

Prospects for genetic technology in salmon breeding programmes

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Abstract

Selective breeding has been very successful in increasing production in Atlantic salmon. Gene technology opens new opportunities to comprehend the nature of the genetic variation underlying production traits. Two major areas in which gene technology may play an important role are (1) production of genetically modified fish and (2) development and utilization of genetic markers. Several studies of transgenic salmonids have shown substantially increased growth rates. However, many different issues are related to whether genetically modified fish should be used or not. Genetic markers can be used for aquaculture purposes and for monitoring wild populations. Construction of genetic maps based on markers enables the identification of quantitative trait loci (QTL) and/or markers linked to them. This will facilitate marker-assisted selection, enabling improvement in economically important traits, in particular traits difficult to breed for, such as food conversion efficiency and disease resistance. Several experiments aimed at mapping QTL in salmonids using genetic markers are ongoing. DNA marker technologies can also be used for identification and monitoring of lines, families and individuals, and for genetic improvement through selection for favourable genes and gene combinations.

Keywords: genetic marker, genetic modified organism, marker-assisted selection, quantitative trait loci, selective breeding programme

Introduction

Until recently, artificial selection to improve domesticated plants and animals has been entirely based on phenotype. While this approach has proven to be successful, the age of genomics offers the prospect of shifting directly to selection based on genotypes or, even more likely, genotypic and phenotypic data that can be used together to estimate breeding values. At first glance, this appears to offer the potential to greatly enhance the selection response by minimizing environmental effects, and has particular interest for traits with low heritability.

Two main areas of gene technology are being developed to improve efficiency of salmon breeding: the production of transgenic fish and the use of genetic markers. Transgenic fish usually have foreign genes inserted into their genome and these will be expressed in the adult individual. Several studies have shown enhanced growth in transgenic fish. Genetic markers are segments of DNA that can be easily and cheaply typed. Genetic markers have been used to search for the location of genes important for good health and high production in so-called Quantitative Trait Loci (QTL) mapping. The purpose of QTL mapping is the use of QTL information in marker-assisted programmes to improve breeding prediction. Another use of genetic markers is to measure genetic variability and relatedness between individuals, strains and lines.

Any attempt aimed at mapping QTL must consider the biology of the species, the conditions of production and the nature of the traits of interest.

Aquaculture species are very different to most domesticated animals. They typically have a very high reproduction rate with very large progeny groups. In the particular case of Atlantic salmon, a long generation interval of 4 years must also be taken into account when planning QTL mapping or when using detected QTL in marker-assisted selection.

A brief overview of past achievements and new approaches and trends for the genetic improvement of farmed salmonids is attempted in this paper. The general objective is to present potential applications of gene technology in salmon breeding programmes. The order of the paper is: (1) to briefly present the main achievements obtained using traditional breeding; (2) to discuss the use of transgenic salmonids; (3) to present the status and use of genetic markers and QTL mapping methods in salmonids; and (4) to outline the prospects for the use of genetic technology in breeding programmes.

Genetic improvement through selective breeding

The ultimate goal of any genetic improvement programme is to increase biological productivity. Genetic evaluation is the process of identifying the best individuals, families or lines to breed for the next generation. Through selective breeding, genetic changes are induced by changing allele frequencies at loci affecting production traits, making use of the additive genetic variation in the population. One important advantage of selection is that the improvement in one generation is retained and the genetic gain in the following generation is added.

Observed phenotype is the simplest form of breeding value and can be quite accurate for highly heritable traits. However, animals need to be compared across environments and many traits have lower heritability. Consequently, most genetic improvement programmes benefit from more sophisticated estimates of breeding values, such as Best Linear Unbiased Prediction (BLUP) (Muir 1997).

High selection intensity can be practised in many fish species because of their high fecundity. This opens up the possibility of sampling an almost unlimited amount of eggs and milt for different population structures. It is also possible to take many samples from each single family to test for different traits included in the selection criterion. This makes it possible to carry out challenge tests for disease

resistance and to utilize specialized equipment to measure flesh quality.

The number of large-scale, selective breeding programmes in aquaculture is still low, although a number of successful examples exist. A pioneering programme was started for Atlantic salmon and rainbow trout in Norway more than 25 years ago (Gjedrem 1983) and was later adopted on a national basis by the Norwegian salmon industry and run by AquaGen AS from 1992. The Norwegian programme has focused on growth rate, age at sexual maturity and, later, disease resistance and quality traits. Today, the selected line developed in the Norwegian breeding programme accounts for approximately 70% of the Norwegian salmon production. Similar breeding programmes to the Norwegian programme are running in Canada (Friars 1993), Iceland (Stofnfiskur H/F), Chile (Genetic SA and Landcatch) and Scotland (Landcatch).

The experience gained from selection in salmonids has resulted in similar programmes in other fish and shellfish species, such as tilapia (Eknath, Dey, Rye, Gjerde, Abella, Sevilleja, Tayamen, Reyes & Bentsen 1998), shrimp (Fjalestad, Carr, Lotz, Sweeney & Gjedrem 1997) and rohu carp (Gjerde, Reddy, Mahapatra, Saha, Ranjit, Meher, Sahoo, Lenka, Govindassamy & Rye 2002).

The selection response for growth rate in salmonids is generally much higher than that obtained for traditional livestock animals (Table 1). The reasons for this are the aforementioned high fecundity and the presence of a much higher genetic variation in fish. According to Gjedrem (1997), the coefficient of variation for growth rate is 7–10% in farm animals, whereas it is 20–35% in most fish species.

Gjerde & Korsvoll (1999) reported realized selection differentials for the year-classes 1980–94 in the Norwegian breeding programme for Atlantic salmon. The average genetic gain for growth rate was around 14% per generation through six generations of selection. These authors also reported that four rounds of selection for delayed sexual maturation has reduced the incidence of salmon becoming sexually mature before they reach market size. Thodesen, Grisdale-Helland, Helland & Gjerde (1999) reported a correlated response in feed conversion efficiency when selecting for growth rate in Atlantic salmon. Therefore, selected fish are better able to utilize feed resources than unselected fish.

In general, high returns on investment are expected in fish breeding programmes compared

with programmes for non-aquatic farmed animals (Gjedrem 1997). However, traits difficult to measure, such as disease resistance and traits with high economical impact on production, would benefit from the use of gene technology.

Transgenic/genetically modified organisms (GMO)

Genetically modified organisms are defined by international agreements, in a very narrow sense, as being essentially transgenic organisms, i.e. organisms that have foreign genes inserted into their genome (see FAO 2000 for more details on definitions).

Several useful genes that can be transferred into different aquatic species have been identified (Table 2). Among the genes identified are those that produce growth hormones for increased growth and efficiency, antifreeze protein for increased cold tolerance and growth, lysozyme for increased disease resistance and prolactin hormones that

influence hatching, osmoregulation, behaviour and general metabolism.

From the mid 1980s, transgenesis with purified gene constructs has been achieved in several fish species: goldfish (Zhu, Li, He & Chen 1985), rainbow trout (Chourrout, Guyomard & Houdebine 1986; Maclean, Penman & Zhu 1987), loach (Zhu, Xu, Li, Xie & He 1986), catfish (Dunham, Eash, Askins & Towes 1987) and Atlantic salmon (Fletcher, Shears, King, Davies & Hew 1988). Most of the research on transgenic salmonids has been conducted with gene constructs designed to influence growth.

Devlin, Yesaki, Biagi, Donaldson, Swanson & Chan (1994) showed that growth hormone over-expression resulted in increased growth rate in transgenic fish. Transgenic F₀ coho salmon that expressed the salmon growth hormone gene under the control of the salmon metallothionein promoter showed a three- to 11-fold increase in weight. There are also other studies demonstrating the superior growth of transgenic fish (Du, Gong, Fletcher, Shears, King, Idler & Hew 1992; Devlin, Yesaki,

Table 1 Response to selection for growth rate in some salmonid species

Species	\bar{x}	Gain per generation (%)	No. generations	Reference
Coho salmon	250 g	10.1	4	Hershberger, Myers, McAuley & Saxton (1990)
Rainbow trout	3.3 g	10	3	Kincaid, Bridges & Von Limbach (1977)
Rainbow trout	4.0 kg	13	2	Gjerde (1986)
Atlantic salmon	4.5 kg	14.4	1	Gjerde (1986)
Atlantic salmon	6.3 kg	14	6	Gjerde & Korsvoll (1999)
Atlantic salmon	3.5 kg	12.5	1	O'Flynn, Bailey & Friars (1999)

Table 2 Transgenic salmonids being tested for use in aquaculture

Species	Foreign gene	Desired effect and comments	References
Atlantic salmon	AFP	Cold tolerance	Reviewed by Devlin (1997)
	AFP salmon GH	Increased growth and feed efficiency	
Coho salmon	Chinook salmon GH + AFP	After 1 year, 10- to 30-fold growth increase	Reviewed by Devlin (1997)
Chinook salmon	AFP salmon GH	Increased growth and feed efficiency	Reviewed by Devlin (1997)
Rainbow trout	AFP salmon GH	Increased growth and feed efficiency	Reviewed by Devlin (1997)
Cutthroat trout	Chinook salmon GH + AFP	Increased growth	Reviewed by Devlin (1997)
Salmon	Rainbow trout lysozyme gene	Disease resistance, still in development	Hew & Fletcher (1997)

Note: The development of transgenic organisms requires the insertion of the gene of interest and a promoter, which is the switch that controls expression of the gene. AFP, antifreeze protein gene (Arctic flatfish). GH, growth hormone gene.

Donaldson & Hew 1995; Hew, Fletcher & Davies 1995; Rahman & Maclean 1999; Saunders, Fletcher & Hew 1998). In general, all these studies have used wild or recently domesticated fish with relatively low growth. More recently, Devlin, Biagi, Yesaki, Smailus & Byatt (2001) investigated the interaction between the genetic background of transgenic fish and growth hormone over expression. Both a slow-growing wild strain of rainbow trout and a fast-growing domesticated strain of rainbow trout were used as recipients for a salmon growth hormone. Growth hormone-expressing transgenic 'wild' trout reached a weight 17.3-fold higher than that of the non-transgenic sibs. However, the fast-growing, non-transgenic domesticated strain displayed a growth rate equal to that of the transgenic wild strain. Introduction of growth enhancement into the domesticated strain did not result in increased growth. The authors concluded that 'the effect of introducing a growth-hormone gene construct into fish to increase growth rates appears to be dependent on the degree to which earlier enhancement has been achieved by traditional genetic selection.' Comparable results have been shown in mice; lines selected for high growth had a higher 8-week body weight than growth hormone transgenic mice (Parks, Eisen & Murray 2000).

So far, no genetically modified salmonids are in commercial production. However, one US/Canadian company has recently sent a proposal to the Food and Drug Administration in the USA to commercialize Atlantic salmon modified to carry growth hormone gene from the Pacific Chinook salmon (Fletcher, Shears, King, Goddard, Kao, Du, Davies & Hew 1992). The company claims that their fish grow four to six times faster than ordinary salmon and also claims that it has a higher food conversion, needing 25% less feed. However, time needed to produce stable lines of desirable and commercially valuable broodstock is considerable. Transgenic fish homozygous for the transgene cannot be produced with certainty until the fourth generation (F₃) (Fletcher, Goddard, Shears, Sutterlin & Hew 2001).

Genetic markers, construction of genetic maps, QTL mapping and marker-assisted selection

Genetic markers

A genetic marker is a section of DNA for which there is an experimental procedure allowing identification

of genotypes. There is a wide array of DNA-based molecular marker types and only a few of them will be mentioned here. Simple sequence repeats (SSR), also known as microsatellites, are the most widely used genetic markers in fish. They are co-dominant, which means that homozygous and heterozygous individuals can be distinguished. Amplified fragment length polymorphisms (AFLPs) were initially developed for plants and are capable of generating a very high density of polymorphic markers, even in species with large genomes and low polymorphism (Becker, Vos, Kuiper, Salamini & Heun 1995). AFLPs are dominant and several markers and alleles are confounded in the same polyacrylamide gel. The advantage of AFLPs is that a large number of polymorphisms can be scored in a single polyacrylamide gel. Our results working with AFLPs in Atlantic salmon indicate that the average number of polymorphisms per primer combination is around 5–10 (unpublished results). This value is low compared with the polymorphism rate observed in plants (Mackill, Zhang, Redona & Colowit 1996; Qi & Lindhout 1997) but is still a fast way to cover the genome of Atlantic salmon with genetic markers.

Genetic maps

Once genetic markers are developed, they are used to construct genetic maps of markers. This means that chromosomal location, order and distance between markers are established. In essence, they provide flag-posts along the genome that allow locations to be identified. They are obtained by analysing the co-segregation of allelic forms of different markers within pedigrees.

A large effort is now being devoted to develop markers and to construct genetic maps for the most important farmed fish species. Genetic maps in salmon and other fish species are not highly developed with the exception of zebrafish (*Danio rerio*) (Woods, Kelly, Chu, Ngo-Hazelett, Yan, Huang, Postlethwait & Talbot 2000).

A collaboration project, with the title 'Generation of highly informative DNA markers and genetic marker maps of salmonid fishes', called SALMAP, funded by the European Commissions FAIR programme, ran from 1997 to 1999 and was aimed at constructing a low-resolution map of Atlantic salmon, rainbow trout and brown trout. As a result of this international effort, a genetic map of rainbow trout comprising approximately 200 microsatellites

was published (Sakamoto, Danzmann, Gharbi, Howard, Ozaki, Khoo, Woram, Okamoto, Ferguson, Holm, Guyomard & Hoyheim 2000). So far, 299 and 232 markers, mostly microsatellites, have been genotyped for Atlantic salmon and brown trout respectively (B. Hoyheim, pers. comm.). Linkage mapping efforts are under way for a number of major aquacultural species including tilapia (Kocher, Woo-Jai, Sobolewska, Penman & McAndrew 1998), channel catfish (Liu 1999), Kuruma prawns (Moore, Whan, Davis, Byrne, Hetzel & Preston 1999a) and black tiger shrimp (Moore, Wilson, Whan, Bierne, Lehnert, Chu, Pongsomboon & Tanssanakajon 1999b).

QTL mapping

QTL mapping is a set of procedures for detecting genes controlling quantitative traits and estimating their effects and genome location. The basis of the methods for QTL mapping is that QTL and relevant markers are located in close proximity in the same chromosome. Co-segregation of alleles at the marker and at the QTL can be evaluated by testing the inheritance of marker alleles with individual performance for the trait in question.

There are two aspects critical for success in a QTL research programme. The first is high heterozygosity at the markers and at the QTL. If the populations at crossing are fixed for alternative alleles at marker loci and QTL, then high heterozygosity is expected in the F_1 . The problem with crossing in species such as Atlantic salmon is that each generation requires 4 years. The second aspect is that the experimental design must make use of enough resources in the most efficient way; this is usually done by computing statistical power. In the context of QTL mapping, this means estimating the probability of detecting a QTL where there is, in reality, a QTL. Designs with poor structure or insufficient data will have low power.

In general, fish offer several advantages for QTL mapping. Their high reproductive capacity means that a large number of individuals are available for performance measurement and marker genotyping. In Atlantic salmon, selective breeding has been successful in identifying growth traits, as explained in the first section of this paper. Consequently, the use of DNA markers in farmed salmon is mainly concentrated towards improving disease resistance. Disease resistance is recorded in challenge experiments in

which full- and half-sibs of the potential breeding candidates are tested for resistance to viral or bacterial diseases commonly found in farmed fish. Two examples of designs that can be used to map QTL for disease resistance in fish are:

1. Selection of high- and low-ranking individuals followed by testing for homogeneity of genotype classes in the two groups. In this approach, the parents are ranked according to the average number of surviving progeny and then the marker is genotyped in the two extremes of the distribution. The allele frequencies in the two groups are compared. This method assumes linkage disequilibrium across families. The problem with this method is that related parents are not accounted for.
2. The transmission disequilibrium test (TDT) for QTL mapping. The rationale behind this technique is that affected offspring would inherit marker alleles in a larger proportion than expected because of linkage with QTL affecting resistance to a disease. Consider the example shown in Fig. 1 where a male heterozygous at both the QTL (Qq) and the marker (Mm) is crossed to a female homozygous at both. The homozygous condition in the dam is not required for the general use of TDT but it is used here for the purpose of illustration. Under the null hypothesis (no linkage between QTL and the marker), the genotyping of the affected offspring of this cross would result in a proportion of 50:50 offspring with genotypes Mm and mm respectively. Departure from the segregation 50:50 would indicate linkage of the marker to a QTL with one allele increasing or decreasing resistance to the disease. The advantage in using TDT is that the genotyping efforts are focused on the affected offspring, which provide most of the information for linkage. TDT is particularly powerful when combined with high selection for the trait in question among progeny. Table 3 illustrates the power achieved for QTL mapping using TDT in a single family when the proportion of selected offspring is 0.50, 0.30 and 0.10. For example, genotyping of 100 progeny when using a proportion selected of 0.10 requires the family size in the challenge experiment to be 1000. The power is high with relatively small genotyping efforts.

The first study of the mapping of quantitative trait loci with disease resistance in fish was recently published (Ozaki, Sakamoto, Khoo, Nakamura,

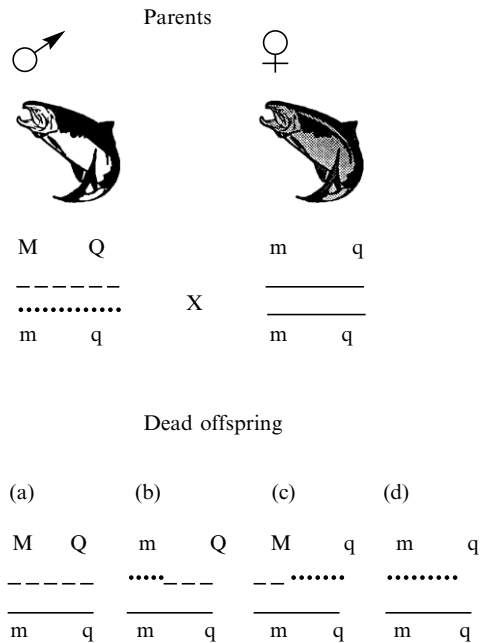


Figure 1 Scheme representing the use of genetic markers to map QTL using a transmission disequilibrium test. The sire is heterozygote for the marker (M/m) and for a gene affecting the disease (Q/q). There are four possible types of offspring according to the alleles inherited from the sire: (a) inheritance of allele M and non-recombinant, (b) inheritance of allele m and recombinant, (c) inheritance of allele M and recombinant and (d) inheritance of allele m and non-recombinant. The test compares if the frequency of alleles m and M is the same among dead progeny in a challenge experiment. For example, if allele Q is making the fish more vulnerable to diseases then a larger frequency of carriers of allele M would be shown among the dead progeny.

Table 3 Power for transmission disequilibrium mapping using selected progeny of 50%, 30%, and 10% when the number of genotyped progeny is 20, 40, 60, 80 or 100

% Selected	Number of genotyped progeny				
	20	40	60	80	100
50	0.12	0.31	0.44	0.54	0.61
30	0.22	0.53	0.71	0.82	0.89
10	0.44	0.84	0.96	0.99	1.00

The significance level is 0.05 and the QTL and marker are assumed fully linked. The QTL effect is 0.5 phenotypic standard deviations.

Coimbra, Akutsu & Okamoto 2001). Two putative QTL, with medium to large effect and with several components of resistance to infectious pancreatic necrosis (IPN), were identified in rainbow trout. These two loci were responsible for a significant portion (27–34%) of the total phenotypic variation in a backcross family (Ozaki *et al.* 2001). Some other studies are developing towards mapping QTL affecting disease resistance: rainbow trout (Palti, Parsons & Thorgaard 1999; Rodriguez, LaPatra, Williams & May 2001) and Atlantic salmon (Grimholt, Stet, Storset, Nordmo, Midtlyng & Rye 1998; Moen, Fjalestad, Munck & Gomez-Raya 2001). Apart from disease resistance, other traits used for QTL mapping are: QTL affecting development rate in clonal rainbow trout strains (Nichols, Robison, Wheeler & Thorgaard 2002), thermal tolerance in outbred rainbow trout (Perry, Danzmann, Ferguson & Gibson 2002), upper temperature tolerance in rainbow trout (Jackson, Ferguson, Danzmann, Fishback, Ihssen, O'Connell & Crease 1998) and spawning time in rainbow trout (Sakamoto, Danzmann, Okamoto, Ferguson & Ihssen (1999).

Marker-assisted selection

Marker-assisted selection (MAS) is the use of genetic markers linked to QTL in genetic improvement programmes. Although the term QTL strictly applies to genes of any effect, in practice it refers only to major genes, as only these are of a large enough size to be detected in mapping experiments. MAS will be more important for the improvement of traits that are difficult or expensive to measure. In fish, resistance to diseases is not measured in selected candidates but in their progeny. Consequently, MAS could be used within families to increase selection response.

Other applications of genetic markers

DNA markers can also be useful tools in stock identification for natural resources protection purposes, for characterizing genetic diversity in founder broodstock, for wild stock management and for use in aquaculture (see review by Ferguson, Taggart, Prodohl, Mcmeel, Thompson, Stone, McGinnity & Hynes 1995). Such genetic profiling is usually called DNA fingerprinting.

DNA fingerprinting has, for example, been used to discriminate between European and North American Atlantic salmon (Taggart, Verspoor, Galvin, Moran & Ferguson 1995) and between wild strains and farmed populations of Atlantic salmon (Mjølnerød, Refseth, Karlsen, Balstad, Jakobsen & Hindar 1997). McGinnity, Stone, Taggart, Cooke, Cotter, Hynes, McCamley, Cross & Ferguson (1997) used DNA profiling to estimate the genetic impact of escaped salmon on native populations.

DNA fingerprinting can be used to test paternity, and this can be done using a variety of genetic markers. Consequently, more families can be kept in the breeding nucleus without the need for using separate tanks at early ages. The development of DNA profiling techniques for family identification can reduce the problem of the introduction of environmental effects common to full sibs. Selective breeding programmes based on a family design require the different families to be kept separately until the fry are big enough to be tagged (5–10 g). Consequently, the length of this period is substantial (several months). The consequences of this delay in tagging are both reduced selection accuracy and lower response to selection. Identification of families by their specific 'fingerprint' allows the families to be kept together from fertilization. This will eliminate the problems related to common environmental effects (Herbinger, O'Reilly, Doyle, Wright & O'Flynn 1999). Additionally, more families can be tested and consequently higher selection intensities can be applied, which would yield a higher selection response.

Prospects for the application of gene technology to salmon breeding

Transgenic fish

Transgenic fish may have potential applications in aquaculture. However, there are still many problems that need to be overcome before transgenic animals can be produced on a large-scale basis. Today, Atlantic salmon farming is based on domesticated strains selected primarily for growth performance. One immediate and important question is if there is anything to be gained with transgenic salmon. If the results comparing transgenic/non-transgenic rainbow trout of different genetic origin (Devlin *et al.* 2001) can be transferred to Atlantic salmon the answer might be no, however such experiments are yet to be carried out. Also, of interest is work with other traits like disease resistance,

however work in this area is still in an early phase. The production of transgenic fish is a 'one-time' improvement and should, in any case, be combined with other genetic improvement methods such as selective breeding.

Most important in considering the use of transgenic fish are the questions raised about environmental issues, human health issues, ethics and, of course, public perceptions. Today, most consumers will not accept genetically modified salmon.

Genetic markers, construction of genetic maps, QTL mapping and marker-assisted selection

One of the requirements for mapping QTL is a linkage map that covers the whole genome (Falconer & Mackay 1996). Consequently, further development of genetics maps will enhance QTL maps and finally gene identification and cloning. Over the next few years, genetic maps of markers for economically important fish species used in aquaculture will be constructed. These genetics maps will be used for QTL mapping. The last step of this process will be the practical implementation of MAS in breeding programmes of farmed species.

There are few examples of breeding programmes in terrestrial farm animals where marker-assisted selection is currently being applied. Some pig and dairy breeding companies claim to actively use marker genotype information for selection purposes. In fish, there are many ongoing studies on genetic markers, mapping and detection of QTL, but to our knowledge there are no large-scale fish breeding programmes using MAS.

It is likely that most of the traits of interest to salmon breeders are controlled by a large number of loci, many of them with a small effect on the quantitative trait and relatively few loci with a large effect. Genes with a small effect are difficult to map and it is unlikely that molecular techniques will replace selective breeding. A combination of 'traditional' selective breeding and MAS would be more beneficial. Traits difficult to measure would benefit from the use of MAS together with phenotypic performance to enable an increased selection response. Today, disease resistance in salmon breeding programmes is only included on a family basis. Individual marker-QTL information together with family information could be used for genetic evaluation of breeding candidates in current programmes. This approach has the advantage of utilizing selection

intensity that is not yet exploited. Predictions of the benefits of MAS are that genetic progress may increase by around 11% under certain circumstances (Gomez-Raya & Klemetsdal 1999).

Other applications of genetic markers

The development of DNA profiling techniques for family identification can reduce the problem of the introduction of environmental effects common to full sibs as described in an earlier section of this paper. The main questions for using DNA fingerprinting are practical issues related to sampling and, most importantly, the costs of genotyping compared with keeping the families in separate tanks. The trend towards fast and cheaper genotyping and sequencing through the use of new technologies, such as DNA chips, would lead to a wider use of the technique in breeding programmes. In addition, this development could increase the selection response substantially.

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