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Microsatellite markers in Aquaculture: Application in Fish population genetics

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Abstract: *Microsatellites or Simple Sequence Repeats (SSRs) represent an abundant source of genetic markers which are highly abundant and dispersed evenly throughout eukaryotic genomes. They have become the markers of choice for a wide range of applications in population genetic, conservation and evolutionary biology. Microsatellites represent ideal molecular markers because they have multiple alleles which are highly polymorphic among individuals. Polymorphism is achieved by having variable numbers of tandem repeat motifs resulting in size variation which can then be visualized by PCR with pairs of locus-specific flanking primers, followed by electrophoresis of the amplification product. Microsatellite motifs occurs once every 10kb in fishes. They are inherited in a co-dominant fashion, and are fast and easy to assay. They are co-dominant in nature with high levels of polymorphism and can reproduce very well. Hence, giving better information than the dominant marker .This makes them a choice maker for estimating population structure and genetic diversity.*

Keywords: *Microsatellites, markers, fish, population, genetics*

I. Introduction

Molecular genetic markers are powerful tools to detect genetic uniqueness of individuals, populations or species (Doveri *et al.,* 2008). Modern sequence based marker systems for genetic analysis such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs) are now predominantly used (Duran *et al*. 2009).

However, Microsatellites have become the marker of choice for application in fish population genetic studies (Beckmann and Soller, 1990). They have multiple alleles which are highly polymorphic among individuals. The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks (Alam and Islam, 2005), population analysis and biodiversity conservation (Romana-Eguia *et al.,* 2004). Simple Sequence Repeat (SSR) markers are preferable because they are potentially co-dominant and highly polymorphic. In addition; microsatellites have a wide distribution in the genome and can be efficiently identified, which is essential in studies about genetic variability of populations (Boris *et al*., 2011).

High cost of developing species-specific markers has been the main challenge of microsatellite markers in the past is the (Castoe et al., 2010). Now, this has been alleviated with the advent of Next-generation sequencing, which allows the detection and characterization of SSR loci easily achievable with simple bioinformatics (Abdelkrim *et al.,* 2009).

Recently, molecular markers have been commonly used for population studies (Al-Atiyat *et al*., 2012). Microsatellites have been used to study the genetic diversity of farmed and wild population's fishes (Norris *et al*., 1999; Boris *et al,* 2011).

In Nigeria, molecular marker based on PCR techniques has been used to determine population structure, and genetic diversity of fish (Ahmad *et al*., 2012; Mojekwu *et al*., 2012). Though there is little information in this area.

Therefore, this write up x-rays the applications and relevance of SSR markers to fish population genetics in aquaculture.

Population Genetics

Population genetics is the study of genetic variation among species, individuals and populations; fundamentally, it shows that distribution of genetic variability is affected by evolutionary forces of mutation, migration, selection, and random genetic drift (Hansen, 2003).

Assessing genetic diversity in aquaculture stocks or wild fish populations is crucial for effective management, interpretation, and understanding and of fish populations or stocks.

Many characteristics and methods have been used to analyze stock structure in fish populations; they include ecological, tagging, parasite distribution, physiological and behavioural traits, morphometrics and meristics, calcified structures, cytogenetics, immunogenetics and blood pigments (Samaradivakara et al., 2012).

Unfortunately, environmental variables often affect the relationship between genes and their phenotypic expression significantly.

Thus, the population geneticists mainly focused on Mendelian traits in species widely used in laboratory studies or on available pure breeds of few species (Hallerman, 2003).

The development of DNA amplification using the PCR (Polymerase Chain Reaction) technique opened up the possibility of examining genetic changes in fish populations over the past years (Ferguson *et al*., 1995). Today many molecular methods are available for studying various aspects of wild populations, captive brood stocks and interactions between wild and cultured stocks of fish and other aquatic species (Yudha *et al*., 2012). The choice of markers for particular method is not straightforward and mostly depends on the experience of the investigators, laboratory facilities and available fund. Thus, there is a need for occasional reviews of the developments in techniques, applications and interpretations of the data gathered.

II. Microsatellite

Microsatellites are highly abundant in various eukaryotic genomes including all aquaculture species studied to date. In most of the vertebrate genomes, microsatellites make up a few percent of the genomes in terms of the involved base pairs, depending on the compactness of the genomes (Zhan *et al*., 2009).

In fish, one microsatellite was found every 1.87 kb of DNA. For comparison, in the human genome, one microsatellite was found every 6 kb of DNA (Beckmann and Weber 1992). It is reasonable to predict that in most aquaculture fish species, one microsatellite should exist every 10 kb or less of the genomic sequences, on average (Wright, 1993; Duran *et al*., 2009).

Their high polymorphism, and PCR based analysis has made them one of the most popular genetic markers ((Duran *et al*. 2009; Boris *et al*., 2011).

Some microsatellite loci have very high numbers of alleles per locus (>20), making them very useful for applications such as parent-offspring identification in mixed populations, while others have lower numbers of alleles and may be more suited for population genetics and phylogeny (Al-Atiyat *et al*.,2012). Primers developed for one species will often cross-amplify microsatellite loci in closely related species (Boris et al, 2011). Genotyping of microsatellite markers is usually straightforward (Castoe *et al.,* 2010).

However, its disadvantage include the requirement for existing molecular genetic information, the large amount of up front work for microsatellite development, and the tedious and labor intensive nature of microsatellite primer design, testing, optimization of PCR conditions and cost (Telles *et al*., 2010).

Applications of Microsatellite Markers in fish population

Microsatellite markers are ideal for many types of applications in aquaculture. They give crucial information in aquaculture fish population, such as: (i) identification of Genetic variability between and within stocks; (ii) monitoring Genetic changes in stocks; (iii) parentage and pedigree analysis in selective breeding ;(iv) Genomic mapping and detection of quantitative trait loci (QTL) (Fjalestad *et al*., 2003;Subasinghe *et al*., 2003; Chistiakov et al.,2005).

Several papers has reviewed the use of Microsatellite markers in aquaculture, in particular their integration into breeding programmes (e.g.Fjalestad *et al*., 2003; Taniguchi, 2003; Cross *et al*., 2004; Boris *et al* 2011; Al-Atiyat *et al*.,2012).

Identification of Genetic Variability between and within Fish Stocks

Molecular markers can be useful tools in stock identification and monitoring potential changes in broodstock, called DNA fingerprinting (Fjalestad *et al*., 2003; Ahmad *et al*., 2012; Mojekwu *et al*., 2012). Almost all major molecular markers from allozymes to microsatellite have been used in determination of between and within genetic variations in hatchery stocks (Sekino *et al*., 2002; Ramos- Paredes and Grijalva-Chon, 2003; Samy-Yehya *et al*., 2012).

Studies have shown loss of genetic variation in hatchery stocks (e.g. in salmonids) as a result of different factors including a low effective number of parents, domestication selection or the mating design (Samaradivakara *et al*., 2012). Sekino *et al*. (2002) assessed genetic divergence within and between hatchery, and wild populations of Japanese flounder (*Paralichthys olivaceus*) by means of microsatellite and mtDNA sequencing analysis. Desvignes *et al*. (2001) studied the genetic variability of French and Czech strains of hatchery stocks of common carp (*Cyprinus carpio*) using allozymes and microsatellites. They detected a more pronounced discrimination between the strains of the two countries by the microsatellite markers. Bartfai *et al*.

(2003) analyzed the whole broodstock of two Hungarian common carp farms (80 and 196 individuals) by using RAPD assay and microsatellite analysis. Microsatellite analysis was more informative than RAPD assay. Microsatellites are the assay of choice for the discrimination of culture stocks (Duran *et al*., 2009; Boris et al., 2011).

Monitoring Genetic Changes in stocks

Microsatellite assays have been very successful in detecting the genetic impact of culture (Zhan *et al*., 2009). The higher sensitivity Microsatellite to phenomena such as genetic drift and founder effect make this marker ideal for monitoring the consequences of founding and propagation in aquaculture than of mtDNA and allozyme (Duran *et al*., 2009).

Microsatellite markers have been used for minimizing inbreeding in rainbow trout (Fishback *et al*., 1999). Analysis of the F1 generation of a Greek gilthead sea bream broodstock revealed a 15% reduction in the number of alleles and a homozygosity increase of 1.5% (Magoulas, 1998).

Thus, SSR markers have important applications in monitoring inbreeding depression. For example, microsatellite markers can be used to locate the specific chromosomal regions responsible for inbreeding depression. This would be most feasible with cultured species where parents and their progeny can be managed and traced within a closed system. It will be possible to use mapped genetic markers to trace the inheritance of specific chromosomal arms in progeny (Morelli, 2007). The polymorphism obtained with microsatellite markers have provided strong information utilized in the management of fish stocks (Alam and Islam, 2005).

Parentage and Pedigree Analysis in Selective Breeding

Selection programmes uses both information on the candidates for selection and their relatives in order to increase the precision of selection and hence selection responses.

One of the applications of Microsatellite markers in brood stock management is in the assessing of the contribution of possible parents in a mass spawning. Typically limited numbers of broods are used in spawning and some putative parents apparently fail to spawn. In this case SSR and other genetic markers, becomes useful in quantifying the relative success of the potential parents. Parentage can be determined using minisatellite or microsatellite markers after spawning (Moran *et al*., 1996; Thompson *et al*., 1998).

One of the most important hindrances to applying effective selective breeding programmes for fish is that newborn individuals are too small to be tagged physically.

Thus, selective programmes making use of family information have needed to keep families separated until the fish are large enough to be individually tagged. This is costly, limits the number of families available for selection and can induce environmental effects common to the members of the same family (Doyle and Herbinger, 1994).

This problem can be resolved by applying Microsatellite and other DNA-based genetic markers. Consequently, more families can be kept in the breeding stock without the need for using separate tanks at early ages. These markers have been used to assess family/parentage identification in many species and can be used to discriminate fish in mixed family groups (e.g. Herbinger *et al*., 1995; Fjalestad *et al*., 2003; Cross *et al*., 2004; Duran *et al*., 2009;Boris *et al.,* 2011; Al-Atiyat *et al*.,2012).

A breeding programme can be initiated with a previously unselected farm raised strain by using a method, termed walk-back selection (Doyle and Herbinger, 1994). In general, this will involve the physical tagging and biopsy of individuals when they are large enough to be marked, with microsatellite analysis based on the biopsy used to assign individuals to family.

Several studies have empirically used microsatellite loci to successfully reconstruct pedigrees in fish populations with families mixed from hatching (Norris *et al*. 2000;Morelli 2007;Zhan et al., 2009; Olivatti *et al.,*2011).

Villanueva, developed deterministic predictions for the power of microsatellites for parental assignment and compared with stochastic simulation results. Their results showed that the four loci that are more informative are enough to assign the offspring to the correct pair up to 99% with 100 crosses that involves 100 males and females respectively. Doyle et al. (1994) used them to discriminate family groups of cod (Gadus morhua). In these cases, offspring assignment was to known parental types.

However, with sufficient levels of variability, family or parental discrimination may also be achievable in the absence of parental information (Norris *et al*., 2000).However, microsatellites appears more popular because of their potential for high variability even among individuals of the same strain (Liu and Cordes 2004).

QTLs of aquatic organisms

A high number of QTL research has been conducted for sex determination and sex linkage in fish using microsatellites because of the great interest in producing monosex populations of certain species.

Sex linked inheritance in fish was first reported by Aida (1921) in medaka. Waldbieser *et al*. (2001) examined 293 polymorphic microsatellite loci in channel catfish, and seven of these loci were closely linked to the sex-determining chromosome region. *Oreochromis* QTLs for sex and colour have been mapped (Kocher *et al*., 2002).

Ozaki *et al*. (2001) used 51 microsatellite markers to identify several chromosome regions containing putative QTL genes that affect resistance to infectious pancreatic necrosis (IPN) in rainbow trout. Tanck *et al*. (2001) utilized 11 microsatellites and found that they were correlated with stress-related plasma cortisol levels and basal plasma glucose levels in common carp.

QTLs for fitness traits and survival have been identified in fish and shellfish. A microsatellite accounted for 7.5% of the variance in thermal tolerance in unselected populations of rainbow trout (Perry *et al*., 2001).

Table of some examples of linkage maps constructed with microsatellite markers in aquaculture species.

Species	Common name	References
Salmo trutta	Brown trout	Gharbi *et al*., 2006
Dicentrarchus labrax	European sea bass	Chistiakov *et al.,* 2005
Oreochromis spp.	Tilapia	Lee *et al.,* 2005
Plecoglossus altivelis Xiphophorus	Ayu	Watanabe et al. 2004, Walter *et al*.,2004
Salvelinus alpinus	Arctic char	Woram *et al*., 2004
Salmo salar	Atlantic salmon	Gilbey *et al*., 2004, Moen *et al*., 2004
Oncorhynchus mykiss	Rainbow trout	Sakamoto *et al*., 2000, Nichols *et al*., 2003
Ictalurus punctatus	Channel catfish	Waldbieser *et al*., 2001
Danio rerio	Zebrafish	Shimoda *et al*.,1999
Crassostrea gigas	Pacific oyster	Hubert and Hedgecock 2004
Crassostrea virginica	Eastern oyster	Yu and Guo 2003
Seriola quinqueradiata and *Seriola lalandi*	Yellowtails	Ohara *et al*. ,2005
Cyprinus carpio	Common carp	Sun and Liang 2004
Paralichthys olivaceus	Japanese flounder	Coimbra *et al*., 2005

Source: Zhanjiang, J. L (2007).

Microsatellite application to fish Population genetics in Nigeria.

The applications of molecular-based knowledge in fish genetics and stock management have been confined to developed and some Asian developing countries.

Several papers has reviewed the use of microsatellite markers in aquaculture and fisheries, (e.g Fegurson et al., 1995;Davis and Hetzel, 2000;Fjalestad *et al*., 2003; Taniguchi, 2003; Cross *et al*., 2004; Alam and Islam 2005;Boris *et al*.,2011; Al-atiyat *et al*., 2012;Yudha *et al.,* 2012).

In Nigeria, quite a number of published work exist on molecular markers in fish characterization using RAPD markers (Ahmad *et al*., 2012; Mojekwu *et al*., 2012; Megbowon and Bombata, 2013; Mojekwu *et al.,* 2013).

However, there are little or no publications and information to the best of my search on the use of microsatellite markers to study fish population in Nigeria.

Though Microsatellite markers have been applied in Nigeria to study population and genetic diversities in livestock; chicken (Olowofeso *et al*., 2005), Insects; malaria mosquitoes and plants (Fatokun, *et al*., 2008; Ogunkanmi *et al*., 2010).

III. Conclusion

Microsatellites have enabled the assessment of genetic variations at much smaller scales than has been possible with other markers (Sunnucks, 2000; Boris *et al*., 2011).

The polymorphism obtained with microsatellite markers has given useful and detailed information in fish stocks management (Alam and Islam, 2005), biodiversity conservation and population analysis (Romana-Eguia et al., 2004). They are optimal for mapping "causal" genes, whether these are responsible for single or multifactorial traits (QTLs). They are also the best markers for determining parenthood in mass spawning and/or rearing even for evolutionarily related genera (Zhan *et al*., 2009) , tracing escapes from cultured to wild populations and estimating coefficients of kinship among individuals drawn from a population (Hansen *et al*., 2001).

Many studies have successfully demonstrated heterologous amplification of a target specie with SSR primer (Barbosa *et al*., 2006; Hatanaka *et al*., 2006, Morelli *et al*., 2007), thereby lowering the costs of future projects.

Nevertheless, their major drawback remains the high cost and labour intensity involved in the development of primers (Telles *et al*., 2010). Another disadvantage is the existence of null alleles that is alleles that do not amplify in PCR reactions (O'Really and Wright, 1995).

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