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## Molecular marker and its application in aquaculture: New opportunities and challenges

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### Abstract

Genetic variation in a species enhances the capability of an organism to adapt to changing environment and is necessary for the survival of the species. Genetic variation arises between individuals leading to differentiation at the level of population, species and higher-order taxonomic groups. The genetic diversity data have varied application in research on evolution, conservation, genetic improvement programmes and management of nature and natural resources. Molecular genetic markers can be used as powerful tools to detect genetic studies of individuals, populations or species. These molecular markers combined with new statistical developments have revolutionized the analytical power, necessary to explore the genetic diversity. Molecular markers and their statistical analysis revolutionized the analytical power, necessary to explore the genetic diversity. Various molecular markers, DNA (mt-DNA or nuclear DNA such as microsatellites, SNP or RAPD) or protein are now being used in aquaculture and fisheries. These markers provide various scientific observations which have importance in aquaculture practice recently such as 1) Species Identification 2) Comparison between wild and hatchery populations 3) Genetic variation and population structure study in natural populations 4) Assessment of demographic bottleneck in natural population 5) Propagation assisted rehabilitation programmes.

**Keywords:** Molecular marker, single nucleotide polymorphism, marker assisted selection (MAS), quantitative trait loci (QTL), molecular breeding

### Introduction

All fishes are caused to undergo mutations due to the regular cellular activity or interactions with the environment, leading to genetic variation (polymorphism). The variation in the genome level of a species enhances the capability of the species to acclimatize to changing the environment and is essential for the survival and overall fitness of the species. In conjunction with other evolutionary forces like genetic drift and natural selection, genetic variation rises between individuals leading to variation at the level of species, population, and higher order taxonomic groups. Molecular genetic markers are powerful tools to detect genetic exceptionality of individuals, populations or species. These markers have revolutionized the analytical power, necessary to explore the genetic diversity (Hillis *et al.*, 1996) <sup>[1]</sup>. Various molecular markers, DNA or proteins are now being used in fisheries and aquaculture, but unfortunately, the terminology used is sometimes confusing. Therefore it becomes crucial that fisheries and aquaculture researchers and managers should have a basic understanding of molecular tools, and their assumptions, strengths, and weaknesses. In fisheries, optimization of the desirable traits such as resistance to diseases, meat quality and body growth is very essential. These traits have a high influence on the profitability of fish farmers. Several generations of selection are needed to improve in these traits through traditional phenotype-based selection method and complex qualitative trait like meat quality is tough to measure for proper selection. In candidate gene approach, some pre-specified genes responsible for the trait of interest are selected. Then genetic variations within these genes are used as a molecular marker to study their association with the desirable phenotypes. It is different from genome-wide association studies (GWAS) where the entire genome is scanned for common genetic variation, but here it only focused on the target genes. Development of Molecular genetic markers has a powerful ability to detect genetic studies of individuals, populations or species. These molecular markers combined with new statistical developments have revolutionized the analytical power, essential to discover the genetic diversity.

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Molecular markers and their statistical analysis revolutionized the analytical power, necessary to explore the genetic diversity. Different molecular markers, protein or DNA (mt-DNA or nuclear DNA such as microsatellites, SNP or RAPD) are now being used in fisheries. These markers provide various scientific observations which have importance in aquaculture practice recently such as 1) Genetic variation and population structure study of natural populations 2) Species Identification 3) Assessment of demographic bottleneck in natural population 4) Comparison between wild and hatchery populations 5) Propagation assisted rehabilitation programs. In this review article, we have concentrated on the basics of molecular genetics, an overview of commonly used markers and their application along with their limitations (major classes of markers) in fisheries and aquaculture studies. In this review, the current status of SNP genotyping is discussed regarding the mechanisms of allelic discrimination, the reaction formats, and the detection modalities.

#### Different Types of Molecular Markers used in fisheries:

Molecular markers are categorized into two categories: type I are markers associated with genes of known function and type II markers are associated with anonymous (unknown) genomic segments (O'Brien, 1991) [2]. Under this classification, RFLP markers belong to type I markers as they were identified from known genes segments. Similarly, allozyme markers are also typed I markers because the function of the protein is known, but the RAPD markers are type II markers as RAPD bands are amplified from unknown genomic regions via the polymerase chain reaction (PCR) using ten bp primer. Due to the development of the anonymous genomic regions, AFLP markers also belong to type II class. Microsatellite markers may belong to both classes. Unless they are linked with genes of known function, and they called type II marker. EST markers are type I markers as they developed transcripts of genes. SNP markers are mostly typed II markers unless they are discovered from expressed sequences (eSNP or cSNP) of genes.

#### Co-Dominant and Dominant Markers

In case of the codominant marker, the both of the alleles of a gene are co-expressed in an individual. So these markers are used to distinguish heterozygotes from the homozygotes individuals and allowing determining the genotype and allele frequencies at target loci. RFLPs are codominant since we amplify only one locus and can see the two alleles separately if they are different lengths. RAPDs and AFLPs amplify multiple loci, so we cannot tell if any given locus is heterozygous or homozygous and so it is known as dominant marker. The Polymorphism Information Content (PIC) value is used to measure the informativeness of a genetic marker for linkage studies. At the molecular level, the term polymorphism is most often used by molecular biologists to define genotypic variation, including single base pair variability (or small nucleotide polymorphisms, SNPs) and more significant variations (such as duplications or deletions) within a gene or the genome. The PIC value will be almost zero if there is no allelic variation and it can reach a max of 1.0 if a genotype has only new allele which is a rare phenomenon. This is mainly to assess the diversity of a gene or DNA segment in a population which will throw light on the evolutionary pressure on the allele and the mutation the locus might have undergone over a period. The helpfulness of

molecular markers can be estimated based on their polymorphic information content value (PIC, Botstein *et al.*, 1980) [3]. PIC denotes to the value of a marker for spotting polymorphism in a population. PIC depends on the number of detectable alleles and the distribution of their occurrences and equals one minus the sum of the square of all allele frequencies. For instance, the PIC of a microsatellite marker with two alleles of frequency 0.5 each should be  $1 - [(0.5)^2 + (0.5)^2] = 0.5$ , while PIC for a microsatellite marker of two alleles with allele frequencies of 0.9 and 0.1 is 0.18. Thus, the higher the number of alleles, the higher the PIC; and for a given number of alleles, the more equals the allele frequencies, the higher the PIC. Comparison of PIC values can give researchers a rough idea of the power of the various marker types discussed below to address specific questions in aquaculture genetics.

#### Use of Single nucleotide polymorphism (SNP) marker in aquaculture

A single nucleotide polymorphism (SNP) is a source of variance in the genome. As suggested by the acronym, an SNP ("snip") is a single base mutation in DNA with a usual alternative of two possible nucleotides at a given position. SNPs are the simplest form and the most common source of genetic polymorphisms. There are four major reasons for an increasing interest to use SNPs as DNA markers for genetic analyses. Firstly, they are prevalent and abundant and thus provide more potential markers near or in any locus of attention than other types of polymorphism such as microsatellites. Genome-wide linkage scans tend to employ high density maps of SNPs because both the theoretical and simulation studies, as well as real data application (Liu *et al.*, 2011) [4], indicate that SNPs can achieve a superior power to detect and localize linkages in comparison to other markers. All these characteristics of SNPs made it suitable DNA variant for association analyses of quantitative traits in beef cattle. Different types of techniques are available to discover the genetic variation within and among stocks such as RFLP, RAPD, AFLP, microsatellites, etc. However, these markers are difficult to use in high throughput genotyping protocols, because they are present in low density and not evenly distributed in the genome. Alternatively, SNPs markers can be used because of their abundance, even distribution across the genome, high genotyping efficiency, data quality and analytical simplicity. The non-synonymous SNPs present in the coding regions of genes are of particular interest because they alter the amino acid sequences of proteins. Such alterations may modify protein function and influence performance traits. A number of SNPs in anonymous genes were reported to be significantly associated with weight gain, growth rate, survival and pathogen resistance in *Litopenaeus vannamei*. In 1996, Lander [5] proposed a new molecular marker technology named single nucleotide polymorphism (SNP). It denotes a sequence polymorphism caused by a single nucleotide mutation at an exact locus in the DNA sequence. Liu & Cordes (2004) [6] defined SNPs as those affected by point mutations giving increase to different alleles containing another base at a given nucleotide position within a locus. This sort of polymorphism includes single base transitions, transversions, insertions and deletions. To be considered an SNP the least frequent allele should have a frequency of 1% or greater. Transitions are the most common (~ 2/3) among all the SNP mutation types. SNPs are

becoming the focal point in molecular marker development since they represent the most abundant polymorphism in any organism's genome (coding and non-coding regions). They have a low mutation rate, adaptability to automation and reveal hidden polymorphisms not detected with other markers and methods (Liu & Cordes, 2004) [6]. Theoretically, one SNP within a locus can produce as many as two alleles, each containing one of two possible base pairs at the SNP site. Therefore, SNPs have been regarded as bi-allelic. SNP markers are inherited as co-dominant markers.

#### **Advantages and limitations of SNP markers:**

The most recently designated type of marker is the single nucleotide polymorphism (SNP, pronounced "snip"). As the name suggests, these are variations at a single base on the chromosome. For example, on some chromosomes a locus might have a C, while on other chromosomes the same locus might have a T. These are the most common markers, with at least three million already labeled, and seem to occur diagonally the entire genome. As with RFLPs, there are nearly always only two alleles at an SNP locus. Separately they suffer the same problem as RFLPS of not being valuable in many of the families. They are being used broadly now because they are very easy to genotype, are very common (happening at least ten times more frequently than the other types of markers) and thus can be used in mixture with each other.

#### **Advantages**

- PCR products can be very small:—Markers will work with extremely degraded DNA samples.
- SNPs are more common in the genome. SNPs are found in coding and (mostly) non coding regions and they occur with a very high occurrence about 1 in 1000 bases to 1 in 100 to 300 bases. The richness of SNPs and the ease with which they can be measured make these genetic variations significant. SNPs close to particular gene acts as a marker for that gene. SNPs in coding regions may alter the protein structure made by that coding region.
- It may possibly multiplex hundreds or thousands on one chip.
- Sample processing may be completely automated.
- No stutter products.

#### **Disadvantages**

- Its PIC (polymorphic information content) is lower than microsatellite markers because, it is a bi-allelic. Each marker is less informative.
- Therefore have to genotype many more SNPs to get the same level of information about DNA sample.
- Mixture interpretation is more difficult
- Multiplexes don't actually work yet. Currently uses more of the DNA sample than STRs use.

#### **Importance of Marker Assisted Selection (MAS) in Fish Breeding**

The correlation between genetic variations in the target gene with performance traits of fishes is used in a candidate gene association study to increase the aquaculture yield, improve incomes of farmers and enhances food security. Many selective breeding programs in fish were carried out, but it takes much time to optimize the target traits (Haldar, 2018) [7]. Selection of the wanted traits and enhancement of fish species

has been a part of the traditional breeding programmes. This is mainly based on the documentation of phenotypes in fishes. It is now an accepted fact that the phenotypes do not necessarily represent the genotypes. Many times the environment may mark the genotype. Thus, the fish genetic potential is not truly reflected in the phenotypic expression for various reasons.

The molecular marker-assisted selection is based on the identification of DNA markers that link to desirable fish traits. These traits include resistance to pathogens and body growth, flesh quality, and various other qualitative and quantitative traits. The advantage with a molecular marker is that a fish breeder can select a suitable marker for the necessary trait which can be detected well in advance.

#### **Molecular Breeding**

With the advancement in genetic engineering and molecular biology, it becomes easy and quick to develop a improve fish species with the desirable trait. The term molecular breeding is used to represent the breeding methods that are coupled with genetic engineering techniques. Over conventional selection methods, molecular breeding is profitable for the aquaculturist for a low heritability trait like growth in fish. Its assistance to detect the existing genetic variation in brooder stock and can be used to improve desirable traits in target fish species. Improvement of performance traits through traditional selection integrated with molecular tools is more accurate, fast and allows us to recognize the genetic mechanism controlling performance traits.

#### **Microsatellites marker in fisheries**

Microsatellites contain many copies of tandemly settled simple sequence repeats (SSRs) that range in size from 1 to 6 base pairs. Rich in all species studied to date; microsatellites have been assessed to occur as frequently as once every 10 kb in fishes (Wright, 1993) [8]. Dependent on the number of base pairs in a repeat unit, microsatellites can be sub-categorized into mono (e.g., C or A), di (e.g., CA), tri (e.g., CCA), tetra (e.g., GATA) repeat unit microsatellites. According, the short repeats of about 10-30 bases are microsatellites, and longer repeats between 10-100 bases are minisatellites. Since microsatellites loci are typically short, it is easy to amplify them using PCR and subsequently analyzes the amplified products. Due to the lead of microsatellites in contrast to other molecular markers, they have gradually replaced allozymes and to some extent mtDNA. For the reason that hyper-variability of microsatellites, these markers regularly show tens of alleles at a locus that differ from each other in the number of the repeats. Microsatellites have a habit of to be evenly dispersed in the genome on all chromosomes and all regions (coding regions, introns, and in the non-gene sequences) of the chromosome. Most microsatellite loci are comparatively small, extending from a few to a few hundred repeats. The moderately small size of microsatellite loci is crucial for PCR-facilitated genotyping. Generally speaking, microsatellites containing a more substantial number of repeats are more polymorphic, through polymorphism has been observed in microsatellites with as few as five repeats. Microsatellites inherited in a Mendelian fashion as codominant markers. Regardless of specific mechanisms, changes in numbers of repeat units can result in a large number of alleles at each microsatellite locus in a population. Microsatellite markers involve a significant amount of up-

front investment and effort. Each microsatellite locus has to be recognized, and its flanking region sequenced to the design of PCR primers. Microsatellite-enriched genomic DNA libraries can be made for most efficient marker development. Because of polymerase slippage during replication, small size alterations between alleles of a specified microsatellite locus (as little as two bp in a locus comprised of di-nucleotide repeats) are possible. Microsatellites recently have become a prevalent marker type in a wide variety of genetic investigations.

### **Quantitative Trait Loci (QTL)**

The rate of the genetic gain through MAS can increase when there is a continuous identification of new QTL for the targeted traits. The extra genetic gain through the MAS may decrease very quickly in successive generation of selection for the same QTL, and also the rate of identification of new QTL is hard to predict. The information developed from the detected QTL in the selection needs developing some selection criteria to connect this molecular information with phenotypic variation. MAS can be powerfully used to detect the major QTLs for a trait and for a phenotypic which is costly to measure. Till date, QTL for traits such as growth and disease resistance, cold and salinity, sex determination have been mapped in fishes. Such studies can provide essential information about outlines and the rate of evolutionary changes besides may use as tools for marker-assisted selection in selective breeding. Mapping genes and QTLs is of fundamental and applied concern in aquaculture species. The purpose of QTL mapping is to assist in selective breeding by understanding the effect and quantity of genes determining a trait. QTL analysis for essential traits like thermal tolerance, spawning time, embryonic development, growth traits, stress, salinity tolerance, infectious hematopoietic necrosis and bacterial cold-water disease have been conducted for more than 20 aquaculture species including finfish, mussels, and crustaceans.

### **Other Applications of DNA Markers in Aquaculture Genetics**

One of the inquiries at the start of any genome research is what type of marker is most appropriate given the project at hand and the species of curiosity. However, with a good understanding of the DNA marker technologies, suitable choices can be reached. In this regard, one has to know if there is already molecular information available when choosing a marker system. RAPD and AFLP markers do not require prior molecular information for the target species. Comparing between RAPDs and AFLPs, levels of polymorphism and power of differentiation are much more significant for AFLPs than for RAPDs. Selecting between these two marker methods is contingent on the tasks involved and the equipment obtainable. If the target is to reveal whether fish are pure species or F1 hybrids, RAPDs are probably sufficient, since the most straightforward way with the least requirement for resources is the best technique if the same objective can be met. If the analysis requires a more powerful approach to reveal higher levels of polymorphism, AFLPs will provide a higher level of differentiation than RAPDs. For genome mapping, AFLPs are highly superior to RAPDs because of their more excellent Use of microsatellites requires the open expanse of the microsatellite markers. If there are time and resources to develop such markers, it is

worthwhile in the long run, especially for genome mapping. However, if genome mapping is the primary goal, consideration should be given from the start to the development of type I microsatellites, i.e., microsatellites associated with genes of known functions. Another technical shortcoming of microsatellites is the difficulty involved in nonspecific amplification (amplification of secondary DNA products other than the targeted microsatellite locus due to nonspecific binding of the PCR primers), although most researchers overcome this problem by optimizing PCR conditions. Also, genotyping with microsatellites (especially those with dinucleotide repeats) is often complicated by the presence of so-called stutter bands. Polymerase slippage forms stutter bands during PCR amplification, which results in secondary products comprising one or more recurrence units less than the main allelic band. Stutter bands can sometimes equal the power of the primary band, making it difficult to describe genotypes, chiefly in population studies precisely. In gene mapping, the genotypes of parents are already identified, so segregation of alleles in the progenies is relatively straightforward. In population studies where understanding is not known, interpretation can prove problematic. For population studies, one other reflection is the high level of microsatellite polymorphism.

### **Species, Strain, and Hybrid Identification**

Genetic identification of species or strains is sometimes compulsory in an aquaculture setting. Because of the significant genetic differences among most species, their identification using DNA markers is relatively straightforward. RFLP, RAPD, AFLP, and microsatellite markers are all applicable, but RAPD analysis probably provides the least expensive, yet reliable identification of species if no prior molecular information is available. Each species will exhibit a RAPD profile with unique binding patterns, and a simple comparison of profiles generated using one or two primers should be sufficient for species identification. Strain documentation is more complicated since fixed, strain-specific markers are not typically obtainable for strains within a species. The amount of genetic variation among strains may be incomplete and may require DNA markers and methods with higher resolution than traditional markers such as allozymes, RFLPs, or RAPDs. Both microsatellites and AFLPs have been shown to provide sufficient power for the determination of strains in aquaculture fish species. The use of allele frequency analysis across multiple microsatellite loci is a robust approach for delineation of individual strains. Allele frequencies for each microsatellite locus are treasured for each strain involved, and those microsatellites that have incredibly differential allele frequencies among strains are used for strain identification.

### **Conclusion**

Molecular markers have revolutionized the biological science and fisheries and aquaculture is not an exception. The markers discussed above show diverse usage in the applied aquaculture and fisheries research. The growing applications are reflected by the frequency of related literature being published to different journals. A range of area in fisheries where these markers can be used as valuable tools are species identification, population size and structure, intra specific relationship, stock enhancement, hatchery management, selective breeding, systematic and evolutionary genetics, legal



applications and many other. These molecular markers can prove fruitful in fields like fish nutrition and disease management programmes.

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