**Molecular Markers: Introduction and its Applications in Aquaculture**

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**Markers**

 Marker can be defined as a heritable trait (phenotypic trait or enzymes or DNA sequences) that can differentiate the individuals/populations/ species. In molecular biology and biotechnology, molecular markers are used to distinguish a specific DNA sequence from a collection of unidentified DNA. There are three major types of markers

(1) Morphological markers, which themselves are linked to the phenotypic characters of a trait

(2) Biochemical markers, which include allelic variants of enzymes called isozymes & allozymes

(3) DNA markers, also called molecular markers, reveal DNA variation sites.

**Morphological markers**

The morpho-meristic characters, i.e., body shape, body colour, size, and other quantitative characteristics, have been used as morphological. However, their application in stock discrimination is restricted by phenotypic plasticity, wherein the observed phenotypic variation might not correspond to the differences at the genome level. The surrounding environment, genetic factors and the interaction between environment and genetic factors also determine morphometric variation. Morphometric characteristics of bivalves are affected by environmental factors like temperature, salinity type of sediment tide level and presence of predators. A study on *Anadara pilula* populations from different regions of Indonesia could find significant differences in all the morphometric traits between sites, indicating that differences may be due to genetic and environmental influences. Morphological markers have limitations as they are based on subjective judgments.

**Biochemical markers (Allozymes)**

Allozymes are protein-based markers coded by a single locus, occurring in different molecular forms. These are distinct allelic forms of enzymes that are separated by charge and, in some cases, by three-dimensional shape on a separating medium, like starch gels or polyacrylamide gels, and are visualized using histochemical stains that show the migration of molecules with specific enzyme activities. Usually, two or sometimes more loci can be distinguished for an enzyme, and these are referred to as “iso-loci.” Therefore, allozyme variation is also termed as isozyme variation. The main strength of allozymes as a molecular markers is their simplicity because allozyme analysis does not require DNA extraction or the availability of sequence information, primers or probes, or any other prior knowledge.

 The importance of allozymes lies in their key feature, i.e., codominant nature and high reproducibility. Allozymes’ banding pattern is called Zymograms and can be readily interpreted in terms of loci and alleles. Biochemical and genetic studies by enzyme electrophoresis have been widely applied to address issues of conservation genetics associated with commercially viable species of marine bivalves. Several studies have observed genetic diversity and population differentiation in mollusks. Nevertheless, allozymes also have limitations, such as low polymorphism, unable to detect silent substitutions and the requirement for fresh tissue. The degeneracy of amino acid codons lessens the extent of genetic variability in proteins compared to nucleotides (Powers, 1991).

**DNA markers**

A genetic marker is a gene or DNA sequence with a known chromosome location associated with a particular gene or trait. Marker can be defined as a variation that may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, like a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long sequence, like mini & microsatellites. Molecular markers offer numerous advantages over conventional phenotypic markers as they are stable and detectable in all tissues regardless of the cell's growth, differentiation, development, or defense status. They are not confounded by the environment, pleiotropic and epistatic effects (Yang et al., 2013).

 Some characteristics of an ideal molecular marker are:

(1) Highly polymorphic and easy to analyze

(2) Highly reproducible.

(3) It must be co-dominant in nature to allow discrimination between homozygotes & heterozygotes.

Nuclear genetic markers like Random Amplified Polymorphic DNA(RAPDs), Amplified Fragment Length Polymorphism (AFLPs), Variable Number of Tandem Repeats (VNTR: minisatellites, microsatellites), Single Nucleotide Polymorphism (SNPs), and mitochondrial DNA markers are the popular genetic markers employed in fisheries and aquaculture. The transmission and evolutionary dynamics of nuclear DNA markers (biparently inherited) differ from that of mitochondrial DNA markers (maternally inherited).

**Random amplified polymorphic DNA (RAPD)**

The RAPD technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. The principle of RAPD is that a single, short oligonucleotide primer (10bp) amplifies random fragments from a complex DNA template. The resulting amplicons may vary in size among the individuals/populations/ species due to the mutations within the primer binding region / between primer critical areas. The RAPD does not require any prior information on the genetic composition of the test species, is less costly, easy to assay, and requires less DNA.

 However, they are dominant markers, and reproducibility is low. RAPD has been widely used in population genetic studies of *Caelatura companyoi* (freshwater bivalve), *Aelatura prasidens* and Cupped oyster, *Crassostrea*.

**Restriction fragment length polymorphism (RFLP)**

RFLP markers are considered the first shot in the genome revolution marking the start of an entirely new era in the molecular genetic sciences. Restriction Fragment Length Polymorphism (RFLP) detects the polymorphism by generating fragments of different lengths after digestion of the DNA samples with specific restriction endonucleases. This results in fragments 10, whose number and size vary among individuals, populations, and species. Traditionally, fragments were separated using Southern blot analysis in which genomic DNA is digested, subjected to electrophoresis through an agarose gel, transferred to a membrane, and visualized by hybridization with specific probes. RFLP loci are inherited as Mendelian markers in a co-dominant fashion.

The advantages of RFLP as molecular markers are:

(1) Restriction enzymes are commercially available

(2) Small amount of templates is required.

(3) The power of RFLP markers in revealing genetic variation is relatively low compared to more recently developed markers and techniques such as SNP and Microsatellite markers etc.

The major disadvantage of RFLP is the relatively low level of polymorphism than the advanced markers. RFLP markers were used in studying genetic variability in populations of freshwater mussels, *Anodonta woodiana*.

**Variable number of tandem repeats (VNTR)**

VNTRs are another type of genetic marker wherein nucleotide motifs are tandemly arranged across the genome. The number of motifs could vary among individuals/populations/species.

 These sequences can be classified based on nucleotide motif size into satellites, minisatellites, and microsatellites. In satellite DNA the motif size is several thousand base pairs, repeated thousands or millions of times. Minisatellites consist of DNA sequences of 9-100 bp in length that is repeated up to 100 times at a locus. Microsatellites have a unique length of 1-6 bp repeated up to 100 times at each locus. The variation in repeat number is due to polymerase slippage and unequal recombination.

**Microsatellites**

Microsatellites are tandemly repeated motifs of variable lengths distributed throughout the eukaryotic nuclear genome in both coding and non-coding regions. They are short tandemly arrayed di-, tri-, or tetranucleotide repeat sequences with repeat sizes of 1–6 bp flanked by regions of nonrepetitive unique DNA sequences. They are also called as “Simple Sequence Repeats” (SSR) or “Short Tandem Repeats” (STR). Microsatellites have been estimated to occur as often as once for every 10 kbp in fishes. A few 11 studies have reported the occurrence of microsatellites within a gene region. Most of the microsatellites are type II markers for which no known function has been established. The advantages of microsatellite markers include locus-specific, high polymorphism, requiring less tissue, less expensive, high reproducibility, amenable to automation, and co-dominant nature. Microsatellites have become the markers of choice for a wide range of applications in population genetics, conservation, and evolutionary biology.

 **Types of Microsatellite Markers**

**Based on the nature of and occurrence of repeat motif, microsatellites have been classified as:**

➢ Perfect microsatellites: Microsatellites, wherein the motifs are tandemly arranged without interruption by any other non-motif region. e.g., (AT) 20

➢ Imperfect repeats: Microsatellites that are interrupted by different non-motif nucleotides e.g., (AT) 12GC (AT) 8

➢ Composite: Microsatellites with two or more different motifs arranged in tandem, e.g., (AT)7 (GC) 6.

 The composite repeats can be perfect or imperfect. The sequences of di-, tri-, and tetra nucleotide repeats are the most common choices for molecular genetic studies.

**Microsatellites can also be categorized into two classes as per the length of the repeat motif.**

Class I microsatellites: perfect SSRs of >20 nucleotides in length

Class II microsatellites: perfect SSRs of >12 nucleotides and 20), are very useful for parent-offspring identification in mixed populations, while others having lower numbers of alleles are suited for population genetics and phylogeny.

 Microsatellite DNA markers have been found to be very powerful in differentiating geographically isolated populations, sibling species, and sub-species 12. The polymorphism obtained with microsatellite markers provides useful and thorough information in fish stock management, biodiversity conservation, and population analysis. Microsatellites are also becoming increasingly powerful in the forensic identification of individuals, and determination of parentage and relatedness, genome mapping, gene flow, and effective population size analysis. Microsatellites, too have some limitations like the appearance of shadow or stutter bands, presence of null alleles (existing alleles that are not observed using standard assays), homoplasy, and relatively large sample size is required to uncover all the alleles of a locus.

### Applications of Molecular markers in Aquaculture

* 1. Fish Species identification.
	2. Research on genetic variation and population structure in naturally occurring populations; thus, it plays a crucial part in population genetics studies.
	3. Comparison between wild and hatchery populations.
	4. Assessment of demographic bottlenecks in natural populations.
	5. Marker-assisted breeding.
	6. Calculating the genetic separation between species and their progeny.

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