POSSIBILITIES FOR MARKER-ASSISTED SELECTION IN FISH BREEDING SCHEMES

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Summary

Aquaculture is an increasing industry, with a total value of 56 billion US$ coming from 210 species. However, only few species have actual selective breeding programs and no fish is selected based on genetic marker-information. The main limitation for marker-assisted selection is the number of genetic marker and QTL maps. Accuracy of selection would be markedly increased for most traits, which today is based on information on sibs.

Keywords

Marker-assisted selection, fish, genetic maps, breeding scheme, selection

Introduction

Aquaculture is an increasing industry, with a total value of 56 billion US$ (FAO statistics, 2001). FAO estimates that there are 210 species in culture, of which the carps and oysters have the largest world-wide production. However, only few species have actual selective breeding programs. Salmonids, shrimps and tilapia have the largest breeding programs, salmonids having the most comprehensible breeding goal.

Marker-assisted selection is not used in any fish-breeding scheme today. The aim of this paper is to shortly review the current status of fish selection programs and evaluate the possibilities of marker-assisted selection for fish.

Fish breeding

Structure of breeding schemes

Most aquaculture species are today bred in mono- or polyculture using mass selection (for growth). Only about 30 family-based breeding programs utilise sib-information in the estimation of breeding values (B. Gjerde, pers. comm). The general structure of such a family-based selection schemes can be split into two parts. The first part is a closed breeding nucleus, with information from sibs coming from test-stations. In the second part, genetic material from the nucleus is used to produce eggs or fry in the multiplier units for the grow-out producers. The limiting factor of these breeding nuclea is the number of tanks, where the fry of a certain full-sib family is kept until individuals are large enough to be physically tagged. The number of offspring per full-sib mating is large, such that very high selection intensity can be achieved. Mostly, each male is mated to two females, such that the tank effect can be estimated. In the following, I will concentrate on the selection within the nucleus.

The high intensity of selection can easily result in high rates of inbreeding. Indeed, there are terrifying examples of unsustainable breeding schemes in fish, where deliberately only fewer than five males and five females constituted the base population. Introduction of unrelated wild stock is often performed to reduce the rates of inbreeding. However, introduction of wild stock also leads to reduced genetic gain, and should therefore be avoided for on-going breeding schemes. Today, methods exist that maximise genetic gain for a certain time horizon while restricting rates of inbreeding (e.g. Meuwissen, 1997). Meuwissen and Sonesson (2003)

presented a method that maximises polygenic and single-gene response for a certain time horizon, while restricting the rate of inbreeding. This method assumed that the gene was known. Output for the two latter methods is the optimum contribution, i.e. the number of matings, per selected fish. It is advantageous for these methods if the use of certain candidates does not have 'practical' constraints. Therefore, a nucleus structure, which is centrally steered, like for the salmon, is optimal for these methods. One practical constraint in some marine species is that matings are natural. Hence, for these species, the number of matings per male or female is restricted for each spawning. However, these species are multi-spawners, such that new mates can be used for next round of selection. Also, it is possible to use frozen milt, when that is available. Currently, milt can be frozen from many species, including salmonids, carp and shrimps, but the use of it is very limited. The large family sizes of fish populations suggest designs that are similar to the top-down and bottom-up marker-assisted selection schemes in dairy cattle. Large family sizes are also an advantage for the genomic selection methods of Meuwissen et al. (2002). Genotyping- costs will be limiting for that method.

Genetic maps

Genetic maps are available for some cultured species, e.g. tilapia, cat fish, tiger shrimp, kuruma prawn, Japaneese flunder, rainbow trout and Atlantic salmon. However, the density of these genetic maps is very low, with the map for rainbow trout having the highest density (Nichols et al., 2003). Most of these genetic maps are made up of lots of AFLP markers, which are anchored to fewer microsatellites. In salmonids, tetraploidi has been found in males. This complicates the marker mapping, in that recombination rates are markedly lower in males than in females (Sakamoto et al., 2000) and therefore also the marker map lengths between males and females differ considerably. There are also differences in recombination rate over the length of the chromosomes in males, i.e. recombination rate is higher in telomeric regions than proximal regions. For the map of tilapia, haploids derived from one single female were used. These are not viable individuals. Hence no phenotypes can be recorded in these individuals.

Double haploid mapping

Different reproductive techniques are used in fish. Sex-manipulation has been done in salmonids and some marine species, e.g tilapia and flounders. The most interesting reproductive technique in outbred populations for marker and QTL mapping are probably gyno- and androgenetic double haploid fish. In gynogenesis, the sperm chromosomes are inactivated by irradiation, and following fertilisation, diploidi is restored by e.g. shortly heating the egg. The result is an individual, which is double haploid with only the female chromosome. In androgenesis, the egg is irradiated and the result is an individual, which is double haploid with only the male chromosome. The power of QTL detection is much higher for double haploid than for full-sib family and hierarchical designs in an outbred population. The superiority of the double haploid population was largest when the size of the experiment is small and when the QTL has a small effect (Martinez et al., 2002). Martinez et al. (2002) considered mitotic double haploid fish, where the two chromosomes are just copies of eachother. In meiotic haploid individuals, the two chromosomes have recombined. The power of QTL detection in these meiotic haploid populations is expected to be even higher and resemble that of selfed populations. Double haploid fish have been used for genetic mapping and QTL mapping. On the other hand, the extremely large full-sib family size that is possible for fish may make the use of special reproductive techniques for marker and QTL mapping studies un-necessary. In for example cod, both males and females produce millions of

gametes at spawning. Cod and halibut are also examples of multi-spawners in contrast to salmon that die after spawning. Multi-spawning makes the mating structure more flexible, e.g. a certain pairing can be repeated. Another objection to using double hapliod fish is that they are fully inbred individuals and may therefore express the trait differently than non-inbred animals. For example, the environmental variance for wing length in *Drosophila Melanogaster* has been shown to be larger for inbred individuals than non-inbred individuals (Falconer and Mackay, 1996). Traits with dominant inheritance may especially have a different expression, because of inbreeding depression.

Sex-manipulation is also done to get all production fish having one sex. All-male production of tilapia is done, because males grow 20-60% faster than females.

Traits
Growth is the most important trait for all aquaculture species under selection. It is recorded on the selection candidates, and can easily be implemented for mass selection. For almost all other traits, accurate measurement techniques for live fish are lacking, such that selection must be based on information on sibs. Marker-assisted selection would be especially valuable for traits that are impossible to measure in the selection candidate. Examples of these important traits are:
1. Disease resistance. Full-sib challenge tests are used on siblings for both viral (e.g. white spot syndrome and taura in shrimps, and infectious pancreatic necrosis in most seawater fish species) and bacterial (e.g. furunculosis and vibriosis) diseases. Because the surving animals are not allowed to enter the nucleus after the test for hygienic reasons, these fish cannot be used as selection candidates, and therefore only their sibs are candidates. Now, simple linear models are used, but work to utilise the non-linear nature of the data is under development.
2. Fillet quality traits. To this group of traits belong colour, texture and different fat measurements (e.g. fat% and fat distribution). Accurate measurements of these traits are being developed for slaughtered fish, but this remains a difficult group of traits to measure on live candidates.
3. Feed efficiency is a trait that is currently only possible to measure at family levels at a young age in the nucleus, but not at individual level. The value of such records is anyway rather limited, because of the unknown correlation with feed efficiency at a later age, when the fish is reared in sea. No selection program for fish is today selecting directly for feed efficiency, but instead indirect selection for feed efficiency is done on growth.
4. Maturation. Sexual maturity leads to reduced growth and fillet quality in several, aquaculture species, e.g. the salmonids. Therefore, selection for late maturity is performed, in that the mature fish will be discarded as selection candidates. In tilapia, late maturity is also wanted, because of excessive spawning, which 'pollutes' the ponds.

In general, all these traits are measured in both males and females.

Quantitative Trait Loci (QTL)
Very few QTLs for production traits have been identified in fish. In tilapia, QTLs have been identified for cold and salinity tolerance (Jackson, 1998; Agresti et al., 2000; Cnaani et al., 2003). These traits are interesting for tilapia to be able to increase the area of production. Now, tilapia is only produced in tropical areas. Allele effects for the growth hormone (GH) gene has been found in coho salmon (Forbes et al. 1994); brown trout (Gross and Nilsson 1995) and chinook salmon (Park et al. 1995). QTLs for disease resistance traits have been found. For example, QTL for infectious hematopoetic necrosis virus was found in a cutthroat

x rainbow trout cross (Palti, et al. 1999). QTLs have also been found for infectious pancreatic necrosis in rainbow trout (Ozaki, et al, 2001). Moen et al. (2003) found QTLs for infectious salmon anemia in Atlantic salmon. No QTLs have been reported for fillet quality traits.

Bottom-up and top-down marker-assisted selection schemes
In livestock populations, the most common within-family marker-assisted selection schemes are the two-generational bottom-up (Kashi et al., 1990) and three-generational top-down schemes (Mackinnon and Georges, 1998). Here, I would like to present these two schemes modified for fish populations.

In the bottom-up scheme (Figure 1), we assume that both sires and dams have genotypic records, and that we have two groups of progeny from these parents. For the group of test progeny, we have phenotypic and genotypic records and for the group of selection candidates, we have only genotypic records. Phenotypic recording implies that the fish cannot be used for breeding, maybe because they have been used in a challenge test, or they have been slaughtered to obtain sib-information. The genetic markers of the sire are denoted M1 and M2 and the dam has M3 and M4, with M1 and M3 having the positive effect. All M1M3 progeny, some M1M4 and M2M3 and none M2M4 would be selected.

Parents with genotypic record

♂ (**M1**M2)   x   ♀ (**M3**M4)

Test-progeny with phenotypic   Candidate progeny with
and genotypic record   genotypic record
to estimate marker effects

M1M3 ⟶ **Select all**
M1M4 ⟶ Select **some**
M2**M3** ⟶ Select **some**
M2M4 ⟶ Select none

Figure 1
Bottom-up marker-assisted selection scheme for fish. Marker genotypes in bold are preferably selected

In the top-down scheme (Figure 2), the grand-parents also only have known genotypic records. Their parents have genotypic record and a mean of the phenotypic records of the test progeny. The mean phenotypic records are used to estimate the marker effects (M1 vs M2 and M3 vs M4) in the parents. We also have one group of test progeny and one group of candidate

progeny. Again, different number of progeny will be selected per group depending on their markers. In Figure 2, these are marked in bold.

In the top-down scheme, fewer genotypic records are taken than in the top-down scheme, which will reduce the cost of the schemes if genotyping is expensive. The top-down scheme is suitable for schemes, where progeny tests are performed. Progeny tests are at the moment not performed in fish, which makes the bottom-up scheme more suitable.

Grandparents with genotypic record

$\male$ (**M1**M2) x $\female$ (**M3**M4)
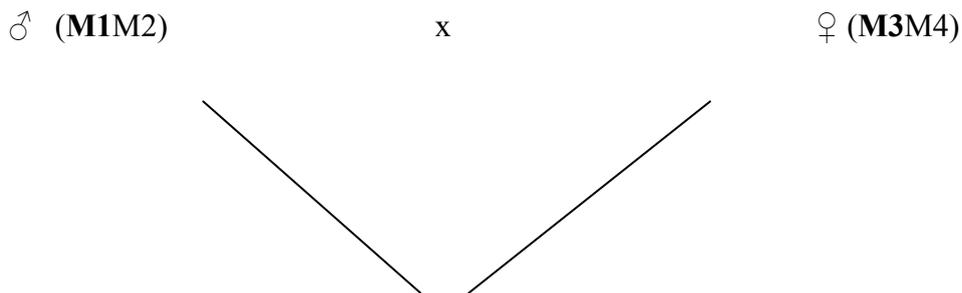
Parents with genotypic record, and the M5 and M6 are taken randomly from the population

$\male$ (**M1M3**, **M1**M4, M2**M3**, M2M4) x $\female$ (M5M6)

Test-progeny with phenotypic mean record to estimate marker-effects in the parent generation

Candidate progeny with genotypic record

	M5	M6		M5	M6		M5	M6		M5	M6
M1	**M1M5**	**M1M6**	M1	**M1M5**	**M1M6**	M2	M2M5	M2M6	M2	M2M5	M2M6
M3	**M3M5**	**M3M6**	M4	M4M5	M4M6	M3	**M3M5**	**M3M6**	M4	M4M5	M4M6

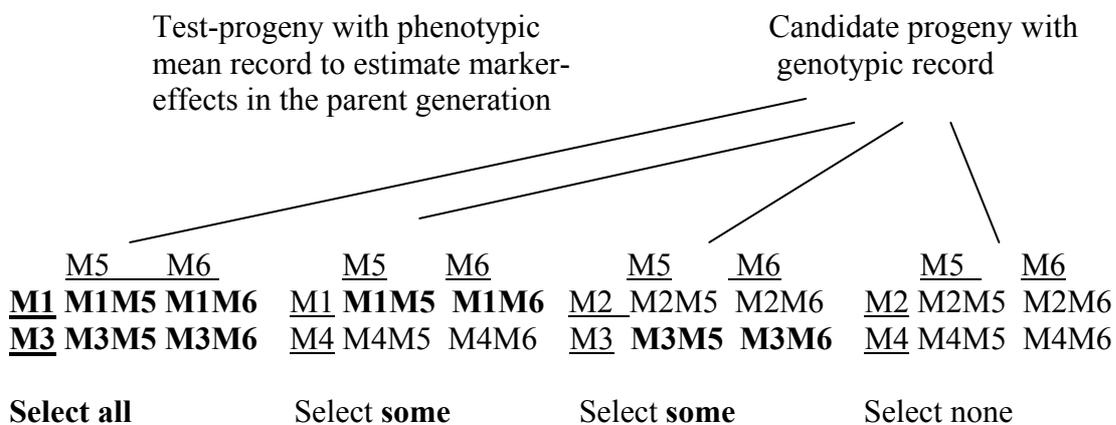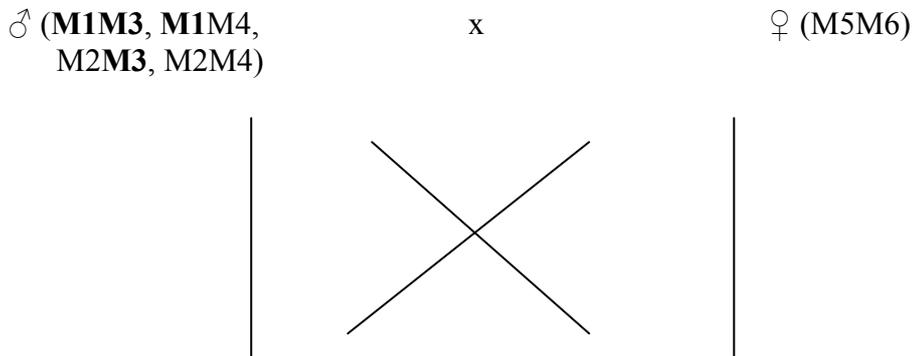**Select all** Select **some** Select **some** Select none

Figure 2
Top-down marker-assisted selection scheme for fish. Marker genotypes in bold are preferably selected

Generation intervals

One advantage with marker-assisted selection is that generation intervals can be reduced, but they can never be lower than the age at which the fish get sexual mature (if the selection scheme does not involve in-vitro steps etc). For the most common cultured species, fish get sexual mature at about two to three years, with the exception of tilapia, which get sexual mature at the age of only one year. In fish, generation intervals are the biologically minimum and it will not be possible to further reduce generation intervals. This reduces the advantage of marker-assisted selection. However, marker-assisted will have other genetic and economical effects. For example, one could pre-select parents based on marker-information for the traits that are measured late in life, and hence saving space and money by only keeping a lower number of fish. Conversely, selection intensity could be increased for the other traits where no marker-information is used. There will be increased accuracy of selection for the traits, where marker-assisted selection is used.

Introgression schemes

Many fish breeding schemes have been started with a relatively narrow base, selecting for only one or two traits from relatively few animals. However, because all fish species still have wild ancestors, introgression of genes, i.e. identified genes or QTLs, from these wild ancestors to the breeding populations, is possible. It would be possible to gain certain properties of the wild population in the breeding population. However, one assumes that all other properties of the wild fish are unwanted in the breeding population, such that only the particular gene of interest should be introgressed, leaving the genome of the breeding population intact. One problem of actually implementing introgression is to actually find the properties of the wild populations, and identify genes or QTL for the trait to be introgressed. This is a costly and time consuming process, especially since many fish species do not even have a genetic map. Methods for simultaneous QTL mapping and introgression would be useful.

Conclusion

In conclusion, marker-assisted selection is at present limited by the lack of high-resolution genetic maps in most aquaculture species. However, marker-assisted selection would markedly increase accuracy of selection for most traits, which today is based on information on sibs only for most traits in family-based fish breeding programs.

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