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CHAPTER 32

Importance of Molecular Taxonomy in Fishery Biology Investigations

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Fitness and survival of diverse organisms to a particular environment is dependent on the unique biological attributes of a species. The inherent genetic variation within species and populations provides the necessary impetus to thrive in the presence of dynamic environmental forces. Understanding the genetic and morphological diversity is the key to ensure sustainability of ocean resources, identify adaptive evolution patterns and manage ocean resources. Molecular taxonomic investigations have undergone tremendous advancements in the last decade due to the advent of sequencing methods. The power and precision associated with molecular taxonomic investigations have contributed immensely to the advancement in knowledge in many areas of biological science.

Several molecular markers are in use for understanding intra- and inter- specific patterns of diversity in marine populations and they can be characterized as Type I and II. When a marker is associated with genes of known function they belong to Type I category and when associated with genes of unknown function, they belong to Type II category.

Type I markers

Allozyme markers are type I markers as they are associated with genes of known functions. DNA encodes allozymes and genetic variation at the level of enzymes can be detected using allozyme electrophoresis. Allozymes are protein variants which originate from allelic variants which differ in electric charge and these variations could be detected using electrophoresis. Allozymes are codominant markers expressed in heterozygous individuals in a Mendelian fashion. Information regarding single locus genetic variation can be gathered using allozyme analysis which can answer many questions regarding intra- and inter specific diversity of fish populations. To detect variations in allozyme pattern, allozymes have to extracted from tissues following standard protocols and variations detected through electrophoresis in an acrylamide or cellulose acetate gel. A single band will be present if individuals are homozygous and double bands when individuals are heterozygous. Allozymes have been extensively studied and used for many investigations due to their simplicity and cost effectiveness as any kind of soluble protein can be used for allozyme analysis. Many numbers of loci can be screened at a time using allozyme markers. Major limitations with the use of allozyme analysis include requirement of large amount of tissue which impedes its use with smaller organisms like larval forms. The tissue sampling method is invasive and hence every time the fish has to be sacrificed and tissue stored cryogenically. Point mutations in nucleotide sequences could not be detected using protein electrophoresis as such mutations may not result in change in the amino acid composition. In spite of all these limitations, allozymes have been widely used for fishery biology investigations like fish systematic, population genetic structure, conservation genetics and forensic applications.

Mitochondrial DNA markers

Mitochondrial DNA is found in the cytoplasm inside organelles called mitochondria and hence they are considered as non- nuclear DNA. Mitochondrial DNA can be considered as a haploid genome which is maternally inherited and transcription takes place as one single unit. Mitochondrial DNA is not subjected to recombination events, selectively neutral and present in multiple copies in each cell. It is easy to isolate mitochondrial DNA from any tissue or blood sample as they are physically separated from the cell's DNA. Detection of population bottlenecks and hybridizations is easy using mitochondrial DNA as effective population size is smaller than nuclear DNA due to its maternal inheritance.

Molecular taxonomic methods using mitochondrial DNA employ either RFLP based length polymorphism or sequence variations to detect differences in patterns of genetic diversity. RFLP is based on the length polymorphisms generated on digesting mitochondrial DNA with restriction enzymes and visualizing these polymorphisms electrophoretically. RFLP has been widely used to understand species specific patterns in many marine fishes.

Sequencing the amplified region of mitochondrial DNA has become very popular now after the emergence of sequencing technologies. Several universal primers are available based on conserved sequence regions so that they can be applied universally to any tissue type. Inter specific comparisons can be carried out using slow evolving gene regions and intra specific comparisons using fast evolving gene regions. The gene, Cytochrome C Oxidase 1 (around 600bp) is being used as the universal barcode for species specific comparisons as it is a slow evolving region. It is highly conserved across a wide range of taxa. D-loop region, the only non-coding region is fast evolving and used for intra-specific or population comparisons. In addition, cytochrome b and ND-1 and ND-5/6 regions are also being used for intra-specific comparisons. Mitochondrial DNA genes find widespread application in fish systematics and population genetics.

Nuclear DNA markers

Nuclear DNA markers can be categorized as arbitrary and specific depending on the gene regions to be amplified.

Arbitrary markers

Arbitrary markers include; RAPD, Random Amplified Polymorphic DNA and AFLP, Amplified Fragment Length Polymorphism. RAPD makes use of an arbitrary marker to amplify regions of genomic DNA and exhibits very high amount of polymorphism. This marker does not require any knowledge of regarding gene or genome and so it is very fast, cheap and efficient. But RAPD markers lack reproducibility and repeatability and many products are simultaneously generated. Homozygous and heterozygous states cannot be distinguished based on these markers and slight changes in amplification conditions bring about variations in band patterns. AFLP markers employ both RFLP and RAPD techniques. Two restriction enzymes are used to digest genomic DNA followed by ligation of double stranded nucleotide adapters to the ends of DNA fragments which will be the primer binding sites for subsequent amplification by PCR. Primers which are complementary to the adapter and sequences at the restriction site with additional nucleotides at the 3' end can be used as selective

agents which can amplify a subset of ligated fragments. Polymorphisms along with presence or absence of DNA fragments are detected on polyacrylamide gels.

Specific markers

Variable number of tandem repeats is parts of DNA repeated tens, hundreds or thousands of times within nuclear genome of eukaryotes. They are repeated tandemly, varying in number at different loci of the genome and individuals. Repetitive DNA can be classified as minisatellite and microsatellite DNA. Minisatellite DNA are loci with repeats of length varying between 9-65bp and microsatellite DNA with repeats 2-8bp. Microsatellites are found abundantly in the genome as compared to minisatellites and they are widely used in population genetic analyses. Minisatellites are of two types; multilocus and single-locus. Multilocus minisatellites consist of tandem repeats of 9-65bp with length varying between 0.1 to 7kb. Minisatellite loci can be used for parentage analyses whereas they are less useful for population genetic analyses. Their mutation patterns are very complex and hence interpretation is very difficult for population genetic analyses. Most of the research works are concentrated on single locus minisatellite probes which are successful in detecting population genetic variations. Other applications of minisatellite loci include forensics, parentage analysis, understanding mating success and confirmation of gynogenesis.

Microsatellites are simple repeated sequences in the genome which are highly variable. These loci can be used as markers and are seen every 10kbp of the genome. They are very useful in genome mapping and population genetic investigations. These loci are highly variable, selectively neutral, do not code for proteins and hence the amount sequence divergence may be directly related to the time since separation. Microsatellites evolve faster at a rate of 10^{-3}-10^{-4} mutation/ generation and are inherited in a Mendelian fashion. They are considered as codominant markers. Due to the high level of polymorphism, they are very popular. Cross amplification with primers developed for closely related species minimizes cost associated with microsatellite detection and characterization. The procedure of microsatellite amplification involves, extraction of DNA, amplification using specific primers and visualization of bands in PAGE gel. Automated genotyping by using labeled primers has made the analysis of size polymorphisms accurate and fast. The presence of null alleles and stutter bands is the major limitation while using microsatellites. Null alleles occur due to mutations at primer binding sites which will decrease accuracy in parentage or relatedness analysis and so discarding loci showing null alleles is the best option. Stutter bands are formed due to slipped strands mispairing during PCR or inadequate denaturation of amplification products. Stuttering is relatively less with tri- and tetra- nucleotide repeats. Microsatellite markers can be used in fisheries and aquaculture for detecting genetic structure of populations, conservation of biodiversity, phylogenetic investigations, phylogeographic studies, understand impacts of stocking and hybridization. It can also be employed for forensic identification of individuals, mapping of genome, determination of kinship and patterns of behaviour.

Single nucleotide polymorphisms arise in the genome due to single point mutations like insertions/deletions and transitions or transversions. Point mutations produce divergent alleles with alternative bases at a specific nucleotide position and these alleles are estimated to understand intra- and inter- specific diversity patterns. SNPs are considered as the most abundant polymorphism

in the genome which can be detected using PCR, microarray chips or fluorescence technology. SNPs are described as next generation markers in fisheries and can be widely applied for population genetics and genomics investigations.

DNA microarrays are small glass microscope slides, nylon membranes or silicon chips which can hold many immobilized DNA fragments in a standard pattern. A reporter probe of known sequence can be matched with DNA from target sample which is of unknown origin. Microarray can also be used for construction of species specific DNA probes which could be subsequently used for identification purposes. DNA of the target samples has to be labeled with fluorescent molecules and hybridized to the DNA of the microarray. A fluorescent signal will be emitted when hybridization is positive which can be detected using appropriate fluorescence scanning/imaging equipment.

Expressed sequence tags can be generated using random cloning of cDNA and they can be used for identification of genes and analysis of expression by means of expression analysis. It is possible to make fast and reliable analysis for the genes expressed in particular tissue types under specific physiological or developmental stage. cDNA microarrays can be used to identify differentially expressed genes in a proper way. In addition, ESTs can also be used for linkage mapping.

Molecular markers in fishery biology investigations

1) To understand inter and intra specific variations

The extent of divergence in DNA or genes is considered as the baseline for species level differentiation and the variability in evolution among taxa should also be considered while making decisions. Mixed catches, larval forms, endangered and threatened animals caught illegally, stranded cetaceans and processed fish products can be identified up to species level using molecular markers as morphological identification is not possible with these samples. Molecular markers can be used for fish stock characterization or identification of sub-species.

2) Phylogenetic and Phylogeograhical studies

Phylogenetic studies focus on historical processes affecting species relationships whereas phylogeographic studies focus on processes affecting geographical distribution. Mitochondrial DNA markers can be utilized effectively for phylogenetic and phylogeographic investigations. Based on mitochondrial DNA information, the evolutionary history of groups of fishes can be reconstructed and vital knowledge on historical demography obtained. In addition, conservation units and ecological patterns also deduced. Mitochondrial DNA has been used as a powerful tool to infer intraspecific phylogenetic patterns in many marine fishes.

3) Identification of genetic structure between and within populations

Identification of stock structure of fish populations is very vital for fisheries management and conservation. Stocks are subpopulations within species which may be reproductively isolated and exhibiting different physiological and behavioural patterns. Mitochodrial DNA as well as microsatellite markers are widely used for inferring genetic stock structure of marine fish populations. Morphological and meristic information should be combined with genetic information so that a comprehensive picture of subpopulation structure is obtained. Identification of subunits from mixed fisheries or origin of stock components is also possible using molecular tools.

4) Genetic tagging/marking

Individual fishes can be marked for tracking movement or migration, understanding population size or contributions of distinct stock to mixed fishery. Since physical tags are not heritable, they cannot be employed for generations. Genetic tagging by tracking a rare allele in individuals of populations over generations will be beneficial to understand the contribution of hatchery programme on harvest and identify migrants from different regions.

5) Forensic investigation

Molecular markers are very effective in identifying dead or stranded fishes and preserved or canned items as morphological identification is not possible in them. Certification of fishery products and detection of illegal trading of fish and fishery products is also possible using molecular forensic technologies. Molecular tools also could be used for monitoring deliberate or accidental release of fishes/organisms to natural waters.

6) Studying the trophic relationships

Determining trophic relationships within ecosystem is very essential for any ecological study and data on diet composition is crucial towards achieving this aim. It is difficult to identify diet components up to species level using morphological features alone as partial or complete digestion will destroy key morphological features. DNA can be extracted from partially digested samples and diet components studied using molecular markers.

7) Ancient DNA to deduce historical evolutionary relationships

Preserved samples in museums, fossil remains, archaeological finds and other unusual sources can be utilized for retrieval of DNA sequence information using several methods. Several such investigations have improved our understanding with respect to evolutionary relationships among different taxa.

8) Applications in aquaculture

Molecular markers can be effectively used in aquaculture for; selective breeding and genetic improvement, finding quantitative trait loci, identifying inbreeding events, genetic identification of hatchery stocks, progeny assignment, marker assisted selection, understanding the effects of ploidy induction and gynogenesis. Molecular markers have wide range of applications in disease diagnosis. PCR based kits are available for detection of many diseases like white spot syndrome virus (WSSV), infectious pancreatic necrosis virus (IPNV), viral nervous necrosis virus (VNNV), channel catfish virus (CCV), infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV).

Conclusion

Molecular markers are excellent tools to enhance fisheries and aquaculture productivity and ensure sustainability. Markers should be selected cautiously so as to improve the quality of the output. Next Generation Sequencing technologies are revolutionizing the field of biology and the future of aquaculture and fisheries will depend on the penetration of these technologies to this vibrant sector.

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