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12

MARKER ASSISTED SELECTION IN SHRIMPS

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This chapter focuses on the principles of marker-assisted selection in shrimp species. Though the chapter specifically highlights shrimps in general, examples from other aquaculture species have been quoted through out the chapter wherever necessary or more appropriate to explain the concepts clearly.

MARKER ASSISTED SELECTION

Selection constitutes the choice of individuals to be used as parents for producing next generation of progeny. Selection is carried out to improve one or more desirable traits/ characters in the population of a species. Genetic improvement through selection has been an important contributor to the dramatic advances in agricultural productivity that have been achieved in recent times (Dekkers and Hospital, 2002). Genetic improvement of any population of a species involves periodic evaluation, selection and culling of animals. Selection of animals is an important aspect of genetic improvement program, because the selected parents will decide the genetic makeup of the progeny and hence their phenotypic performance.

Most of the economically important traits (body weight, disease resistance etc.) in shrimp are quantitative traits that are controlled by protein products of fairly large number of genes (Breese and Mather, 1957) called minor genes or polygenes, wherein, very few genes have relatively large effects with many others having smaller effects in the overall expression of the trait (Spickett and Thoday, 1966). These polygenes interact among themselves and also with the environment before being expressed as observable phenotype. The polygenic inheritance coupled with a large array of environmental factors contributes to slow improvement of quantitative traits under conventional selection (Togashi *et al.*, 2004). The number, locations and size of effects of each polygene are never known. One positive facet in this complex situation is that every single polygene influencing a quantitative trait follows Mendelian rules of inheritance.

Conventionally, as we do not know which genes contribute to phenotype, selection of animals is being carried out on the basis of observed phenotypes recorded on (1) the

animals themselves (individual selection or mass selection) or (2) their direct relatives (pedigree selection, progeny testing) or (3) their collateral relatives (family selection, within family selection, sib selection). This method has given good results for many traits in several livestock species. Now, we understand that some genetic variants (or alleles) of specific genes are associated in a positive way with a given trait. It is therefore possible to genotype an animal using a DNA-based genotyping test and select individuals carrying the preferred genetic variant. When we use information on animals DNA (mostly genetic marker information at selected loci) for selection of individuals to become parents in the next generation, such a selection procedure is called as Marker Assisted Selection (MAS). The word "assisted" implies that the selection is also influenced by other sources of information such as animal's observed performance. Synonyms for MAS in literature include 'marker assisted breeding' and 'marker aided selection'.

THEORY UNDERLYING MAS

DNA, the most talked molecule in this universe transmits genetic information from parents to progeny and also stores information required for the synthesis of proteins essential for the structure and function of organisms. The DNA present in each cell of an organism is organized in to sets of chromosomes (e.g. 44 pairs in *P. monodon*) and constitutes genome of that organism. In a diploid bi-sexual individual where chromosomes are arranged in pairs, there exist two copies for every gene, one inherited from each parent. A gene is a stretch of DNA that contains information required for synthesizing one polypeptide molecule and polypeptides are the building blocks of proteins. Thousands of proteins synthesized in the body interact and determine the visible characteristics or phenotype. The genetic makeup of the organism is called genotype. It is possible for the DNA sequence that makes up a gene to differ between animals. The difference could be as small as a single nucleotide. These alternative gene sequences called genetic variants or alleles may result in differences in the amount or type of protein being produced by that gene. In a diploid individual, the two alleles of a gene make up the genotype for that gene. In a population where, there are only two alleles, say A and B for a gene, three different genotypes are possible i.e. AA, BB and AB. The phenotype of the animals carrying different genotypes could also differ as, a change in gene may result in addition or deletion or change in protein responsible for phenotype. Animals carrying alleles that are encoding proteins having beneficial effect on the phenotype are to be selected based on markers that are linked to these alleles and should be used as parents for producing next generation so that the beneficial alleles pass on to the progeny. MAS improve the accuracy of selection and increase the rate of genetic progress by identifying animals carrying desirable alleles for a given trait at an earlier age. As MAS could be practiced as early as the embryo stage in animals, there is no longer a need for the animal to develop to a stage at which the trait can be recorded.

SITUATIONS WHERE MAS IS BENEFICIAL

1. When heritability of the target trait is low, there by not permitting improvement through conventional selection. Genetic variability in the population (estimated as heritability) is the instrument in the hands of breeder that helps him in improving the population. Conventional methods of selection fail to significantly improve traits that are having low heritability values. When the proportion of the additive genetic variance explained by the marker loci exceeds the heritability of the character, selection on the marker loci alone is more efficient than selection on the individual phenotype (Smith 1967). Also, as trait heritability increases, the benefit due to marker information decreases as it becomes easier to select superior animals based on performance or phenotypic records.
2. When the desired trait cannot be measured on one sex. For sex-limited traits, conventional selection based on the phenotypic records can be practiced in only one sex in which the trait is expressed whereas, MAS could be practiced in the opposite sex also. Even for a sex-limited trait with higher heritability marker information greatly increases the efficiency of selection.
3. When the desired trait cannot be recorded before animal attains sexual maturity (e.g. age at sexual maturity, age at first spawning etc.). Sexual maturity leads to reduced growth and reduced feed conversion efficiency in most of aquaculture species. Therefore, there is a need to select those animals that mature late than others.
4. When the target trait is difficult or expensive to measure (e.g. disease resistance, feed conversion efficiency etc.). Challenge tests are used to identify the families of shrimps that are relatively resistant to certain viral and bacterial diseases. But as the surviving shrimp cannot be used in breeding programs, their sibs only are the candidates for selection. Whereas in MAS, all the shrimp are the candidates for selection and there is no need to carry out challenge tests, once the marker for disease resistance is identified and validated.
5. When the target trait cannot be measured in the lifespan of the animal (e.g. head-to-tail ratio).
6. When no conventional selection program exists for a character, it is better to start with MAS. An advantage with MAS is the reduced generation interval there by increasing the genetic gains per unit time. But the generation interval cannot be lower than the age at which shrimp attains sexual maturity. Biologically the generation intervals in shrimp species are lower i.e. about 2 years in common cultured species and cannot be decreased further. This reduces the advantage of MAS in exploiting generation interval. However, increased selection intensity, increased accuracy of selection and pre-selection of parents at an early age there by saving time, money and space give edge to MAS over conventional schemes of selection.

7. When the trait under selection (say 'X') is genetically correlated with another trait (say 'Y'), that we do not wish to change. In this case, we need to find a marker that is associated with trait 'X' but not associated with trait 'Y'.

8. When the species you are dealing with is not fully domesticated and controlled breeding cannot be practiced. This situation is probably specific to some aquaculture species that are difficult to be domesticated completely. A worth mentioning example is the difficulty faced globally in breeding pond reared tiger shrimp inside a hatchery and getting progeny successfully. Unless the animals selected in a conventional selection program breeds and produce next generation of progeny, the genetic merit for which the animals are selected is not carried to the next generation. In this situation, if markers are available for desired traits, MAS could be practiced in every generation to decide the parents (from the lot of wild caught animals that breed in a hatchery) that are carrying beneficial alleles.

CAN MARKER ASSISTED SELECTION REPLACE CONVENTIONAL SELECTION

The answer is NO. Marker assisted selection allows for the accurate selection of animals with specific DNA variations (or markers) that have been found associated with complex quantitative traits. Quantitative traits are controlled by several polygenes. It is important to realize that we may find out only those markers having significant influence on the complex trait leaving out many other markers which also contribute towards the final expression of the trait along with environment. Therefore, even when a marker is available for a quantitative trait, breeding values estimated for the trait should be utilized for making selection decisions as they consider all the genes that contribute to a given trait. Marker assisted selection should be seen as a tool to assist with, and not as a replacement for traditional selection methods.

Conventional selection methods bring out a general increase in the frequency of beneficial alleles controlling the target trait in the population, generation after generation. Whereas, MAS directly increases the frequency of specific alleles, selected for in the population.

Identification of Quantitative Trait Loci (QTL)

A stretch of DNA sequence in the genome that contains genes controlling a particular quantitative trait is called as quantitative trait locus (quantitative trait loci in pleural sense). In some aquaculture species, linkage maps were utilized to find out the QTLs for quantitative traits. This QTL mapping process involves screening of markers already mapped on to linkage maps for their association to traits of interest, in specialized populations called 'mapping populations'. The positions of the chromosomes harboring the associated markers and the near by regions in the genome are the QTLs for the concerned trait having

candidate genes. The markers found associated with quantitative traits would be utilized for MAS after validation.

The resources required for identification of QTL are polymorphic DNA markers, high resolution linkage maps and mapping populations that are segregating for the polymorphic markers. The basics, principles and procedures involved in this entire process of QTL mapping and MAS are briefly discussed below.

DNA Markers

An important by-product of extensive genomics research in the past decade is the availability of large number of molecular markers. DNA or molecular markers represent genetic differences between individual animals. DNA markers are the most widely used type of markers primarily due to their abundance and are not influenced by environment unlike morphological or biochemical markers. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996). DNA markers are mostly located in non-coding regions of DNA and therefore are selectively neutral (Collard *et al.*, 2005). DNA markers that are commonly used for QTL mapping have been listed in Table 1, though other markers such as minisatellites (Jeffreys *et al.*, 1985), expressed sequence tags (Adams *et al.*, 1991) and single nucleotide polymorphisms (Kwok *et al.*, 1994) are also widely used for several other applications. O'Brien (1991) has classified genetic markers in to two types, Type I markers associated with a gene of known function and Type II markers associated with anonymous gene segments of one sort or another.

Table 1. Commonly used DNA markers in QTL mapping studies.

Marker	Detected as	Type of Loci	Reference
Restriction Fragment Length Polymorphism (RFLP)	Codominant	I & II	Botstein *et al.*, 1980
Random Amplified Polymorphic DNA (RAPD)1990;	Dominant	II	Welsh and McClelland Williams *et al.*, 1990
Amplified Fragment Length Polymorphism (AFLP)	Dominant	II	Vos *et al.*, 1995
Microsatellites or Simple Sequence Repeats (SSR)	Codominant	II > I	Tautz, 1989

Markers are of two different kinds, direct and indirect. Specific loci or markers may be part of the gene and directly influence a quantitative trait. Sorensen and Robertson (1961) explained the basic theory for incorporating direct markers in to selection index for

practicing selection. Marker loci with no direct effect on the character of interest also could be utilized in selection by virtue of statistical associations or linkage disequilibria between alleles at the marker loci and quantitative trait loci (Soller, 1978).

How Linkage Disequilibria Arise

Linkage disequilibria (linkage disequilibrium in singular tense) between loci are produced by three factors, hybridization, random genetic drift and epistatic selection. In a large population created by hybridization between genetically differentiated groups, after T generations of random mating, substantial linkage disequilibria are likely to be maintained between selectively neutral loci with recombination rates, $r < 1/T$ (Kimura and Ohta, 1971). In a randomly mating population of effective size Ne, genetic drift is expected to produce substantial associations between polymorphic loci with recombination rates $r < 1/(4Ne)$ (Hill and Robertson, 1968). In domesticated populations, except those of very small size, the number of generations since the last hybridization event usually will be smaller than four times the effective population size i.e. $T < 4Ne$. Therefore, hybridization is more powerful mechanism for generating useful linkage disequilibria than is random genetic drift.

A major limitation with these associations is that the linkage disequilibrium diminishes over generations by recombination during meiosis. Therefore it is mandatory to identify such indirect markers that are tightly linked to QTL or closely placed along with QTL on the chromosome so that the association is not disturbed by recombination.

Linkage Maps

A linkage map of a species has molecular markers placed in an order along the length of the chromosomes in the genome of that species. Linkage map indicates the position and relative genetic distances between markers along the chromosomes. The distance between markers is estimated by the principles explained for the first time by Arthur Sturtevant (Sturtevant, 1913). Sturtevant assumed that crossing over is a random event and hence has equal probability of occurring at any position along the length of the paired chromosomes during meiosis. Therefore, the markers that are closely placed are less likely to be separated during meiosis than distantly placed markers. Further, the frequency with which the markers are separated during meiosis (recombination frequency) is a direct estimate of the distance between markers. Recombination frequencies for different pairs of markers could be utilized to construct a map showing relative distances between markers on the chromosome. Recombination frequencies between any pair of markers range from 0 % (complete linkage) to 50 % (no linkage).

Two mapping functions, Kosambi mapping function and Haldane mapping function are commonly used for converting recombination frequency in to map units called centiMorgans.

When map distance is less than 10 centiMorgans (cM), recombination frequency between a pair of markers equals map distance between markers. For map distances of more than 10 cM, the relationship between map distance and recombination frequency is not linear (Hartl and Jones, 2001). Kosambi function assumes existence of interference and Haldane function assumes no interference during crossing over (Kearsey and Pooni, 1996). The readers should note that the distance between markers on a linkage map is not directly related to the actual physical distance of DNA between markers.

The genotype information of polymorphic markers in mapping populations (described later in the chapter as a single mapping population could be used for both construction of linkage map and QTL detection) is subjected to linkage analysis for the construction of linakge map. Popular software packages used for construction of linkage maps in aquaculture species include CARTHAGENE (Givry *et al.*, 2005), CRIMAP (Green *et al.*, 1990), JOINMAP (Stam, 1993; Van Ooijen and Voorrips, 2001), LINKMFEX (Danzmann and Gharbi, 2001), MAPCHART (Voorrips, 2001), MAPINSECT 1.0 (http://www.dpw.wau.nl/pv/pub/), MAPMAKER (Lander *et al.*, 1987) and MAPMANAGER (Manly and Olson, 1999; Manly *et al.*, 2001). Except JOINMAP, all others are freeware. Linkage between markers is calculated as odds ratio i.e. the ratio of linkage versus no linkage and is expressed as logarithm of odds (LOD) value or LOD score (Risch, 1992). Generally a pair of markers with a LOD score of >3 would be utilized for the construction of linkage map. A LOD score of 3 between markers indicates that presence of linkage between markers is 1000 times more likely than no linkage.

After linkage analysis, linked markers are grouped together in to linkage groups, each of which represents an entire chromosome or segment of chromosome. The accuracy of estimated recombination frequencies and the subsequent distances between markers is directly related to the number of individuals (or the number of informative meioses) studied in the mapping population. Mapping population sizes used in shrimp for constructing linkage maps range from 43 to 283. An important use of linkage map is to identify the chromosomal regions containing QTLs associated with traits of interest in a QTL analysis. Such linkage maps with QTL positions marked on them could be referred to as QTL maps. Most of the genetic linkage maps in shrimp species (Table 2) are constructed using AFLP and microsatellite markers. The linkage maps currently available are of low resolution with markers placed unevenly. Sex-specific linkage maps were constructed for all the shrimp species, as recombination rates are different between sexes. Because, the choice of appropriate mapping function (either Kosambi or Haldane) depends on the recombination frequency (Liu, 2007). Salmonid fish have the largest reported sex-specific differences in recombination ratios for any known vertebrate (Sakamoto *et al.*, 2000). In some species like Arctic char (Woram *et al.*, 2004) and Atlantic salmon (Moen *et al.*, 2004) females have higher recombination rates than males whereas in others like Japanese flounder (Coimbra *et al.*, 2003) males have higher recombination rates over females.

Table 2: Details of linkage maps developed for shrimp species.

Species	Marker used	Number of linkage groups ♂	♀	Map coverage (cM) ♂	♀	Software used	Mapping population	Mapping function	Reference
Litopenaeus vannamei	AFLP	51	47	2116	2771	Map Manager QTX, Mapmaker /EXP 3.0 & Mapinspect 1.0	1 family (n = 43)	Kosambi	Perez, *et al.*, 2004
Penaeus monodon	AFLP	19		1412		MAPMAKER/exp families	3 full-sib (n = 144)	Haldane	Wilson, *et al.*, 2002
Penaeus monodon	AFLP	44	43	2378	2362	Join Map 3.0	3 full-sib families (n = 283)	Kosambi	Staelens, *et al.*, 2008
Marsupenaeus japonicus	AFLP	43	31	1780	1026	MAPMAKER/EXP	Single F_2 family (n = 102)	Haldane	Li, Y. *et al.*, 2003
Litopenaeus vannamei	AFLP & microsatellites	45	45	3220.9	4134.4	MapMaker/Exp 3.0	Single full-sib family (n = 96)	Kosambi	Zhang, *et al.*, 2007
Fenneropenaeus chinensis	AFLP	36	35	1737.3	2191.1	MAPMAKER/EXP 3.0MAPCHART	Single F_1 family (n = 100)	Kosambi	Li, Z. *et al.*, 2006
Fenneropenaeus chinensis	AFLP	35	28	1617	1090	MAPMAKER/ EXP 3.0	Single F_2 family (n = 110)	Haldane	Tian, *et al.*, 2008
Penaeus monodon	SSLP, EST & SCAR	9		103.6		JoinMap 2.0	F_1 inter-cross family	Kosambi	*Wuthis-uthimet havee, et al., 200*

Mapping Populations

A single mapping population could be used for both linkage map construction and QTL mapping. The phenotype for the traits of interest should be recorded on the individuals of mapping population that was used for linkage map construction, if same population is considered for QTL mapping also. Mapping population consists of individuals that are segregating for the polymorphic markers available on the linkage map of that species. A segregating population could be (1) an F_2 family produced by inter-crossing of F_1 hybrids or (2) a back cross family produced by mating F_1 hybrids back to one of their parents or (3) double haploid families produced by either androgenesis (Stanley, 1976) or gynogenesis (Chourrout, 1984). For successful detection of QTL, the F_1 families should be produced by mating individuals having extreme phenotypes for the trait concerned. This increases the heterozygosity at QTL, an important factor that determines the power of detection of a QTL. Benefits of mating divergent lines with extreme phenotypes have been demonstrated in rainbow trout while mapping QTL for upper thermal tolerance (Perry *et al.*, 2001). QTL mapping designs for aquaculture species have been thoroughly reviewed by Massault *et al.*, (2008). The mapping populations used, the QTL detecting methods followed and the traits for which QTLs are identified in some aquaculture species have been summarized in Table 3.

Sample Size

Large family sizes are required for detecting QTL of small effects. When the QTL explains 10 percent of the phenotypic variance in the target trait, the optimum family size appears to be 50 individuals per family for a QTL mapping experiment in outbred populations. When the total population size (individuals from all families) reaches 1000 individuals, family sizes of 25/50/100 give similar power of detecting a QTL that is explaining 10 % of phenotypic variance (Martinez *et al.*, 2002).

For a given sample size, there is an inverse relationship between the heritability of the character and the proportion of additive genetic variance that can be detected, even with a very large number of marker loci. For detecting additive genetic variance in characters of low heritability, a population composed of large families is less efficient than a population of the same size composed of unrelated individuals, unless a large fraction of the phenotypic variance is caused by genetic dominance and common family environment.

Number of Markers

Lande and Thompson (1990) in their classical paper gave a simple formula to arrive at minimum number of molecular markers (N) required for the likely detection of associations with important QTLs which is calculated as,

$$N = 2TL + C$$

Table 3. List of some QTLs detected in aquaculture species.

Species	QTL for traits	Mapping population	QTL detecting method	Reference
Tilapia(*O.mossambicus* X *O. aureus*)	Cold tolerance & fish size	2 unrelated F_2 families of interspecific hybrids (n = 114)	ANOVA, regression interval mapping	Cnaani *et al.*, 2003
Arctic charr (*Salvelinus alpinus*)	Body weight, condition age at sexual maturation	2 full-sib families (n = 188)	Single marker and interval mapping	Moghadam *et al.*, 2007
Kuruma shrimp (*Marsupenaeus japonicus*)	Growth traits	A F_2 full-sib family from high and low growth parents (n = 102)	Interval and composite interval mapping	Li *et al.*, 2006
Rainbow trout (*Oncorhynchus mykiss*)	Resistance to IPNV	One back-cross family from resistant and susce -ptible strains (n = 52)	χ^2 test and interval mapping	Ozaki *et al.*, 2001
Oncorhynchus mykiss	Meristic traits	Double haploid individuals from an F_1 family (n = 99)	Composite interval mapping	Nichols *et al.*, 2004
European seabass (*Dicentrarchus labrax*)	Morphometric traits	26 full-sib families derived from several sires and a single dam (n = 449)	Half-sib interval mapping	Chatziplis *et al.*, 2007
Rainbow trout (*Oncorhynchus mykiss*)	Pyloric caeca number	Double haploid progeny from an F_1 hybrid (n = 54)	Composite interval mapping	Zimmerman *et al.*, 2005
Tilapia(*O.mossambicus* X *O. aureus*)	Stress response, body weight & sex determination	An F_2 family derived from inter-specific hybrid (n = 114)	Genome scan using ANOVA	Cnaani *et al.*, 2004

Where, T = Number of generations since last hybridization event in the population
L = Map length in Morgans
C = Haploid chromosome number

This minimum number of markers may not be sufficient to detect QTLs in partially inbred populations. The power of mapping a QTL on to a linkage map with a marker spacing of 10 cM is same as that of mapping a QTL on to a linkage map having infinite number of markers (Darvasi *et al.*, 1993).

QTL Analysis

The basic principle involved in QTL mapping is detecting an association between phenotype of the trait and genotype of markers. Phenotypic records are available for the individuals in mapping population for the trait of interest. A polymorphic marker locus has 2 or more alleles in the population, though only 2 alleles are present in any diploid individual. A mapping population can be divided in to different genotypic groups on the basis of the genotype at the marker locus. A significant difference between phenotypic means of the groups for the trait of interest indicates that either marker itself is the QTL for the trait of interest or marker is linked to the QTL controlling the trait of interest. It should be noted that QTLs can only be detected for traits that segregate between the parents used to construct the mapping population.

Single marker analysis, simple interval mapping and composite interval mapping are the three methods widely used for QTL detection (Tanksley, 1993; Liu, B. 1998). Single marker analysis is the simplest method considering single marker locus at a time and uses statistical methods like *t*-test, ANOVA and linear regression. Coefficient of determination (R^2) from linear regression will tell us the amount of phenotypic variation explained by the marker locus linked to the QTL. Simple interval mapping considers a pair of adjacently placed linked markers at a time and try to find out whether QTL exists or not in the interval between marker pair. Composite interval mapping combines simple interval mapping with linear regression and considers additional genetic markers along with adjacent pair of linked markers. Both the interval mapping methods try to find out likely sites for a QTL between a pair of adjacent linked markers. The position on the linkage map where highest LOD values are obtained in QTL analysis corresponds to the region of the chromosome harboring the QTL. We could be able to say that, QTL exists between these two markers on the linkage map. The confidence intervals for QTLs are calculated by either 'one-LOD support interval' method (Lander and Botstein, 1989) or 'bootstrapping' (Visscher *et al.*, 1996).

The QTL identified could be (1) a functional mutation itself or (2) a marker (or multiple marker haplotype) that is in population-wide linkage disequilibrium with functional mutation or (3) a marker (or multiple marker haplotype) that is in population-wide linkage

equilibrium with the functional mutation. Application of MAS for genetic improvement varies with each of these three cases, first case being the easier and strait- forward to apply. The second and third cases should lead to the identification of functional mutation responsible for phenotype.

Markers identified should be validated for their reliability in predicting the phenotype in different populations. Validation is necessary because false-associations between markers and the trait of interest can arise. It is also true that an association in one particular experimental study could occur by chance alone, and the verification of previous results has always been one of the prime motivators driving scientists to repeat experiments. Markers that cannot be validated have no value as tools for marker-assisted selection.

Power of QTL Detection

Family structure of the mapping population i.e. number of families and family size; and also the heterozygosity of the QTL have major impact on the power of QTL detection. The power of QTL detection is more affected by increasing the number of the progeny than by increasing the number of families (Kolbehdari *et al.* 2005). If the heterozygosity is low, the QTL will be segregating only for a limited number of individuals and will reduce the amount of information (Hayes *et al.*, 2006; Verhoeven *et al.*, 2006). If we take a few large families, it may so happen that none of the parents is heterozygous for the QTL. Therefore a balance between family size and number of families needs careful consideration. The heritability of the QTL has more impact on the power to detect QTL than the heritability of the trait.

Experiment designs that would have an 80% power to detect a QTL of moderate effect (explaining between 1.5 and 5% of the trait variation) by genotyping 1000 or fewer individuals are demonstrated for aquaculture species by simulation studies (Massault *et al.*, 2008). More the interval between markers less is the power of detecting a QTL. Marker density does not greatly affect the power of detecting a QTL when the distance between markers is less than 10 cM, but ideally marker spacing should not exceed 20 cM (Massault *et al.*, 2008). Some aquaculture species have linkage maps with much lower average distance between markers (5 cM for sea bass, Chistiakov *et al.*, 2005; and 9 cM for oysters, Hubert and Hedgecock, 2004).

Cost-effectiveness of MAS

Marker assisted selection offers potential savings compared to conventional breeding methods when (a) phenotypic screening is expensive, (b) when a breeder could not detect the presence of multiple alleles related to a single trait based on phenotype (Melchinger, 1990), (c) when it is necessary to screen for traits whose expression depends on seasonal or geographical considerations, (d) when it allows alleles for desirable traits to be detected early, well before

the trait is expressed, and (e) when it allows breeders to identify heterozygous animals that carry an allele of interest whose presence cannot be detected phenotypically.

The question of time savings is extremely important, because over the long run the greatest benefit offered by MAS will be its ability to reduce the time required for animal breeding.

Development and Implementation of MAS

The whole approach of MAS can be summarized in to following activities.

1. Identify parents having extreme phenotypes for the trait of interest.
2. Develop a mapping population of animals segregating for the trait.
3. Record phenotype for the trait on the animals of mapping population.
4. Screen the mapping population for polymorphic DNA markers.
5. Construct high resolution linkage maps by linkage analysis.
6. Identify molecular markers that are co-segregating with the trait of interest.
7. Validate the reliability of markers for use in MAS.
8. Produce clear and simple protocols for genotyping the markers.
9. Study the cost-effectiveness of MAS over conventional tools of selection and other bio-techniques.
10. Implement MAS into the genetic improvement programs.

Current Status in Shrimp

Sex-specific linkage maps are available for almost all commercial species of shrimp. The existing linkage maps must be tagged with some more markers to increase the map resolution. Recently, several EST markers have been developed for shrimp species (Maneeruttanarungroj, *et al.*, 2006; Tassanakajon, *et al.*, 2006; Tong *et al.*, 2002). These EST sequences would be utilized to find out additional microsatellite markers which can be anchored on to the existing linkage map to make it denser. An EST database developed from Thailand for *P. monodon* has about 40,000 EST sequences (http://pmonodon.biotec.or.th). NCBI has huge collection of EST database for commonly cultured shrimp species. Quantitative trait loci for total length and carapace length have been mapped on to the male linkage map of *M. japonicus* using interval mapping method (Li, Y *et al.*, 2006). A microsatellite marker is found to be associated with WSSV susceptibility in *Penaeus monodon* (Mukherjee and Mandal, 2009). The broodstock not

possessing this particular marker should be selected as WSSV resistant broodstock and to be utilized for producing progeny. An allele at microsatellite locus RS0622 (AY132778 in NCBI database) was found as associated with resistance to WSSV in *Fenneropenaeus chinensis* (Dong *et al.*, 2008). Major reasons for low paced QTL detecting programs in shrimp species include high costs and difficulties involved in domesticating the species, selective breeding, broodstock maintenance and phenotypic recording.

CONCLUSIONS

Most of the economically important traits are quantitative, polygenic, influenced by environment and generally complex natured making genetic improvement of these traits more laborious and time consuming. Therefore these traits are exciting targets for application of MAS. Marker assisted selection is likely to accelerate genetic progress in some traits better than other. Hence, target traits for MAS should be selected wisely. Disease resistance is supposed to be the trait that gets maximum benefit from MAS as it has low heritability and also difficult to measure.

Markers should not be seen as a replacement to conventional tools of selection. Instead of bypassing conventional breeding methods, markers should be used as tools to complement them. For high heritable traits MAS may be less useful than conventional selection, as it is easier to select superior animals based on performance records. Selection decisions taken after considering both the breeding values and markers are always superior to the decisions taken on either breeding values (consider numerous genes) or markers (consider single gene) alone. So the real challenge with MAS will be to determine the weight that should be given to the marker information in selection process.

Efforts in to QTL mapping and MAS are low paced in aquaculture compared to plants and animals. The inability of practicing MAS reflects lack of investment in QTL detection programs and existence of less number of genetic improvement programs. MAS in shrimp species is at present limited by the lack of abundant availability of genetic markers and high resolution genetic maps. Single nucleotide polymorphism is the answer for the abundance of molecular markers. Recent technical advances made in to linkage analysis and QTL analysis made the whole process of QTL identification easier than before. The technology itself is no longer limiting. The issue is the construction of high resolution linkage maps, initiating QTL detection programs, detection of QTL and cost-effective application of MAS on large scale. Existing genetic improvement programs should be designed to accommodate QTL information in cost-effective and sustainable manner. The potential impact of intellectual property rights (IPRs) on the development and application of MAS is to be addressed.

Although the existence of QTL for quantitative traits is convincingly agreed beyond doubt, their value for breeding programs and commercial applications in plants, livestock, forestry and aquaculture species is yet to be proven. Everything that [illegible] MAS an

attractive enterprise to many researchers still holds true. But we need to identify the applications in genetic improvement programs in which molecular markers offer real advantages over conventional breeding methods. MAS should not be seen as a cure for every breeding problem. Conventional breeding methods still provide a cost-effective option for improving several traits and they will continue to be attractive in the future.

Breeder should make sure that the costs and benefits from MAS should be considered just like any other input in the culture system. If benefits overweigh expenses then only one should include markers along with conventional tools for selection of animals. For marker assisted selection to be profitable, the increased economic returns from greater genetic gain as a result of using the markers must outweigh the cost of genotyping.

One should remember that good genetics can never overcome bad management or environment. Whatever the better genes an animal has for a trait, it is never going to perform better, unless good environment is provided. So, irrespective of the efficient selection strategy followed in the population, proper management of animals is vital for improving any species.

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