**Aquaculture Revolution: The Role of Molecular Markers**

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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 and allozymes.

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

**Morphological markers**

Morphological attributes have been defined as morpho-meristic traits, such as size, colour, shape, and other quantitative traits. But phenotypic plasticity—the possibility that phenotypic variation seen does not match genetic differences—restricts their use in stock discrimination. The genetic component, the surrounding environment, and the interaction of these elements also influence morphometric variation. Environment variables such as temperature, predator presence, sediment type and salinity, and tide level impact the morphometric features of bivalves. Significant variances were found in all morphometric parameters between sites in research on *Anadara pilula* populations from several regions of Indonesia, suggesting that genetic and environmental factors may be responsible for local variations. Morphological markers have limitations as they are based on subjective judgments.

**Biochemical markers (Allozymes)**

Allozymes are protein-based indicators that exist in several molecular forms and are encoded by a single locus. These unique allelic versions of enzymes are seen by histochemical stains that display the migration of molecules with certain enzyme activity. The enzymes are separated by charge and, in certain situations, by three-dimensional shape on a separating medium, such as starch gels or polyacrylamide gels. For an enzyme, two or occasionally more loci can be identified; these are known as "iso-loci." For this reason, isozyme variation is another name for allozyme variation. Since primers, probes, sequence information, DNA extraction, and other prerequisite knowledge are not needed for allozyme analysis, its primary advantage as a molecular marker is its simplicity.

Two key features that make allozymes noteworthy are their codominance and remarkable reproducibility. The banding pattern of allozymes is called a zymogram, and it is easily understood in terms of loci and alleles. Enzyme electrophoresis has been widely used in biochemical and genetic studies to address genetic conservation issues pertaining to marine bivalves that are economically sustainable. Numerous studies have reported on the genetic diversity and population differentiation of mollusks. Allozymes do have certain disadvantages, though, such as low polymorphism, the difficulty in detecting subtle changes, and the requirement for freshly harvested tissue. Amino acid codons are less degenerate than nucleotides, which lowers the genetic diversity of proteins (Powers, 1991).

**DNA markers**

The most widely used nuclear genetic markers in aquaculture and fisheries are mitochondrial DNA markers, random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), variable number of tandem repeats (VNTR: minisatellites, microsatellites), single nucleotide polymorphism (SNPs), and VNTR. In contrast to mitochondrial DNA markers, which are inherited maternally, nuclear DNA markers are transmitted and evolve differently. 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 and heterozygotes.

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**Random amplified polymorphic DNA (RAPD)**

The polymerase chain reaction (PCR)--based RAPD methodology has been a widely employed molecular method for creating DNA markers. The idea behind RAPD is that random segments from a complicated DNA template can be amplified using a single, short (10 bp) oligonucleotide primer. Due to mutations occurring within the primer binding region or between primer crucial areas, the resultant amplicons may differ in size among individuals, communities, or species. The RAPD is less expensive, simpler to assay, and requires less DNA than other methods. It also does not require prior knowledge of the genetic makeup of the test species. 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) A 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 are 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 varying lengths that are found in both coding and non-coding areas of the eukaryotic nuclear genome. These are sections of nonrepetitive unique DNA sequences surrounded by short tandemly arrayed di-, tri-, or tetranucleotide repeat sequences with a repetition length of 1-6 bp. They are also known as "Short Tandem Repeats" (STR) or "Simple Sequence Repeats" (SSR). In fish, microsatellites have been reported to occur as frequently as once every 10 kbp. Microsatellites have been found within a gene region in just 11 investigations. The majority of microsatellites are type II markers, meaning that their exact function is still unknown. Microsatellite markers have several benefits, including being locus-specific, having a high polymorphism, requiring less tissue, being less expensive, having good repeatability, being automatable, and co-dominant nature. Microsatellites have become the markers of choice for various 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.

 The ability to distinguish between geographically isolated populations, sibling species, and sub-species has been demonstrated by microsatellite DNA markers 12. In fish stock management, biodiversity conservation, and population analysis, the polymorphism discovered using microsatellite markers offers comprehensive and practical information. In addition, microsatellites are becoming increasingly useful for forensic person identification, parentage and relatedness analysis, genome mapping, gene flow, and accurate population size estimation. The emergence of shadow or stutter bands, the existence of null alleles (alleles that exist but are not detected by conventional assays), homoplasy, and the need for a comparatively large sample size to detect all of a loci' alleles are some of the drawbacks that microsatellites share with other technologies.

### 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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