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Review

MOLECULAR TAXONOMY: USE OF MODERN METHODS IN THE IDENTIFICATION OF A SPECIES

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The field of biology that deals with the theory and practice of classification of organism is called as taxonomy. Taxonomy term was proposed by de Candolle in 1813 which means law of arrangements (taxis- arrangement or order; nomos- law). It is the science of biological classification. It consists of three separate but interrelated parts i.e. classification, in which we arrange organisms into groups or taxa based on their mutual similarity or evolutionary relatedness; Naming or nomenclature and identification which involves process of determining a particular isolate belonging to a recognized taxon. A species is a group of interbreeding natural populations that is reproductively isolated from other such groups. To identify all such types that lived or exist on this earth planet, several criteria are followed. Initially morphological features formed the basis of identification but then other features were also taken into consideration for this purpose.

So far, more than 1.5 million species of animals, plants and microorganisms have been reported and it is estimated that the number of undescribed living species could be more than 3 million. Taxonomists claim that at least 50 million species of different organisms have become extinct in this long course of evolution. Every species of animal and plant may exist in numerous different forms, for example, they may be found in two distinct sexes, age groups, seasonal forms and other phenotypic variations .We have noted the possible present forms of life on land but a large number of beautiful creatures are found to inhabit deep in the oceans. Still we find certain remote narrow regions in the oceanic zones where human endeavor to explore life through modern technology do not yield fruitful.

The different characters accumulated with reference to animals provide the basis to establish relationships between taxa. Two animal species are considered to be closely related if they share more common characters. In taxonomic practice, however, if two animal species have to be considered different, it becomes essential to find actual differences between them. According to Mayr a taxonomic character is any attribute of an organism by which a member of a taxon differs or may differ from a member of another taxon. Any attribute of an organism may be useful as a taxonomic feature if it show difference from the equivalent features in members of another taxon. Every group of organism e.g. moths, butterflies, mollusks, starfishes, fishes or birds possess different taxonomic characters. Thus a taxonomist has to be well trained to be familiar to deal with organism of a particular taxon.

The present form of classification which we adopt to classify the organisms is the outcome of the efforts of biologists especially taxonomists. Taxonomy helps us to organize huge amounts of knowledge about organisms because all members of a particular group share many characteristics. It allows us to make predictions and frame hypotheses for further research based on knowledge of similar organisms. It places them in useful groups with precise names so that researchers can work with them and communicate efficiently. Study of taxonomic features allows the construction of databases that can be used for a further rapid identification of organisms in the laboratory work.

Taxonomic Characters

Several features of the organisms are of taxonomic importance. Morphological, cytological, ethological, ecological and biochemical characters are the major criteria which are looked into to scrutinize the organism under consideration (Mayr, 1969,1971). Bacterial species are also considered for their specific characters for example their cell shape and size, staining reactions, presence or absence of spores or reproductive forms, type of motility, cultural characters (e.g. nutrients. oxygen, temperature, etc.) and the way growth occurs in liquid media, and particularly on solid media (e.g. colony form), biochemical characters such as metabolic end-products and the presence or absence of a particular enzyme or pathways, serological characters i.e.

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the nature of the surface antigens as revealed by suitable specific antibodies and molecular characters (the sequences of bases in the DNA, GC ratios and nucleic acid hybridization).

Molecular techniques in the field of biology have helped us 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 most 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 vertebrate that the taxonomists realized significance of molecular data and this made them understand that other tradional methods are although important but molecular evidences could be final or confirmatory evidences.

The molecular techniques now have become less time consuming that even thousands of base pairs of nucleotides or polypeptide chains with hundreds of amino acids could be sequenced within few hours. In 1964 Davis reported that the giant Panda is a bear and is not related to the raccoons. His this explanation was based on morphological features. At that time some scientist could not believe him but the molecular analysis in this regard proved to be correct (Mayr, 1986). More interestingly the cheetah (Aconyx) which has been considered by morphologist to be the most aberrant of all the cats, but molecular studies have revealed that it is the close relative of lion-tiger group (Colliear and O'Brien, 1985). Taxonomists have observed that each organ or organ system may have its own specific rate of evolutionary change. This concept is equally valid for molecular characters also.

DNA (nuclear as well as mitochondrial) segments and proteins separated from the tissues of the organisms and are subjected to further analysis so that the linear order of nucleotide or amino acids can be deciphered. Gel electrophoresis can separate segments of DNA/ proteins in the gel on the basis of their sizes, charges and weight and form a useful method to characterize organisms of different taxa.

Restriction site mapping of ribosomal DNA helped taxonomist to establish affinities among the species of genus Rana (an amphibian). Field et al., (1988) selected 18S rRNA to compare and classify the animals of invertebrate phyla. Thus the sequence coding for eukaryotic rRNA has been useful material to envisage relationship among the lower forms of organisms and the prokaryotic forms. By comparing the base sequences of specific genes, one can determine the exact number of mutational variations (Gordman, 1982). The similarities and dissimilarity in the arrangement of amino acid sequences among the members of different taxonomic groups can also be done. Amino acid sequencing tells the number of substitutions in the polypeptide chain and therefore one can also correlate the changes which might have resulted in the DNA in the course of evolution. A large number of proteins have been sequenced now and the degree of relationship among several taxa have been inferred. These studies have revealed that different proteins undergo changes at varying rates, for example, four proteins i.e. histones, cytochrome c, globins and fibrinopeptides of vertebrates were analyzed for their amino acids sequences and it was found that they have undergone changes in different organisms with specific rate in the long course of evolution (Brooker, 1999; Narendran, 2006; Snustad and Simmons, 2010). Out of these proteins, histone proteins have undergone least changes indicating that the DNA segment coding for them are more conserved.

Cytochrome -c is a respiratory molecule and it is found in the mitochondria of eukaryotes. A large number of animal species have been examined for their amino acid sequence for this protein. It is relatively small protein of a chain lenghth slightly over 100 amino acid residues. This ubiquitous protein has been extracted, purified and sequenced in about 100 eukaryotic species. Cytochrome c has 104 amino acids in vertebrates and a few more in certain lower forms of organisms. It has been observed that the amino acids of this protein undergo very slow evolutionary changes. The amino acid sequence of human and chimpanzee are found to be identical and humans and rhesus monkeys with only one amino acid change. This shows that lines leading to humans and monkeys diverged from a common ancestor. We also observe an increasing change in

Summary of the methods presently employed in identification of the species

Methods	Explanation
Hybridization	Genetic materials from two different species is subjected to hybridize. Closely related species show higher percentage of hybridization
DNA sequencing	DNA segments of two species are sequenced from one end to the other and the sequences of the two form the basis of establishing similarity or dissimilarity between them
Restriction mapping	Segments of DNA are isolated from different species and subjected to restriction mapping. Closely related species will have more similar restriction map
Chromosome banding	The chromosomes of different species are examined through microscope. Banding of chromosomes are also done for taxonomic purposes
Amino acid sequencing	Like DNA sequencing protein sequencing is also done. The amino acid sequence of a given protein will be more similar between closely related species.
Immunological methods	Antibodies that recognize specific macromolecules, usually on the cell surface are tested on different secies. Antibodies that recognize macromolecules form one species will often recognize closely related species, but not from distantly related species

the number of amino acids when other groups of animals are taken into consideration. Our cytochrome-c differs in 10 amino acids from that of dogs, 12 amino acids from horses, in 24 amino acids from that of moths, and in 38 amino acids from that of a fungus (yeast).

The alpha globin polypeptide chain of vertebrate has been selected for taxonomic purpose. There are 141 amino acids in this polypeptide chain. When humans amino acids sequence of alpha globin was compared with other vertebrate animals, it was found that it differed in 79 amino acids from sharks, followed by carp(68), newt (62), kangaroo(27) and cow(17). This also reveals the time that has elapsed since the evolutionary lines diverged from a common ancestor. Fibrinopeptides on the other hand seem to be highly modifiable as a number of amino acid substitutions could be observed in a single group of animals, for example, mammals.

Molecular Techniques Employed In Taxonomic Studies

The advent of molecular biology in the 1980s contributed a set of powerful new tools that have helped biologists to detect the smallest variations within species and even within individual strains. Molecular methods are now central for determining phylogeny and nomenclature. In the molecular taxonomy, one can research both DNA and RNA, and the main techniques that have been used in the systematics comprises of restriction maps construction and restriction analysis through RAPD, RFLP,AFLPs (Amplified fragment length polymorphisms), DNA-DNA hybridization, DNA-RNA hybridization and sequencing of DNA.

Indirect analysis of the genome can also be performed through ELISA (Enzyme Linked Immunosorbent Assay), Electrophoretic mobility of total protein extract ribosomal proteins pattern obtained by two-dimensional PAGE and HPLC. Multilocus enzyme electrophoresis is used widely in the investigations of genetic heterogeneity in bacterial populations and for clarifying the intra-species diversity of the prokaryotes. Estimation of the melting temperature of the DNA forms one of the methods to establish strain homology among prokaryotic forms. Higher G + C gives a higher melting temperature. GC Ratios are usually of minimal value in the

overall taxonomic characterization of an organism because two organisms can have identical GC ratios yet unrelated both taxonomically and phylogenetically. The number of fragments into which a given restriction enzyme cuts a bacterial genome depends on the frequency of restriction sites. The length of a given fragment depends on the distance between restriction sites. A restriction enzyme digesting a given genome generates a reproducible pattern of bands; each band corresponds to a restriction fragment of a certain molecular weight. The pattern usually varies slightly between gels. Since every organism will be giving a different RFLP patterns, its pattern of bands obtained corresponds to its DNA fingerprint. Because standard agarose gel electrophoresis fails to efficiently resolve fragments that are more than 50,000 bases long, new methods have recently been developed that separate the very large fragments generated by enzymes that cut at rare sites.

RFLP technique is regarded as the most sensitive method for strain identification and several organisms have been widely studied using this technique. RFLP is a technique that exploits variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules that come from differing locations of Restriction enzyme sites.The basic technique for detecting RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest. The resulting DNA fragments are then separated by length through Agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure. Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe. An RFLP occurs when the length of a detected fragment varies between organisms. Each fragment length is considered an allele, and can be used in genetic analysis.

PFGE (Pulse Field Gel Electrophoresis) and PCR (Polymerase Chain Reaction) metods are employed to characterize animal and plant species. In PFGE, one uses DNA nicking enzymes- restriction enzymes to digest the DNA molecule to generate a characteristic pattern. To compare two different species, one employes same types of restriction enzymes to cleave the DNA of both species and the comparison of the two may reveal the similarity or dissimilarity between them. In PCR identification, a single segment of DNA is amplified. Such DNA segment can be a particular protein coding gene or rRNA gene or a portion of it. The amplified fragment is then sequenced and the sequence compared to that of sequences in the databases. The rRNA gene sequences are very much conserved and thus slight changes in the nucleotide sequence can be used for species identification.

DNA/DNA Hybridization is an experiment in whichthe radio-labeled sample is reannealed with unlabeled DNA from a different source. The degree of reassociation is measured and members of same species show highest hybridization than distant one which show less percentage of hybridization. In 1961, McCarthy and Bolton presented a means of comparing genetic material through DNA-DNA hybridization a method for bacterial systematics. DNA-DNA hybridization or DNA-DNA reassociation technique is based on a comparison between whole genome. If the strands are heated, they will separateand as they cool, the attraction of the nucleotides will make them bond back together again. To compare different species, scientists cut the DNA of the species into small segments, separate the strands, and mix the DNA together. DNA from two organisms is sheared into small segments, and a small amount of radiolabeled DNA from one organism is added to a large amount of unlabeled DNA from the other organism. Since the concentration of labeled DNA is low, complementary strands of labelled DNA do not "find each other"; however, they do "find" complementary segments of unlabeled DNA. The amount of radioactivity incorporated into double strands is compared with that incorporated when the organism's own unlabeled DNA is used in excess.
RNA sequencing also provide basis for the identification of two separate species. 16S rRNA and 18 SrRNA which are part of ribosomal subunits of prokaryotic and eukaryotic cells are isolated and sequenced. These rRNA being small in size and characterized with species specificity form a useful tool for systematic studies.

CONCLUSION

Presently every existing species of plant and animals is being considered as precious boon of our nature which has taken very long time to come and exist in the present form. Preserving biodiversity is just like to preserve valuable asset, which have been gifted by our nature. Taxonomy is the subject which is so operational that it cannot be learned merely by reading book of this field. Molecular collections are thus being made for their suitable analysis. Such materials consist of whole specimen, tissues, individual cells or even body fluid. These materials are preserved for their future studies. Proteins, nucleic acids or other bio-molecules of such studies provide information for their affinities with other group of organisms.

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