematics which deals with the grouping by numerical methods of taxonomic units based on their character states. It aims to create a taxonomy using numeric algorithms like cluster analysis rather than using subjective evaluation of their properties. The concept was first developed by Robert R. Sokal and Peter H. A. Sneath in 1963 and later elaborated by the same authors .They divided the field into phenetics in which classifications are formed based on the patterns of overall similarities and cladistics in which classifications are based on the branching patterns of the estimated evolutionary history of the taxa. In recent years many authors treat numerical taxonomy and phenetics as synonyms despite the distinctions made by those authors. According to Heywood the numerical taxonomy may be defined as the numerical evaluation of the similarity between groups of organisms and the ordering of these groups into higher ranking taxa on the basis of these similarities.

Numerical taxonomy involves two aspects: (a) Construction of Taxonomic Groups: i. In numerical taxonomy, first, individuals are selected and their characters spotted out. There is no limitation to the number of characters to be considered. However, the larger the number of characters better is the approach for generalization of the taxa. ii. The resemblances among the individuals are then established on the basis of character analysis, which can often be worked out with the help of computers, the accuracy of which depends on the appropriateness in character. The best way to delimitate taxa is, to utilize maximum number of characters, with similar weightage given to all of them. (b) Discrimination of the Taxonomic Groups: When the taxonomic groups chosen for the study show overlapping of characters, discrimination should be used to select them. Discrimination analysis can be done by various techniques, specially devised for such purposes. Numerical taxonomy is thus, based on certain principles, also called neo Adansonian principles. Numerical taxonomy has been successfully applied in the following studies:

a. Study of similarities and differences in bacteria, other micro-organisms and several animal groups.

b. Delimitation of several angiospermic genera like Oryza, Sarcostemma Solarium, and other groups including Farinosae of Engler and a few others.

c. In the study of several other angiospermic genera including Apocynum, Chenopodium, Crotalaria, Cucurbita, Oenothera, Salix, Zinnia, wheat cultivars, Maize cultivars, etc.

d. Phytochemical data from seed protein and mitochondrial DNA RELP studies has been numerically analyzed to study the interspecitic variations among eight species of cassia L . Based on the results of electrophoretic patterns, the degree of pairing affinity (PA) or similarity index was calculated by the following formula, according to the method of Sokal & sneth and Romero Lopes et al.:

$$PA = \frac{\text{Bands common to species A and B}}{\text{Total bands in A and B}} \times 100$$