Potential Applications of Reproductive and Molecular Genetic Technologies in the Selective Breeding of Aquaculture Species

Nguyen Hong Nguyen, Alphis. G. Ponniah and Raul W. Ponzoni
The WorldFish Center
PO Box 500 GPO, 10670 Penang, Malaysia.

Abstract

The use of reproductive and genetic technologies can increase the efficiency of selective breeding programs for aquaculture species. Four technologies are considered, namely: marker-assisted selection, DNA fingerprinting, in-vitro fertilization, and cryopreservation. Marker-assisted selection can result in greater genetic gain, particularly for traits difficult or expensive to measure, than conventional selection methods, but its application is currently limited by lack of high density linkage maps and by the high cost of genotyping. DNA fingerprinting is most useful for genetic tagging and parentage verification. Both in-vitro fertilization and cryopreservation techniques can increase the accuracy of selection while controlling accumulation of inbreeding in long-term selection programs. Currently, the cost associated with the utilization of reproductive and genetic techniques is possibly the most important factor limiting their use in genetic improvement programs for aquatic species.

Introduction

Selective breeding in aquaculture species has been very successful, averaging a genetic gain of 10 to 20 per cent per generation (Ponzoni et al. 2005). Such progress has been achieved through the application of quantitative genetics and statistical methods, whereby genetically superior animals are identified, based on their own performance or that of their relatives. Recently, the advent of molecular genetics has opened possibilities for direct selection of animals on genotype or, alternatively, selection based on linkage associations between markers and quantitative trait loci (QTL). During the last decade, efforts have been made to enable the incorporation of molecular genetic information in practical genetic improvement of both plants and animals. However, the benefits from the use of these technologies will not be fully realized unless the cost of genotyping is reduced (Dekkers and Hospital 2002). By contrast, reproductive technologies, especially artificial insemination and in-vitro fertilization (IVF), have significantly increased the rate of genetic improvement and have had a large impact on the breeding structure of livestock species (e.g. Nicholas 1996; Kinghorn et al. 1999; van Arendonk and Bijma 2003). For aquaculture species, these areas of research have been barely touched upon, and their application to selective breeding programs has been very limited. The objective of this paper is to present some thoughts on four technologies that are considered to have potential for current breeding programs in carps and tilapia, namely: marker-assisted selection, DNA fingerprinting, in-vitro fertilization, and cryopreservation.

Application of molecular information

Marker-assisted selection

The usefulness of molecular information in genetic improvement programs depends on advances made in four main areas of research: molecular genetics (genetic markers and linkage maps), genes and quantitative trait loci (QTL) detection, genetic evaluation systems, and marker-assisted selection. So far, genetic maps have been constructed for tilapia (Kocher et al., 1998; Lee et al. 2005), common carps (Sun and Liang 2004), rainbow trout (Young et al. 1998; Sakamoto et al. 1999; Nichols et al. 2003), Atlantic salmon (Moen et al. 2004a), kumara prawn (Moore et al. 1999; Li et al. 2003), *Peneaus monodon* (Wilson et al. 2002) and catfish (Lui et al. 2003). However, only a limited number of studies have found QTL affecting cold tolerance (Cnaani et al. 2003) and salinity tolerance in tilapia (Lee 2003), cold tolerance in common carps (Sun and Liang 2004), infectious pancreatic necrosis in rainbow trout (Ozaki et al. 2000), infectious salmon anemia in Atlantic salmon (Moen et al. 2004b), 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 of our knowledge, 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. By contrast,

several direct DNA tests have been developed in plants and animals; in both cases, the application has focused on direct genetic 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 (COMB).

MAS for crosses between inbred lines: As firstly proposed by Lande and Thompson (1990), Zhang and Smith (1992) 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 an F2 generation population, with 100 markers in a 2000 cM genome. Genetic gain was the highest with combined selection on both QTL-EBV and polygenic-EBV (COMB), followed by BLUP, and the lowest with MAS (Figure 1). The rate of response to MAS decreased over generations because recombination (crossing-over during meiosis) caused an erosion of the association between markers and QTL in the low density map used. 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 seldom available and when they are, they have very low fitness. Hence, at present, this approach is not of practical value in aquatic animal improvement programs.

MAS within strains: This method has potential of selection for traits that are measured on slaughtered animals (flesh quality) or traits that are recorded in only one sex (sexual maturity in female) (Table 1). 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 selection, however, decreases 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 prior to selection in order to accurately estimate QTL effects. In addition, prior knowledge of QTL regions that segregate within the population limits its application to the currently existing breeding programs in tilapia or carps because QTL mapping studies need to be conducted prior to implementation of MAS. In freshwater finfish, flesh quality is not a trait of a primary emphasis and the fish are priced on live body weight in all countries participating in International Network on Genetics in Aquaculture (INGA). 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, especially tilapia, are generally disease free if well managed, and highly adapted to local conditions. Nevertheless, epidemics occasionally occur in these species. Improvement of survival rate can be achieved by modification of environmental factors, such as better management, feeding or water quality. The benefits of including these hard-to-measure traits (flesh quality or disease resistance) by either conventional selection or MAS depend largely on their economic values.

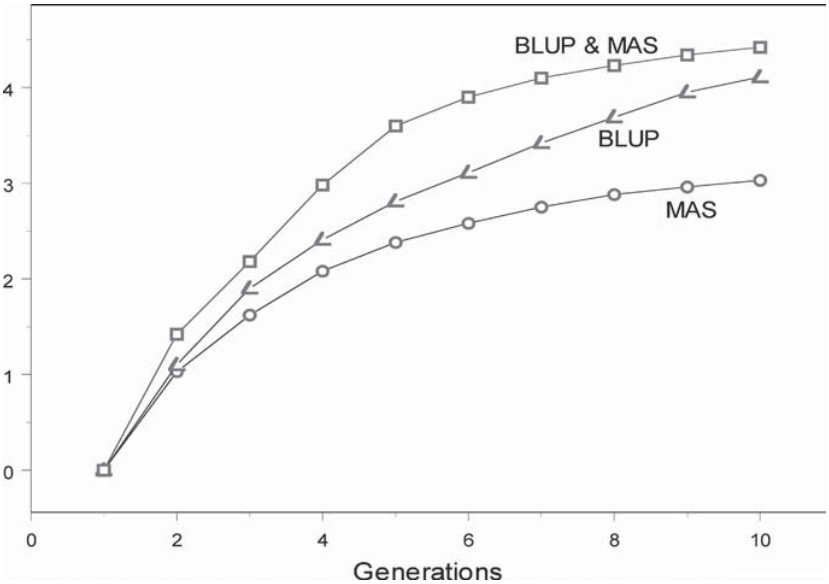

Figure 1. Genetic gain from three different selection strategies in an F2 population created by crossing between two inbred lines for a trait with heritability of 0.25 (Data plotted were extracted from Zhang and Smith 1992).

Table 1. Percentage of genetic gain from index selection combining EBVs for both QTL and polygenes relative to conventional BLUP selection (Derived from Meuwissen and Goddard 1996).

Generations	Types of traits measured				Heritability for BS and AS				% variance explained by QTL			
	CT	SL	BS	AS	0.1	0.27	0.1	0.27	6.7	13.3	26.7	46.7
1	64	38	38	9	21	9	45	38	5	13	29	47
2	62	37	30	5	17	5	36	30	5	12	23	40
3	55	31	25	4	13	4	34	25	4	10	19	33
4	39	21	15	2	6	2	23	15	4	7	12	25

CT = carcass traits, SL = sex limited traits, BS = traits measured before selection and AS= traits measured after selection

DNA fingerprinting

DNA fingerprinting (Figure 2) can be used for genetic tagging and parentage verification, control of inbreeding, elimination of deleterious recessive genes, 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. First, both 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. Second, the number of tested families can be increased without the need for increasing facilities (e.g. tanks, ponds). As a consequence, the use of DNA markers is expected to increase genetic gain without a rapid accumulation of inbreeding. In breeding programs carried out under field conditions, tag losses may be as high as 5 to 35 per cent. An example of this is on GIFT tilapia (Ponzoni, pers. com). 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 per cent (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 relatives' performance than an individual's own performance.

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 per cent chance of correct assignment of offspring to pairs of parents in mating designs involving 92 to 240 parental pairs (Estoup et al. 1998; Fishback et al. 2002 and Vandeputte et al. 2004). This result is supported by Villanueva et al. (2002) in a deterministic simulation study where four highly polymorphic loci developed for salmon are sufficient

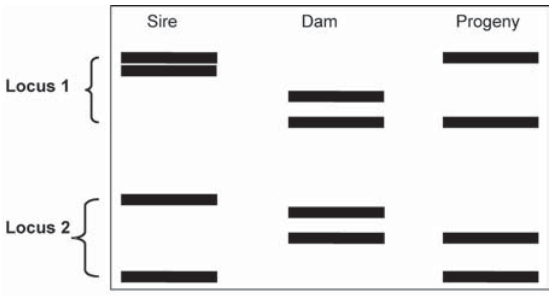

Figure 2. Analysis of DNA fingerprint for 2 loci.

to assign 99 per cent of the offspring to the correct pair of parents with 100 crosses involving 100 males and 100 females. An additional marker is required for correctly assigning 99 per cent of the offspring when 100 crosses are produced from 10 males and 10 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 (cost of genotyping at 15 to 30 Euro per sample, Vandeputte 2003). Hence, cost-benefit analysis should be carried out to assess the economic desirability of incorporating this technology into breeding programs. For cattle and sheep in Australia, the acceptable price for a wide adoption of the DNA test by the industry is from 10 to 15 AUD[a] while the current rate is about 35 AUD (Kinghorn et al. 1999). It should be noted also that genetic tagging does not completely replace the need for physical tags, because the individuals need to be associated with both the parentage and the performance information for genetic evaluation and selection purposes. This would add extra costs to selection programs. The cost of genetic tag is expected to go down with the development of DNA chips, by which many SNP (single nucleotide polymorphisms) markers can be genotyped simultaneously and cheaply.

[a] 1 USD = 1.39 AUD

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 diallel cross design. However, results with electrophoresis analysis in fish and terrestrial animals have shown that, despite the high level of homogeneity at molecular levels that are typical of many crustaceans, there are still markedly differences in production characteristics between the strains (e.g. Jones et al. 2000). A typical example is the large genetic variability in a performance trait such as live weight in the GIFT fish population undergoing several generations of selection (Ponzoni, unpublished results), although the observed heterozygosity ranged only from 0.026 to 0.071 in the founder strains (Macaranas et al. 1995). It is, therefore, 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

In-vitro fertilization (IVF)

IVF has been successful in several aquaculture species and the technique is well developed and can be applied in the current breeding programs in carps and tilapia. The main advantage of IVF is that it allows design of different mating schemes in selection programs. In particular, the use of a factorial (complete, incomplete, by set, rectangular) design enables separation of additive, dominance, and maternal components of variance. This would give unbiased prediction of breeding values and would result in greater accuracy of selection. Compared with hierarchical mating, the factorial design results in greater genetic gain at the same level of inbreeding (Dupont-Nivet et al., in press). It should be noted that the increase in genetic gain with IVF is a result of an increase in the accuracy of selection and a decrease in the level of inbreeding, but not generation interval because in-vitro maturation of oocytes (immature eggs) has not yet been developed. Hence, the generation interval for females cannot be lower than the age at which the female fish reach sexual maturity. In practice, the cost of setting up an artificial incubator system to implement IVF in selective breeding is generally reasonable (Danting, personal communication). It is, therefore, recommended that the IVF technique could be incorporated in current genetic improvement programs for both carps and tilapia.

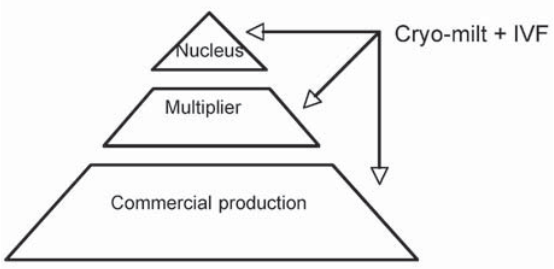

Figure 3. Roles of cryopreserved milt and IVF in a pyramid breeding structure.

Cryopreservation of milt

To date, preservation of eggs and embryos has not yet been possible in aquatic species (except for oysters and seabream). The species in which successful cryopreservation of milt has been achieved are listed in Table 2. The roles of cryopreserved milt in a traditional pyramid breeding structure are illustrated in Figure 3. In selection programs at nucleus level, cryopreserved milt and embryos may be 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 (multiplier) or producers (commercial grow-out production). 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), 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 the 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 herds (country) or years can be directly compared, ranked and selected as parents; thus, genetically superior broodstock can be identified and widely used. This approach has significantly increased the genetic gain in performance traits of farm animals.

Table 2. Successful milt cryopreservation in aquatic species.

Freshwater Finfish	Marine Finfish	Crustaceans	Mollusc	Invertebrate
Carps	Atlantic cod	Prawn	Oyster*	Sea urchin
Tilapias	Herring			
Catfish	Grouper			
Pike	Mullet			
Salmonids	Plaice			
	Turbot			
	Striped bass			
	Red snapper			
	Sea perch			
	Pacific bluefin tuna			
	Gilthead seabream*			

Embryo cryopreservation successful

In the GIFT project, the gene bank for cryopreserved milt was established and is still being maintained. Given availability of facilities and resources, a similar program should be initiated for the carps. Despite the potential benefits of the technique, commercial cryopreservation protocols for practical production are still uncommon in farmed aquaculture species, except for Atlantic salmon (Salte et al. 2004) and oysters (Adams et al. 2004).

Limitations to the application of genetic and reproductive technologies in genetic improvement programs

At this stage of development in molecular genetics, two major issues that limit application of genetic markers are 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, false-positive detection of marker and gene association also results in low efficiency of MAS. In our current existing breeding programs for tilapia and carps, MAS should be used only when there is a tight linkage between markers and the gene of interest. 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. This creates a need to develop new generation markers (e.g. SNP), physical and comparative maps, and to integrate them into linkage maps to increase the ability to identify functional mutations or candidate genes in aquaculture species.

Costs of MAS: Currently, the cost of utilizing markers is possibly the most important factor that limits implementation of MAS in genetic improvement programs. At various stages of MAS development, areas that represent large costs include laboratory equipment, consumables, infrastructure, marker development, genotyping, data recording and labor. The question of whether these costs can be compensated by economic returns from genetic gain using MAS still remains open. In addition, several constraints and limitations for the application of molecular genetic information include intellectual property rights, joint research collaborations among international institutions, the lack of manpower, and research funding.

Concluding remarks

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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