

Progress of DNA Marker-Assisted Breeding in Maricultured Finfish

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Abstract : The marine products industry has developed based on direct catch from natural resources. However, because of the depletion and gradual restriction of aquatic resources, only recently has breeding been considered as an important research area. With the depletion of aquatic resources, the expectation regarding aquaculture research is increasing. Also genetic improvement of traits for improved culture is leading to superior varieties because we can improve the phenotype to suit aquaculture conditions by domestication of artificially produced fish with each generation.

The essential conditions for DNA marker-assisted selection (MAS) is development of useful resource families to evaluate phenotypes and information about genetic linkages and a large number of polymorphic genetic markers. At the beginning of the study, we mainly aimed to use improved technologies on salmonid fish (Ozaki *et al.*, 2001). But now the research and development are being applied to other kinds of maricultured fish. Some of the cases have already reached a practical stage and have been used in genetic improvement production. Two cases of MAS programs have succeeded in developing Japanese flounder resistant to lymphocystis disease (Fuji *et al.*, 2007) and Atlantic salmon resistant to infectious pancreatic necrosis (Moen *et al.*, 2009). These cases indicate the validity of the methodology.

MAS breeding is applicable to other target species. However, there still remain problems and potential to further improve the methodology. One is markers-assisted introgression (MAI) or quantitative trait loci (QTL) pyramiding. MAI is the next phase of MAS. This method uses DNA markers of the responsible region for specific traits, and introgression hybridization to obtain several economic traits on one strain. We are carrying out research and development about MAI using Japanese flounder (*Paralichthys olivaceus*) as a target species. The other is effective utilization of natural genetic resources. At this point, aquatic resources have advantages because wild species have not been selected and still maintain high genetic diversity. Individuals have high potential for genetic breeding regarding phenotypic characters. We are researching practical applications about selection of economic important traits from natural genetic resources using Japanese amberjack (*Seriola quinqueradiata*) as a target species.

Keywords : marker-assisted selection (MAS), markers-assisted introgression (MAI), DNA marker, economic traits

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Introduction

The marine products industry has developed based on direct catch from natural resources. However, because of the depletion and gradual restriction of aquatic resources, only recently genetic breeding has been considered as an important research area. With the depletion of aquatic resources, the expectation regarding aquaculture research is increasing. Also genetic improvement of traits for improved culture is leading to superior varieties, because we can improve the phenotype to suit aquaculture conditions by domestication of artificially produced fish with each generation.

The essential conditions for DNA marker-assisted selection (MAS) is development of useful resource families to evaluate phenotypes and information about genetic linkages and a large number of polymorphic genetic markers. At the beginning of our research, we mainly used improved technologies on salmonid fish. But now the research and development are being applied to other kinds of maricultured fishes. Some of the cases have already reached a practical stage and have been used in genetic improvement production. Two cases of MAS programs have succeeded in developing Japanese flounder resistant to lymphocystis disease and Atlantic salmon resistant to infectious pancreatic necrosis (IPN). These cases indicate the validity of the methodology.

MAS breeding is applicable to other target species. However, there remains the potential to further improve the methodology. One is markers-assisted introgression (MAI) or quantitative trait loci (QTL) pyramiding. Markers-assisted introgression is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits and introgression hybridization to obtain several economic traits in one strain. We are carrying out research and development on MAI using Japanese flounder (*Paralichthys olivaceus*) as a target species.

The other improvement is effective utilization of natural genetic resources. At this point, aquatic resources have more advantages because wild species are not selected and still maintain high genetic diversity. Individuals have high potential

for genetic breeding with respect to phenotypic characters. Aquaculture species have those genetic resources. We are researching practical applications for selection of economic important traits from natural genetic resources using Japanese amberjack (*Seriola quinqueradiata*) as a target species.

First Case of Identified Loci Associated with Disease Resistance and Pilot Examination of Marker-assisted Selection

IPN is a well-known acute viral disease of salmonid species. We have identified QTLs associated with resistance to this disease in rainbow trout (Ozaki *et al.*, 2001). We searched for linkage among 51 microsatellite markers used to construct a framework linkage map in backcrossed families of rainbow trout (*Oncorhynchus mykiss*) produced by crossing IPN-resistant (YN-RT201) and IPN-susceptible (YK-RT101) strains. Two putative QTLs affecting disease resistance were detected on chromosomes A (IPN R/S-1) and C (IPN R/S-2; Figure 1), suggesting that this is a polygenic trait in rainbow trout. These markers have great potential for use in MAS for IPN resistance and provide the basis for cloning of IPN resistance genes. Clarification of the genetic bases of complex traits has broad implications for fundamental research, but will also be of practical benefit to fish breeding.

In our analysis of IPN disease resistance/susceptibility, we showed the possibility of MAS; therefore, we performed MAS in a candidate locus (IPN R/S-1) that was related to IPN disease resistance in the analysis family. Progeny obtained from the crossbreeding were tested with artificially induced infection. As a result, the combination of crossbreeding that had the positive allele of YN-RT201 showed significantly higher resistance compared to the negative allele (Figure 2).

Marker-assisted Selection and MAS-lymphocystis Disease Resistant Flounders Are Now Marketed in Japan

An allele of a microsatellite, Poli9-8TUF (Figure 3), has a dominant effect at a single major locus and is responsible for resistance to lymphocystis disease

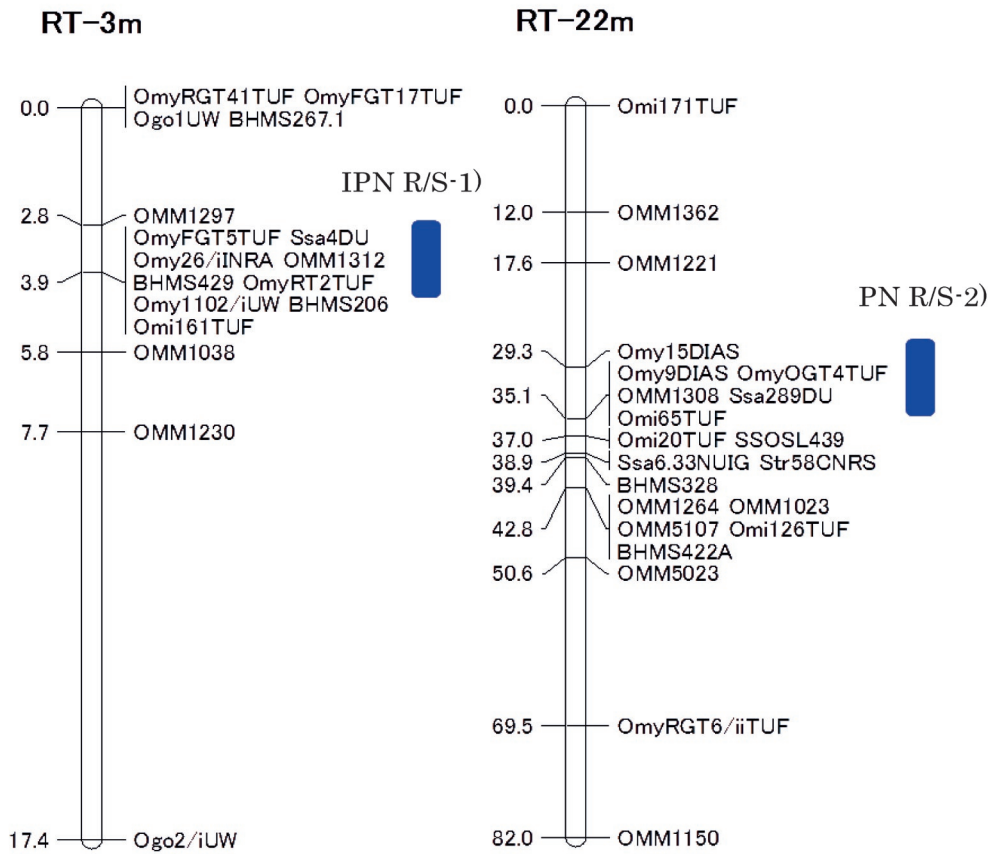


Fig. 1. Genetic linkage maps of significant loci in linkage group RT-3 and RT-22 in rainbow trout. Map distances between markers are shown in centimorgans (cM). A LOD of 3.0 was used to create linkage groups of each family. Map figures were drawn using MapChart ver.2.0.



Fig. 2. Five days after initiation of the infection experiment with infectious pancreatic necrosis virus (IPNV) in rainbow trout. The positive allele on IPN R/S-1 clearly showed IPN disease resistance. Fish on the left have the positive allele; those on the right have the negative allele.

(LD-R) in Japanese flounder. A new population of Japanese flounder was produced by marker assisted breeding using this allele. A female that originated from the KP-B inbred line with LD-R that was homozygous for the favorable allele (B-favorable) and a male from a commercial stock bred for higher growth rate and good body shape were selected as parents. A female was selected as the LD-R-bearing parent because the recombination rate of females is lower in the region where the LD-R locus is located. As expected, the B-favorable allele was transmitted as a heterozygote to the progeny (LD-R+ population). The LD-R+ population, when tested at two commercial fish farms that had LD outbreaks, showed no incidence of LD at either farm, while a control population without B-favorable alleles (LD-R-) had incidences of 4.5% and 6.3% at the two farms. These results show that marker-assisted breeding using molecular markers linked to an economically important trait is an efficient strategy for breeding (Fuji *et al.*, 2007). MAS-lymphocystis disease resistant flounder are now selling in marketplace and have a market penetration rate 35% in Japan (Figure 4).

Linkage Analysis of Resistance to *Streptococcus iniae* Infections in Japanese Flounder (*Paralichthys olivaceus*)

Streptococcal disease caused by *Streptococcus iniae* is a serious bacterial disease in Japanese flounder. Developing streptococcosis-resistant Japanese flounder will reduce the number of outbreaks of the disease as well as reduce the need for antibiotics and vaccines. Genetic linkage analysis is an effective method for identifying QTL associated with resistance to a disease. In this study, 159 microsatellite markers selected from genetic linkage maps of Japanese flounder and F1 progeny from crosses between disease-resistant and disease-susceptible parents were used for detection of QTL associated with resistance to the disease. Some loci associated with disease resistance were found in the JF-7, JF-10, JF-11 and JF-17 linkage groups (Ozaki *et al.*, 2010). These QTL regions are candidates for disease resistance against streptococcal infection.

MAS breeding has the potential to further improve the methodology through MAI or QTL pyramiding. MAI is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits and introgression hybridization to obtain several economic traits in one strain. We are carrying out research on and development of MAI using Japanese flounder as a target species, to make the strain disease resistant and to incorporate other positive traits (Figure 5).

MAS Programs Have Succeeded in Atlantic Salmon Resistant to IPN in Norway

IPN is an economically devastating disease in Atlantic salmon (*Salmo salar*) farming worldwide. The disease causes large mortalities at both the fry and post-smolt stages. Family selection for increased IPN resistance is performed through the use of controlled challenge tests, where survival rates of sib groups are recorded. However, since challenge-tested animals cannot be used as broodstock, within family selection is not performed and only half of the genetic variation for IPN resistance is being exploited. DNA markers linked to QTL affecting IPN resistance would therefore be powerful selection tools. The aim of this study was to identify and fine-map QTL for IPN-resistance in Atlantic salmon for use in MAS to increase the rate of genetic improvement for the trait (Moen *et al.*, 2009).

The QTL confirmed in this study represents a case of a major gene explaining the bulk of genetic variation for a presumed complex trait. QTL genotypes were deduced within most parents of the 2005 generation of a major breeding company, providing a solid framework for linkage-based MAS within the whole population in subsequent generations. Since haplotype trait associations valid at the population level were found, there is also a potential for MAS based on linkage disequilibrium. However, in order to use MAS across many generations without reassessment of linkage phases between markers and the underlying polymorphism, the QTL needs to be positioned with even greater accuracy. This will require higher marker densities than are currently available.

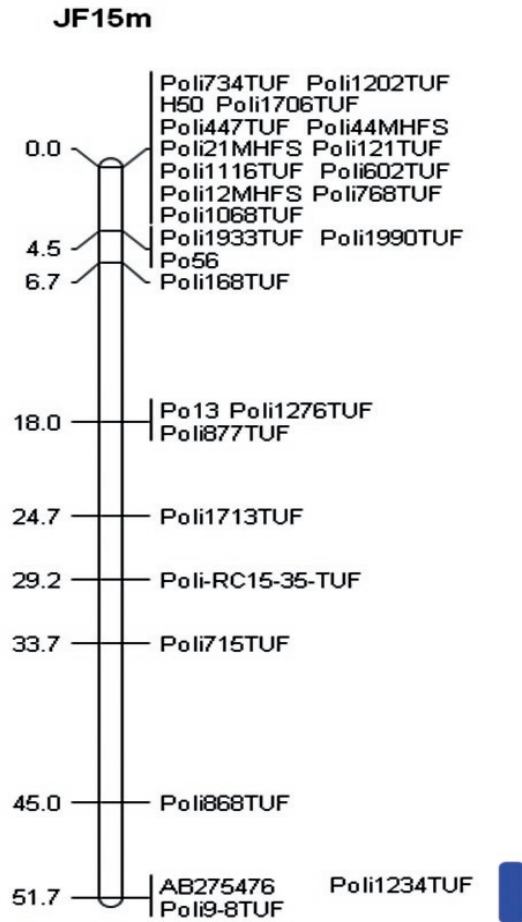


Fig. 3. Genetic linkage maps of significant loci in linkage group JF-15 in Japanese flounder. Map distances between markers are shown in centimorgans (cM). A LOD of 3.0 was used to create linkage groups of each family. Map figures were drawn using MapChart ver.2.0.



Fig. 4. MAS-lymphocystis disease resistant flounder have a market penetration rate of 35% in Japan.

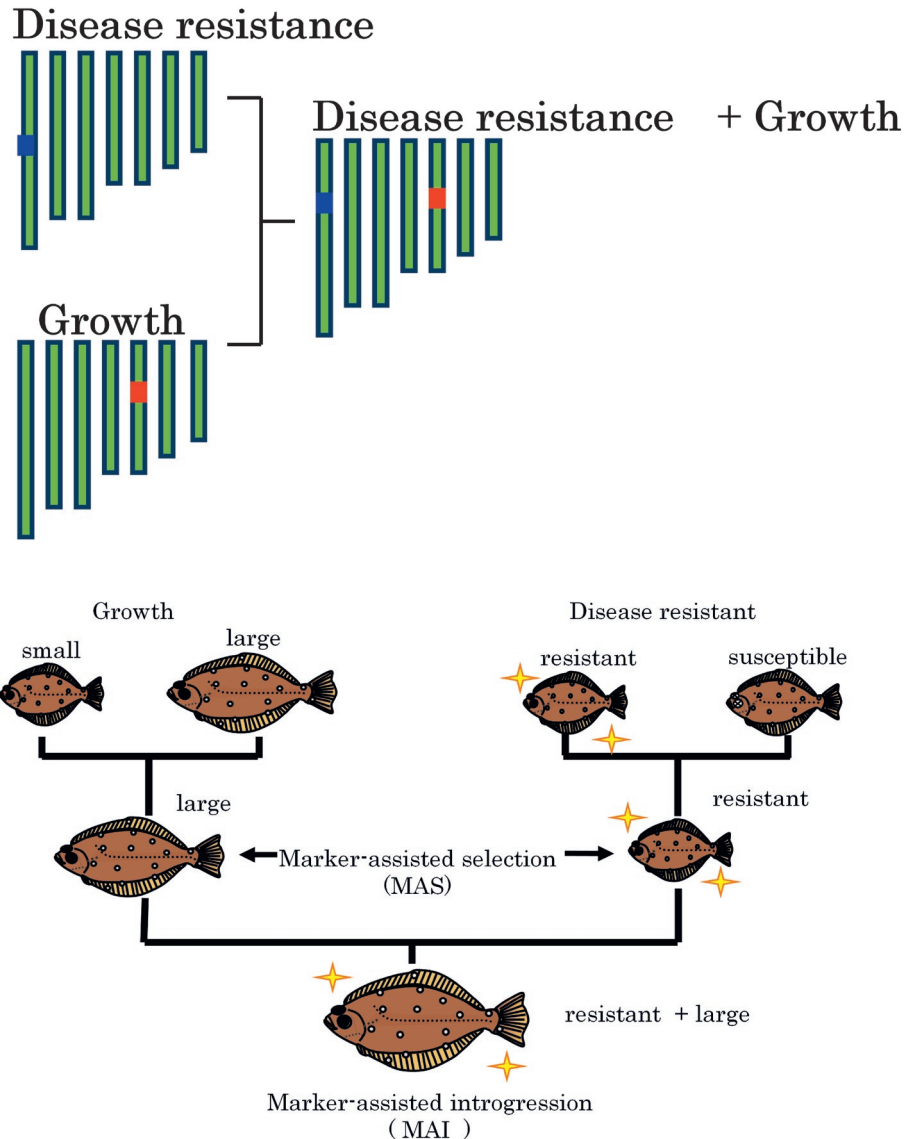


Fig. 5. Markers-assisted introgression is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits, and introgression hybridization to obtain several economic traits on one strain.

Developing the Infrastructure of New Molecular Tools and Information Using Single Nucleotide Polymorphism and Parasitic Disease Resistance QTL Analysis in Yellowtail

Yellowtail (*Seriola quinqueradiata*) is one of the most important commercial aquaculture species in Japan. However, most of the production facilities use captured juveniles. In particular, establishment of hatchery-produced juveniles are desired for

sustainable aquaculture production systems. That can make discovery of economically important traits from genetic resources possible. We are trying to develop the infrastructure of new molecular tools and information using single nucleotide polymorphism (SNP).

The sex-linked locus in yellowtail has been identified using microsatellite markers. We characterized the sex determining system by genetic linkage analysis conducted on fish of both sexes from

a single family. The associations between phenotypic sex and genotypic data of microsatellite markers selected from a yellowtail genetic linkage map were tested. The putative sex-determining locus is located between locus Sequ21 and locus Sequ17 in LG12, and the sex-linked alleles were inherited from the female parent (Fig. 6). This result suggests that yellowtail has a ZZ – ZW sex determining system, and that it would be possible to use these sex-linked markers to discriminate the sexes (Fuji *et al.*, 2010).

The parasitic disease benedeniasis is a serious problem in *Seriola* species caused by *Benedenia seriolae*. The parasite can cause growth reduction and external injuries in yellowtail, increasing the risk of secondary viral or bacterial infections. We produced F₁ hybrids in yellowtail and also constructed genetic linkage maps using microsatellite markers. In addition, we are trying to identify QTLs associated with resistance to *B. seriolae* by those molecular markers in F₁ and F₂ hybrids.

Genetic analysis is performed to detect loci associated with phenotype as a step toward new genetic breeding programs. Also, marker-assisted and marker-introgression breeding programs have attracted a great deal of attention for their use in improving multiple economically important characters. Haplotype genetic selection has just started as is limited to a few fish species for which control of the life cycle has been completed. It will make it possible to do tailor made genetic breeding using genotype selection and will help produce a stable supply of high quality fish.

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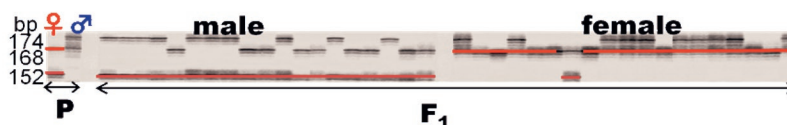


Fig. 6. Electrophoresis image of Sequ21 in linkage group 12 in yellowtail (*Seriola quinqueradiata*). The lower allele (152 bp) from the female parent is a unique allele that was found in the males.