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FORESIGHT PROJECT ON GLOBAL FOOD AND FARMING FUTURES

Application of genetics and genomics to aquaculture development: current and future directions

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SUMMARY

Global aquaculture production continues to grow rapidly yet a small proportion of the animals and plants being used come from managed breeding and improvement programmes. The biology of aquatic organisms offer many opportunities for rapid genetic gains as new genetic and genomic techniques make the management of improvement programmes feasible in a wider range of species. The current paper describes the application of a wide range of techniques, many unique to aquatic organisms, and their potential to secure aquaculture production in the future.

INTRODUCTION

Aquaculture production has grown from less than 1 million tonnes (t) in the 1950s to nearly 60 million t in 2008 (FAO 2009). However, production of many species still depends on wild-caught fry or broodstock: 0·86 of the 69 farmed Chinese freshwater species (Honglang 2007) come from wild-caught fish. For domesticated stocks, there is growing evidence that traditional breeding approaches seriously degrade the genetic quality without continuous replacement by wild or managed fish (Mair *et al.* 2007). Less than 0·05 of production was estimated to come from scientifically managed breeding programmes (Gjedrem 2005).

The history of fish genetics has been reviewed by Dunham (2004) and Gjedrem (2005). A range of attributes has been targeted, including growth, maturation, environmental tolerance and disease resistance. Advantages for genetic gain include external fertilization and high fecundity, allowing a large number of gametes to be collected and fertilized under controlled conditions. Breeding designs with large family sizes can be constructed, allowing better estimates of genetic parameters and high-selection pressures, and improvements can be passed on quickly to industry. Many species are still close to being wild organisms and display high levels of genetic variation; heritabilities are medium to high across a wide range of traits. Aquatic organisms are susceptible to environmental manipulation and a single genotype can potentially have many phenotypes. Single-sex populations can dramatically boost production. The phenotypic sex of many species can be changed by administering sex hormones during the sexually labile period of development, single-sex stock being generated directly or through controlled breeding of sex-reversed individuals. Other major traits such as the onset of sexual maturity can be advanced or delayed by the manipulation of daylength.

The new '-omic' (e.g. genomic and proteomic) tools have also been applied in a wide range of species, though with few practical applications as yet. More widely in the development of productive and cost-effective aquaculture, genetic applications for developing high-quality feed inputs have great significance, progress in which is described in the final section of the present review.

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SELECTIVE BREEDING

The species that have seen the most improvement are common carp, rainbow trout, Atlantic salmon and tilapia, channel catfish and ornamental fishes. Modern programmes for carp were established in Central and Eastern Europe (Kirpichnikov 1981), and improved strains contribute to the bulk of European and Asian production, with programmes to further develop these in Vietnam and India (Penman *et al.* 2005). Recent studies have shown that cross breeding and pedigree-based selection can still produce improvements (Vandeputte 2009).

Rainbow trout has also had a long history of domestication, in the USA (Gall & Huang 1988), Norway (selected for seawater production) (Gjedrem 1992), Finland (Kause *et al.* 2003) and Denmark (Henryon *et al.* 2002). But the uptake of selected strains by the industry has been slow; for instance, the largest commercial US egg supplier, Trout Lodge, only started a family-based breeding programme within the last 5 years. Short-term improvements such as all-female production or all-female triploids have been adopted but most eggs still come from mass-selected domesticated strains.

Norwegian Atlantic salmon programmes were initially government funded, but are now run by private companies (Aquagen and Salmobreed; Gjedrem 2005). Individual and family performance is assessed for a range of commercial traits (growth, sexual maturation and body conformation) and, more recently, disease resistance (infectious pancreatic necrosis virus (IPNV), *Aeromonas*, infectious salmon anaemia (ISA)). Estimated gains are between 8 and 10% per generation (Gjoen & Bentsen 1997). Similar programmes have also been developed by Landcatch Natural Selection in Scotland and Chile, Stofnfiskcur in Iceland and Aquachile in Chile. Most farmed eggs now come from scientific breeding programmes, with significantly improved performance. Genomic technologies, including quantitative trait loci (QTL) and marker-assisted selection (MAS) approaches, are already benefiting improved virus resistance (Houston *et al.* 2008; Moen *et al.* 2009).

Tilapia have become the second most important group of farmed fish in the world (>2·7 million t in 2008) with *Oreochromis niloticus* becoming the dominant species in fresh water. Early selection work was hindered by the degraded nature of many of the commercial strains being used. The Genetically Improved Farm Tilapia (GIFT) programme, starting in 1987 in the Philippines, systematically compared wild and commercial strains in a variety of aquaculture environments and began family-based selection for growth. The work was further developed by Worldfish (CGIAR, see Acosta & Gupta 2010). Other programmes have either used the GIFT strain as a starting point (Genomar 2008) or have constructed new synthetic populations using a number of selected lines (Rutten *et al.* 2005). The bulk of tilapia production, however, does not come from improved sources and producers use direct hormone sex-reversal of mixed sex fry to produce phenotypic all-male populations to overcome problems of excessive fry production during growout (see Beveridge & McAndrew 2000).

Selective breeding programmes have been more recently applied to other marine high-value species: European sea bass, Gilthead sea bream and Turbot. By 2005 it was assumed that as much as 0·80 of the European sea bass production already came from commercial populations which had undergone some level of genetic improvement and at least one major Greek company has initiated (in 2002) a large-scale family-based selective breeding programme on sea bream. Programmes on other high-value species, such as cod, halibut and tuna, are also under way.

HYBRIDIZATION

The ease of gamete collection in fish means that there are many references to inter-specific and inter-generic hybridization and the potential for using hybrids in aquaculture (Bartley *et al.* 2001). Hybrids have become important at the national level in several countries, e.g. *O. niloticus* × *Oreochromis aureus* in Israel because of the skewed male sex ratio and cold tolerance of the hybrid (Hulata *et al.* 1993). In the USA, the sunshine bass (*Morone chrysops* × *Morone saxatilis*) grows faster and has better overall culture characteristics than either parental species under commercial culture conditions (Smith 1988). However, despite the large numbers of reported hybrids few have been successfully cultured for extended periods because of the added complexity of production or because of introgression of the hybrid back into the parental species and loss of beneficial characteristics.

CHROMOSOME SET MANIPULATION

A range of genetic and environmental manipulations can be applied to aquatic species. If normally fertilized eggs are heat or pressure shocked at the 2nd meiotic division, a triploid (3n) embryo, containing three chromosome sets, is produced. Protocols are now available for over 30 different fish and shellfish species (Dunham 2004). Triploids are effectively sterile and are used in the production of larger rainbow trout, usually from all-female strains (Bye & Lincoln 1986), channel catfish (Wolters *et al.* 1991), common carp (Basavaraju *et al.* 2002) and in other species whose maturation slows growth. They are also used to improve growth rate and flesh quality in oysters (Guo *et al.* 1996). Triploids can be used to reduce the risk of stocked or escaped farmed strains interbreeding with native populations (Kozfkay *et al.* 2006) or exotic

species, such as grass carp for aquatic weed control, becoming established (Wattendorf 1986). Triploid sterility is also proposed to stop impacts from escaped transgenic fish (Mair *et al.* 2007). A shock at first mitotic division will produce a tetraploid embryo with four sets of chromosomes (4n). Male 4ns produce diploid sperm and can be crossed with normal fish to produce 3n directly avoiding having to shock large numbers of eggs, e.g. for rainbow trout (Myers *et al.* 1986).

Techniques for parthenogenesis can derive offspring from wholly maternal or paternal origins (Komen & Thorgaard 2007). These use gamma or ultraviolet radiation to destroy the nuclear DNA in eggs or sperm; the treated gamete is then fused with an untreated sperm or egg to produce a haploid embryo, which can be made diploid by inhibiting the second meiotic (meiotic gynogenetic) or first mitotic division (mitotic gynogenetic or androgenetic). Although still mainly used as research tools they can generate unique genotypes for analysing complex traits or generating new strains. Containing sufficient DNA, haploid embryos can be used for gene-mapping (Kocher *et al.* 1998), generating unusual genotypes such as YY male tilapia broodstock used to produce all-male XY tilapia offspring by crossing with normal XX females (Myers *et al.* 1986). Meiotic gynogenetic silver barb (*Puntius goniontus*) are all-female, can be produced in large numbers and can be sex-reversed to generate neo-males without the need for progeny testing; they are now used in large numbers as broodstock for commercial production of all-female fry (Pongthana *et al.* 1999). Gynogenesis was similarly used in the development of all-female turbot (Cal *et al.* 2006).

Double haploid individuals from either a mitotic gynogenetic or androgenetic background can also be used to generate clonal or isogenic lines after a second round of parthenogenesis. Apart from model species such as zebrafish (Streisinger *et al.* 1981), clonal lines have been produced in tilapia (Müller-Belecke & Hörstegen Schwark 1995; Hussain *et al.* 1998; Sarder *et al.* 1999) rainbow trout (Scheerer *et al.* 1986; Thorgaard *et al.* 1990; Quillet *et al.* 2007) and common carp (Bongers *et al.* 1997). These are useful for disease and vaccine studies and an important resource for whole genome sequencing.

GENE TRANSFER TECHNOLOGIES

Transgenic fish containing the human growth hormone gene (hGHg) have been produced in goldfish (Zhu *et al.* 1985), rainbow trout (Penman *et al.* 1990), channel catfish (Dunham *et al.* 1987) and Nile tilapia (Brem *et al.* 1988). However, less than 0·05 integrated a copy of the construct into the host genome, and a number of other problems arose. Improved techniques resulted in growth enhancement in rainbow trout (Penman *et al.* 1991), common carp (Zhang *et al.* 1990), channel catfish (Dunham *et al.* 1992) and tilapia (Rahman & Maclean 1999). The most dramatic results were with total piscine constructs using either an ocean pout antifreeze promoter (ocAFP) controlling a chinook salmon growth hormone (GH) cDNA or a sockeye salmon metallothionein (MT) promoter controlling a full-length sockeye GH gene. Transgenics showed a 5–30-fold increase in growth up to 1 year of age (Devlin *et al.* 1995) and individuals successfully passed this performance onto their offspring. The same construct had similar effects when used in other salmonid species (Du *et al.* 1992; Devlin *et al.* 1995; Devlin 1997; Cook *et al.* 2000).

Salmonids have a very positive response to GH (Dunham & Devlin 1998), while warm water species such as tilapia and carp, growing rapidly throughout the year, are probably not as reliant on GH regulation. Effects are also greater on wild fish than selected strains; Devlin *et al.* (2001) found a 17-fold improvement of transgenics over their wild sibs in rainbow trout, but only 4·4% gain with selected domestic strains.

Quite apart from the difficult issue of public acceptability and environmental risk, a range of practical constraints means that transgenic fish are unlikely to become a commercial reality in the immediate future (Mair *et al.* 2007). However, functional genomic work is already identifying candidate transgenic genes in the area of improved disease resistance.

MOLECULAR TECHNIQUES

Genetic markers

Liu & Cordes (2004) and Liu (2009) provide reviews on molecular markers in aquatic organisms. Markers are defined as Type 1 or actual genes of known function and Type 2 or anonymous DNA segments. To date, Type 2 and in particular microsatellite markers (Tautz 1989) have had a notable impact and large numbers of microsatellite loci (small tandem repeat DNA sequences) have been generated in salmonids, tilapia, sea bass, sea bream and carp.

Microsatellites have immediate application in defining stock parentage (Norris *et al.* 2000), particularly for marine species where it is difficult to develop single family rearing. Studies in mass spawning species such as sea bream (Brown *et al.* 2005) and cod (Herlin *et al.* 2008) and in manually spawned halibut (Jackson *et al.* 2003) have shown that parentage assignment is critical to avoid the offspring of a few individuals dominating replacement broodstocks. This technology also removes the need for physical tags, increasing potential family numbers in commercial environments and avoiding the risk of tags entering the human food chain, and can also reduce the cost of maintaining breeding nucleus backup populations.

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Genetic markers have many other potential uses in managing farmed and wild stocks, for example identifying origin of escaped farm stock in Atlantic salmon, rainbow trout and Atlantic cod (Glover 2010) and defining potential introgression of genes from farm escapes into wild populations of salmon (Skaala *et al.* 2006).

Genetic mapping

Genetic linkage maps (Danzmann & Gharbi 2007) enable identification of QTL or genome sections containing genes influencing important traits. The QTL data for species such as Atlantic salmon, tilapia and common carp are steadily increasing (Korol *et al.* 2007), though most maps require further precision. However, using the large differences in recombination rate between sexes (Hayes *et al.* 2006), it has been possible to speed up QTL identification for resistance to infectious pancreatic necrosis (IPN) and incorporate the QTL into a commercial Atlantic salmon breeding programme (Houston *et al.* 2008).

The majority of QTL described are for easily measured traits. Future programmes will benefit by identifying QTL or more focused MAS for traits that are more difficult to measure, e.g. those with low heritability, identifying candidates early before maturity, traits only observed in one of the sexes and a range of post-harvest traits such as flesh quality (e.g. colour, fillet conformation and adiposity; Lande & Thompson 1990). The use of MAS technology will increase in aquaculture, and De Santis & Jerry (2007) have listed a number of candidate genes derived from what is known in terrestrial livestock.

Rapid developments in identifying expressed sequence tags (EST) are opening prospects for mapping Type 1 markers. For stock in different developmental stages or environments it will be possible to see which genes are expressed and how a given gene or subset of genes is affected. Mapping of ESTs is easier if a radiation hybrid (RH) mapping panel is available. The first panel was constructed in a commercial species, gilthead sea bream *Sparus auratus* (Senger *et al.* 2006), and work is in progress for species such as sea bass. Type 1 markers allow comparative use of genomic approaches to help identify possible loci for less well-studied commercial species Sarropoulou *et al.* (2008).

This area is developing very rapidly (Rexroad 2007), with work on single nucleotide polymorphisms (SNP) (Liu 2009) and bacterial artificial chromosome (BAC) libraries to more densely map species (He *et al.* 2007). Several BACs are now available: for Atlantic salmon (Davidson, 2007), tilapia (Katagiri *et al.* 2001), sea bass (Whitaker *et al.* 2006) and Pacific oyster (*Crassostrea gigas*; Cunningham *et al.* 2006). Physical mapping projects based on BAC are under way in Atlantic salmon (Ng *et al.* 2005), tilapia (Katagiri *et al.* 2005) and Channel catfish (Xu *et al.* 2007). New generation sequencing (NGS), particularly restriction site-associated DNA (RAD tagging; Miller *et al.* 2007) offers the opportunity to rapidly and cost-effectively identify and analyse thousands of SNPs and should speed up the discovery rate of QTL, particularly in species with poor genomic resources.

Functional genomics

The construction of microarray chips, containing thousands of ESTs derived from the whole animal or more focused subsets of genes specific to a tissue or a biological function, allows more specific assessment of characteristics. Work in salmonids has progressed the furthest (Rise *et al.* 2007), studying gene expression for a range of traits including disease-related responses, such as for *Piscirickettsia salmonis* (Rise *et al.* 2004), *Aeromonas salmonicida* (Ewart *et al.* 2005), Ameobic gill disease (Morrison *et al.* 2006), immune response to a lipopolysaccharide challenge (MacKenzie *et al.* 2006), live bacterial vaccines (Martin *et al.* 2006), DNA vaccination (Purcell *et al.* 2006) and *Gyrodactylus* species (Fast *et al.* 2006; Lindenstrom *et al.* 2006). Other traits include response to growth in transgenic salmon (Rise *et al.* 2007) and stress associated with handling (Krasnov *et al.* 2005), temperature (Vornanen *et al.* 2005) and highly unsaturated fatty acid lipid metabolism (Taggart *et al.* 2008).

With large numbers of genes being monitored for expression under a range of different conditions, it is likely that integrating QTL mapping with global gene expression may well identify patterns correlating with differences in key traits (Haley & de Koning 2006).

THE POTENTIAL FOR ENGINEERING PLANT SUBSTRATES FOR OMEGA-3 STOCKS IN FISH FEEDS

A further area of genetic exploration in aquaculture is that of providing feeding sources, particularly to replace or supplement high-quality inputs currently derived from fishmeal and oil, increasingly seen as a limitation for future growth in aquaculture production. Sourcing of key components, particularly omega-3 fatty acids in plant substrates, could offer great advantages of supply and cost. The reproductive tissues of higher plants store significant amounts of neutral lipids (predominantly as triacylglycerols), providing a convenient and renewable source of useful fatty acids. Over 400 different fatty acids have been identified in seed oils although, remarkably, none have been found to contain the very long chain omega-3 polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaneoic acid (EPA) and docosahexaenoic acid (DHA).

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Thus, there exists the possibility of genetically engineering plants to modify seed oil composition to include omega-3 PUFAs. Its feasibility has already been demonstrated with the 'stacking' of multiple genes in a single transgenic plant, and also the identification from marine microbes of the biosynthetic genes which direct the synthesis of EPA and DHA. As outlined below, considerable progress has been made in the last 5 years towards producing an alternative, sustainable source of fish oils from transgenic plants.

The first landmark 'proof of concept' demonstration of the accumulation of the C20 omega-3 LC-PUFA EPA in transgenic plants was achieved in 2004 by several groups, using genes from PUFA-synthesizing micro-organisms to direct the synthesis of this fatty acid in either leaves or seeds of different plant species (*Arabidopsis*, Qi *et al*. 2004; tobacco and linseed, Abbadi *et al*. 2004). Both studies reported low but significant levels of EPA (0·01–0·03 of total fatty acids). Building on these foundations, further work was carried out in *Brassica juncea* (Indian mustard) in which up to nine algal and moss genes were expressed, resulting in the accumulation of 0·15 EPA but < 0·01 DHA in seed oils (Wu *et al.* 2005). Similar attempts to produce DHA in transgenic *Arabidopsis* seeds resulted in < 0·01 DHA in total fatty acids (Robert *et al.* 2005), whereas further fine-tuning resulted in accumulation of up to 0·25 EPAs in *Brassica carinata* (Cheng *et al.* 2010). However, while transgenic accumulation of the C20 omega-3 PUFA EPA is achievable at levels similar or greater to those found in marine organisms (fish, algae or diatoms), the synthesis of DHA is still a considerable challenge, with no current demonstration of accumulation above 0·03–0·04 (Venegas-Calerón *et al*. 2010). However, since DHA is a direct metabolite of EPA, it might be expected that the recently reported high levels of EPA will serve as a superior platform with which to increase transgenic DHA levels.

In addition to EPA and DHA, efforts have been focused at transgenic production of the omega-3 surrogate stearidonic acid (SDA): SDA is not a *bona fide* omega-3 LC-PUFA, being only 18 carbons long, but has been demonstrated to undergo *in vivo* conversion to EPA in animals and could serve as an alternative source of fish oils. Only a very few plant species accumulate SDA, such as *Echium*, a non-agronomically adapted and low-yielding species. Transgenic soybeans accumulating significant SDA (up to 0·29 of total seed fatty acids) have been reported (Eckert *et al.* 2006), and has transgenic linseed enriched with up to 0·13 SDA (Ruiz-Lopez *et al.* 2009). Interestingly, although SDA levels in transgenic linseed were lower than that reported for soybean, the C18 omega-3 content was superior, as was the absence of the omega-6 fatty acid dihomo-gamma-linoleic acid (GLA; Ruiz-Lopez *et al*. 2009). Stearidonic acid has been reported to be effective as a fish oil replacement in aquaculture, though this has been disputed.

Collectively, these data confirm the promise of transgenic plants as sources of omega-3 LC-PUFAs. However, while levels of EPA (and SDA) achieved are equivalent to marine sources, DHA still represents a challenge (most likely due to its more complicated biosynthetic pathway and possibly also its reduced oxidative stability). However, given recent progress in elevating EPA it seems likely that higher DHA levels will be achievable, most likely some 0·05–0·10 of total seed oil. Such levels, especially in conjunction with moderate (0·10–0·15) levels of EPA would represent a very significant and useful source of omega-3 LC-PUFAs for aquaculture. Alternatively, an EPA-containing plant oil (lacking DHA) could provide a convenient feedstock, particularly as such material has already been produced. It will also be important to evaluate the performance of different farmed fish species when provided with these novel sources of omega-3 LC-PUFAs.

For applications in aquaculture, several factors need to be highlighted. Firstly, these oils should be considered as 'enhanced' with omega-3 LC-PUFAs, rather than a direct replacement, as plant oils contain significant oleic, linoleic and linolenic acids not usually found in marine oils. Thus, they might require blending to ensure optimal composition. Cultivation and use of genetically modified (GM)-derived products are also widely regulated and positive consumer responses would also need to be assured. Another consideration is the volume of GM crops to be grown – aquaculture demands could require considerable area just to provide sufficient omega-3 LC-PUFA oils to substitute for the many thousands of tonnes of fish oils consumed in the UK.

CONCLUSIONS

The current review has outlined genetic applications related to aquaculture and associated activities, ranging from immediately practical, commercially developed approaches already delivering tangible improvements to emerging techniques which will inform potential for a range of stock, environmental and husbandry interactions. There is substantial scope for using existing practical techniques; widespread adoption of broodstock management and improvement programmes would have significant impacts on sector performance, and routine application of genetic principles to hatchery-based fishery stocking programmes, and to defining and managing the aquaculture impact on biodiversity would bring about important resource and environmental gains. With a mix of strategies based on the more experimental techniques, and with more quantitative rigour the

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costs and benefits to the sector and to the wider environment and resource base can be better defined.

While the application of GM technologies to stocks themselves remains highly controversial, other areas may have more immediate potential. The possibility of using transgenic plants to synthesize omega-3 LC-PUFAs is within sight of providing a terrestrial feedstock unconstrained by current concerns for marine sources (Napier & Graham 2010). With suitable controls and consumer trust, genetically modified oilseed crops could become more widely grown, providing further potential for a wide range of aquaculture production.

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