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APPLICATIONS OF MOLECULAR TECHNOLOGIES IN SELECTIVE BREEDING OF IMPORTANT AQUACULTURE SPECIES

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Abstract: Fisheries management is getting difficult due to over utilization of fish stocks, pollution and various anthropogenic activities resulting in reduction of genetic resources and its variations. The use of reproductive and genetic technologies can increase the efficiency of selective breeding programs for aquaculture species. Marker-assisted selection can result in greater genetic gain, particularly for traits difficult to measure, than the conventional. DNA fingerprinting is the most useful tool for genetic tagging and parentage verification. Both *in-vitro* fertilization and cryopreservation techniques can increase the accuracy of selection by controlling the accumulation of inbreeding.
Keywords: Selective breeding, MAS, Fingerprinting, Cryopreservation.

INTRODUCTION

About 71 % of the globe's surface is covered by seawater and 1 % percent by fresh water. In 1990 global food production of all types were approximately 4.6 billion metric tons of gross tonnage and about 2.4 billion tons of edible dry matter. Aquaculture production is limited compared to that from land-based animals. In 1966 the production of fish and shellfish was estimated to 1 mill. tons and in 1975, 5 million tons (Bakos *et al.*, 1979). The production increased to 7.7 mill. tons in 1985 to 63 mill. tons in 2014 (FAO, 2015). Expansion has therefore been rapid in the last decade. According to New (1991), the aquaculture production must, be 63 mill. tons in year 2025 to meet the demand for fish and shellfish. To reach this goal the production must increase by 4.75 percent per year. Efficient breeding programs will be crucial to this development, not only to reach the production goal but also to reduce production cost, improve disease resistance, improve utilisation of feed resources and improving product quality (Gjedrem, 1997).

Charles Darwin published his book "The origin of species" in 1859 and focused on the idea of selection to improve quantitative characters which is a central conception in quantitative genetics which includes improvement of production traits in livestock and aquaculture

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species (Charles Darwin, 1859). Selective breeding gives 10-20% genetic gain in aquaculture species per generation (Ponzoni *et al.* 2015). Such progress has been achieved by application of quantitative genetics, whereby superior animals are identified and selected based on their performance or that of their relatives. Recently, the advent of molecular genetics has opened possibilities for direct selection of animals on genotype selection based on linkage associations between markers and quantitative trait loci (QTL). However, the benefits from the use of these technologies will not be fully realized unless the cost of genotyping is reduced. By contrast, reproductive technologies, like artificial insemination and *in-vitro* fertilization, have significantly increased the rate of genetic improvement and have a large impact on the breeding structure (Dekkers and Hospital 2012).

For aquaculture species, these areas of research have been barely touched upon, and their application to selective breeding programs has been very limitedinspite of selection response is usually higher in fish and shellfish than in farm animals (Olesen*et al.,* 2003). The objective of this paper is to discuss some thoughts on potential technologies that can be considered for current breeding programs in carps, tilapia, shrimps and other economically important species for better management and conservation of genetic diversity.

APPLICATIONS OF MARKER-ASSISTED SELECTION (MAS)

The usefulness of molecular information depends on advances made in four main areas of research: molecular genetics (genetic markers and linkage maps), genes &quantitative trait loci (QTL) detection, genetic evaluation systems, and marker-assisted selection. So far, genetic maps have been constructed for tilapia (Lee *et al.* 2005), common carps (Sun and Liang 2004), rainbow trout (Nichols *et al.* 2003), Atlantic salmon (Moen *et al.* 2004), *Penaeusmonodon* (Wilson *et al.* 2002) and catfish (Lui *et al.* 2003).However, only a limited number of studies are found on QTL affecting cold tolerance and salinity tolerance in tilapia (Cnaani*et al.* 2003), cold tolerance in common carps (Sun and Liang, 2004), thermal tolerance (Perry *et al.* 2001), development rate (Sundin *et al.* 2005) and pyloric caeca number (Zimmerman *et al.* 2005) in rainbow trout. To the best, there have not been any causative mutations or candidate genes controlling performance and production traits reported in aquatic species. Hence, the potential for direct Genotype- Assisted Selection (GAS) or Introgression Assisted Selection (IAS) cannot be realized at this stage, although in theory the IAS method could be carried out with informative markers. Based on linked markers published for aquaculture species in the literature, there are two possible uses of Marker-Assisted Selection (MAS): in cross populations between inbred lines, and within strains

(Dekkers 2004). For each of these methods, three strategies can be employed, namely: 1) Selection on Estimated Breeding Values (EBV) derived from markers alone (MAS), 2) Selection on markers-based EBV first and then on polygenic EBV, and 3) Index selection combining both QTL-EBV and polygenic EBV.

MAS for crosses between inbred lines:

As firstly proposed by Lande and Thompson (1990), compared three strategies: selection on marker score alone (MAS), BLUP selection (only polygenic EBV) and index selection combining both markers-based EBV and polygenic EBV (COMB) in F2 generation population. Genetic gain was the highest with combined selection on both QTL-EBV and polygenic EBV (COMB), followed by BLUP, and the lowest with MAS. The rate of response to MAS decreased over generations because of crossing-over during meiosis, caused an erosion of the association between markers and QTL. The MAS strategy has potential for selection of traits that are difficult to measure (e.g. flesh quality) because it does not require extensive phenotypic recording. For aquaculture species, inbred lines are infrequently available having very low fitness.

MAS within strains:

This method has potential of selection for traits that are measured on flesh quality or traits that are recorded in only one sex. The efficiency of MAS within strain is largely dependent on heritability of the interested traits, size of QTL effects and recombination rate, increasing for lowly heritable traits and with the proportion of the variance explained by the QTL (Meuwissen and Goddard 1996). The advantage of MAS selectiondecreases over generations due to fixation of QTL and loss in polygenic response. Despite high efficiency expected from theoretical prediction (2 to 60%), this method of selection requires extensive recording of both phenotypic and genotypic data for several generations in order to accurately estimate QTL effects. In freshwater finfish, flesh quality is not only a trait of a primary emphasis but the weight of the fish also plays an important role. Hence, breeding objectives for farmed finfish have mainly focused on improvement of body weight at harvest or growth related traits.

For disease resistance, most of the species are generally disease free if well managed, and adapted to the local conditions. Improvement of survival rate can be achieved by modification in managements like feeding, water quality, etc.

DNA FINGER PRINTING

DNA fingerprinting can be used for genetic tagging, parentage verification, control of inbreeding, and prediction of heterosis. Genetic tagging and control of inbreeding are of practical significance in aquaculture breeding programs. The posterior assignment of parents and tracking origins of family allow pooling of all families from incubation, thus enabling communal testing very soon after hatching. This overcomes two major problems encountered in aquaculture species *viz.*1) Maternal genetic and common environmental effects (caused by separate rearing of full sibs until they reach the size at which they can be physically tagged) can be avoided. 2) The number of tested families can be increased without the need for increasing facilities (e.g. tanks, ponds).

Consequently, the use of DNA markers is expected to increase genetic gain without a rapid accumulation of inbreeding. Although loss in genetic gain is yet to be quantified in aquaculture breeding programs. Results in dairy cattle indicate that pedigree errors may reduce genetic gain by 3 to 10% (Spelman *et al.* 2002). The loss in genetic gain is greater for lowly heritable traits than for highly heritable ones because the accuracy of EBV for traits with low heritability relies more on information from relative's performance than an individual's. Pedigree analysis using microsatellite markers in general has a very high degree of accuracy. The use of between 8 and 14 microsatellites gives a 90 to 95% chance of correct assignment of offspring to pairs of parents in mating designs involving 92 to 240 parental pairs (Fishback *et al.* 2002 and Vandeputte *et al.* 2004). Villanueva *et al.* (2002) support this result in a deterministic simulation study where four highly polymorphic loci developed for salmon are sufficient to assign 99% of the offspring to the correct pair of parents with 100 crosses involving 100 males and 100 females. Both these experimental and theoretical results indicate that parentage identification is possible with the DNA markers currently available in several fish species.However, the technology is still expensive for aquaculture species (Vandeputte 2004). Hence, costbenefit analysis should be carried out to assess the economic desirability of this technology into breeding programs.

GENETIC CHARACTERIZATION OF STRAINS

The identification of populations or strains with superior characteristics is one of the most critical steps before the commencement of selective breeding programs, especially of new aquaculture species. DNA markers can be used to identify genetically distinct populations. Characterization of genetic variation among populations in this way aims to group and to help determine strains to be included in strain evaluation trials. A mixed (synthetic) base

population may then be established from the best performing individuals involving all strains in a diallelcross design.

However, results with electrophoresis analysis in fish and terrestrial animals have shown that, despite the high level of homogeneity, there are still differences in production characteristics between the strains (Jones *et al.* 2000). A typical example is the large genetic variability in a performance trait such as live weight in the GIFT (Genetically Improved Fish Tilapia) population undergoing several generations of selection although the observed heterozygosity ranged only from 0.026 to 0.071 in the founder strains (Macaranas *et al.* 1995). Therefore, it is concluded that genetic characterization of strains before assembling breeding population may be of some use in establishing that two or more populations are likely to be closely related, but it is of no value in terms of ascertaining performance or genetic variation for performance traits.

REPRODUCTIVE TECHNOLOGIES

Cryopreservation of Milt

To date, preservation of eggs and embryos has not yet been at the stage in aquatic species. In selection programs at nucleus level, cryopreserved milt and embryos canbe used as a control to measure genetic gain with minimum bias. This is mainly because the frozen material can present a wider genetic base than a random unselected control of limited size, and there is no accumulative genetic drift over time. The improved genes of superior sires proven from the selection programs are then transferred to either hatcheries or producers. In a number of species, e.g. Atlantic salmon (Salte *et al.* 2004) or oysters (Adams *et al.* 2004), cryopreserved sperm can be applied in practice. It is a safe way to disseminate the improved genes between herds or populations. As some species spawn once during their life (e.g. salmonids, eels), or in the case of pink salmon that all spawn at two years old, cryopreserved milts can be introduced between populations to reduce the risk of inbreeding.

In future, once large-scale genetic evaluation is underway, cryopreserved sperm can be used to create genetic connectedness through a “reference sire” scheme. In this way, the genetic merit of all animals across globe or years can be compared, ranked and selected as parents. This approach has significantly increased the genetic gain in performance traits of farm animals.

LIMITATIONS TO THE APPLICATION

At this stage of development, a major issue that limits the application of genetic and reproductive technologies in genetic improvements is as follows:

Technical issues:

There has been a lack of high-resolution linkage maps in most of the aquaculture species. The efficiency of MAS is low if markers are located far from the target gene. Even when molecular markers are closely mapped, falsepositive detection of marker and gene association also results in low efficiency of MAS. Experiences in both plant and animals indicate that MAS is successful with traits controlled by single gene with major effects, but little progress has been made with traits controlled by multiple genes. Thiscreates a need to develop new generation markers (e.g. SNP), physical and comparative maps to increase the ability to identify functional mutations or candidate genes in aquaculture species.

CONCLUSION

Based on the currently available knowledge in aquatic species and the lessons from plants and animals, one possibility of utilizing molecular markers in aquaculture practical breeding programs is genetic tagging. The feasibility of cryopreserving spermatozoa and the conduct of *in-vitro* fertilization offer opportunities for increasing the rate of genetic improvement while constraining level of inbreeding.

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