

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/260147349>

Recent advances of genome mapping and marker-assisted selection in aquaculture

ARTICLE *in* FISH AND FISHERIES · SEPTEMBER 2014

Impact Factor: 8.76 · DOI: 10.1111/faf.12020

CITATIONS

21

1 AUTHOR:



[Gen Hua Yue](#)

Temasek Life Sciences Laboratory

126 PUBLICATIONS 1,861 CITATIONS

SEE PROFILE



Recent advances of genome mapping and marker-assisted selection in aquaculture

Gen Hua Yue

Molecular Population Genetics Group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore, 117604, Singapore

Abstract

Aquaculture is the fastest growing sector in agriculture. Substantial genetic gains have been achieved in a few cultured species using conventional selective breeding approaches. However, the majority of fish and shellfish species remain in their wild state. Due to the recognition of the enormous potential of marker-assisted selection (MAS) to speed up genetic gain through early selection, aquaculture scientists have constructed linkage maps in over 40 species and mapped quantitative trait loci (QTL) for important traits in over 20 species since the 1990s. Although MAS and genomic selection (GS) have not been widely used in aquaculture, their application in breeding programmes is expected to be a fertile area of research. In this paper, I summarized the recent advances of linkage and QTL mapping, as well as MAS in aquaculture species. I also discussed the potentials of genome-wide association studies (GWAS) and GS in aquaculture species.

Correspondence:

Gen Hua Yue,
Molecular Population
Genetics Group,
Temasek Life Sci-
ences Laboratory,
1 Research Link,
National University
of Singapore,
117604 Singapore,
Singapore
Tel.: 65-68727405
Fax: 65-68727007
E-mail: genhua@tll.
org.sg

Keywords Fish, genome mapping, GS, GWAS, MAS, QTL

Received 29 May
2012
Accepted 30 Dec
2012

Introduction	377
Linkage mapping	377
DNA markers and genotyping	377
Reference families for linkage mapping	378
Linkage analysis of markers	379
Status of linkage mapping	380
Mapping of quantitative trait loci	380
Important traits in QTL analyses in aquaculture species	380
Families for mapping QTL	381
Methods to detect QTL	382
Software for QTL mapping	382
Factors affecting QTL analysis	383
Status of QTL mapping in aquaculture species	383
<i>QTL mapping in tilapia</i>	383
<i>QTL mapping in Atlantic salmon</i>	384
<i>QTL mapping in rainbow trout</i>	385
<i>QTL mapping in Asian seabass</i>	386
<i>QTL mapping in Japanese flounder</i>	386

<i>QTL mapping in Kuruma prawn</i>	387
<i>QTL mapping in Pacific oyster</i>	387
Marker-assisted selection	388
Conclusions and future directions	388
Acknowledgements	390
References	390
Supporting Information	396

Introduction

There are over 30 000 teleost fish and hundreds of thousands of shellfish species on earth. According to the recent FAO estimate, only <400 species have been cultured, of which carps, tilapia and oysters have the largest worldwide production. Fish genetics programmes became more prevalent in the 1900s due to the development of knowledge of breeding and inheritance. Selective breeding programmes for genetic enhancement began in the 1960s (Gjedrem and Baranski 2009). Until now, selective breeding has been conducted for over 60 fish and shellfish species (Gjedrem and Baranski 2009). However, some important traits, such as disease resistance, feed conversion rate (FCR), fatty acid profiling and flesh quality are difficult to measure on selection candidates, but have major effects on the productivity and profitability of many aquaculture species.

With the rapid development of sequencing technologies, it is now easy to detect and characterize a large number of DNA markers in species of interest using next-generation sequencing and polymerase chain reaction (PCR). Large numbers of codominant DNA markers, such as microsatellites, have been identified in major aquaculture species (Liu 2011). Linkage maps have been constructed for some important aquaculture species since the first report of the linkage map of tilapia (*Oreochromis spp.*, Cichlidae) in 1998 (Kocher *et al.* 1998). The availability of linkage maps makes it possible to identify quantitative trait loci (QTL) for important traits on the whole genome of the species of interest to assist the selection of desired traits. QTL for important traits (e.g. cold and salinity tolerance, sex determination, growth traits and disease resistance) have been mapped in over 20 aquaculture species. A recent paper of marker-assisted selection (MAS) in fish breeding schemes (Sonesson and Meuwissen 2009) demonstrated that MAS would be especially valuable for traits

that are difficult to record on the candidates for selection such as disease resistance, fillet quality, feed efficiency and sexual maturation.

Aquaculture genetics (Liu and Cordes 2004), breeding programmes (Hulata 2001), DNA makers and their applications (Liu and Cordes 2004), aquaculture genomics (McAndrew and Napier 2010) and the principle of marker-assisted breeding schemes (Rothschild and Ruvinsky 2007) have already been reviewed in detail. However, the recent rate of advances of linkage and QTL mapping, and MAS in aquaculture species has been rapid and has not been summarized yet. The aim of this paper is to summarize the recent advance of genome mapping and MAS in important aquaculture species and discuss the future directions.

Linkage mapping

A linkage map is an ordered listing of genetic markers located along the length of the chromosomes in the genome. Construction of a linkage map requires four major components: polymorphic markers, genotyping platforms, reference families and software for analysis of linkage between pairwise markers and among markers.

DNA markers and genotyping

DNA markers are variable DNA sequences in a genome that can be differentiated using biochemical methods. Currently, two types of DNA markers, microsatellites (Weber 1990) and single nucleotide polymorphism (SNP) (Wang *et al.* 1998), are the most widely used in linkage mapping due to their abundance, ease and high throughput of scoring. The advantages and disadvantages of the two types of markers for mapping can be found in the review of Liu and Cordes (2004). More recently, a new type of DNA polymorphism, copy number variation (CNV) was discovered in humans (Sebat *et al.* 2004). The

application of CNV in aquaculture just came into sight recently (Shirak *et al.* 2008; Bai *et al.* 2011).

Microsatellites are short tandemly repeated (1–6 bp) DNA sequences found throughout a genome (Weber 1990). Currently, due to the advent of the next-generation sequencing technologies (Metzker 2009), resequencing of a genome of three giga bases (Gb) costs <1000 USD using the Illumina's HiSeq 2000. Microsatellites can be easily identified in genome sequences using software, such as RepeatFinder (Volfovsky *et al.* 2001), SciRoko (Kofler *et al.* 2007) and many more (see review Sharma *et al.* 2007). There are a number of approaches for genotyping microsatellites (Guichoux *et al.* 2011). Currently, most laboratories are using DNA sequencers (e.g. ABI3130 and ABI3730xl) for genotyping microsatellites. The ABI3730xl genotyping platform is currently the most-high-throughput platform for genotyping microsatellites.

SNP describes polymorphisms caused by point mutations at a given nucleotide position within a locus (Sachidanandam *et al.* 2001). A number of methods for identifying SNPs in non-model organisms have already been described in detail (Du *et al.* 2010). Although whole-genome sequences for the majority of aquaculture species are not available yet (Liu 2011), it is now possible to develop a large number of SNPs using next-generation sequencing technologies (Miller *et al.* 2007, 2012; Davey *et al.* 2011), such as restriction site-associated DNA sequencing (i.e. RAD-seq) (Fig. 1). RAD markers are mainly SNPs which can be used for linkage mapping (Le Bras *et al.* 2011). To use the RAD makers for linkage mapping, it is important to isolate RAD tags. RAD tags are the genomic DNA sequences immediately flanking each instance of a particular restriction enzyme cutting site on the whole genome (Miller *et al.* 2007). Once RAD tags have been isolated, they can be analysed using bioinformatic tools to genotype DNA sequence polymorphisms (Houston *et al.* 2012). Recently, the draft genome sequences of two aquaculture species (i.e. Atlantic cod, *Gadus morhua*, Gadidae and Pacific oyster *Crassostrea gigas*, Ostreidae) have already been published (Star *et al.* 2011; Zhang *et al.* 2012). The genomes of at least 11 aquaculture species are being sequenced. These species are salmon (*Salmo salar*, Salmonidae), catfish (*Ictalurus punctatus*, Ictaluridae), Nile tilapia (*Oreochromis niloticus*, Cichlidae), rainbow trout (*Oncorhynchus mykiss*, Salmonidae), common

carp (*Cyprinus carpio*, Cyprinidae), grass carp (*Ctenopharyngodon idella*, Cyprinidae), large yellow croaker (*Pseudosciaena crocea* Sciaenidae), orange-spotted grouper (*Epinephelus coioides*, Serranidae), half-smooth tongue sole (*Cynoglossus semilaevis*, Cynoglossidae), Japanese flounder (*Paralichthys olivaceus*, Paralichthyidae) and Asian seabass (*Lates calcarifer*, Latidae). From these sequencing efforts, a large number of SNPs is expected to be identified. There are over 30 different methods from six technologies that can be used in genotyping SNPs (Perkel 2008). In aquaculture species, several platforms have been applied in genotyping SNPs (Liu 2011). Genotyping SNP using next-generation sequencing technologies (Fig. 1) could be the most cost-effective and high-throughput approach for genotyping large number of SNPs and samples. However, the total cost for genotyping a larger number (e.g. 10 000) of SNPs in many (e.g. 200) individuals is currently still quite substantial (>75 000 USD). With declining sequencing costs, it is expected that next-generation sequencing will replace other platforms for genotyping of a large number of SNPs in the near future.

CNV is a form of structural variation (Sebat *et al.* 2004). CNV corresponds to relatively large regions of the genome that have been deleted or duplicated on certain chromosomes. As CNVs in human were associated with a number of human diseases, they are also potentially associated with important traits in aquaculture species (Shirak *et al.* 2008; Bai *et al.* 2011). Next-generation sequencing enables the rapid identification of CNVs (Mills *et al.* 2011). CNVs can be genotyped using Taqman technology (Graubert *et al.* 2007), microarray (Komura *et al.* 2006; Wang and Bucan 2010) and next-generation sequencing (Mills *et al.* 2011). Next-generation sequencing will be the most attractive method for genotyping a large number of CNVs in the next few years.

Reference families for linkage mapping

Constructing a linkage map requires one or more reference populations/families where DNA markers segregate (Wang *et al.* 2007). Population sizes used in constructing a linkage map ranges from dozens to a few hundred individuals. However, for high-resolution mapping, a large number of individuals (>500 individuals) are required to detect rare recombinants. F₁ families generated by crossing genetically diverse individuals can be used for

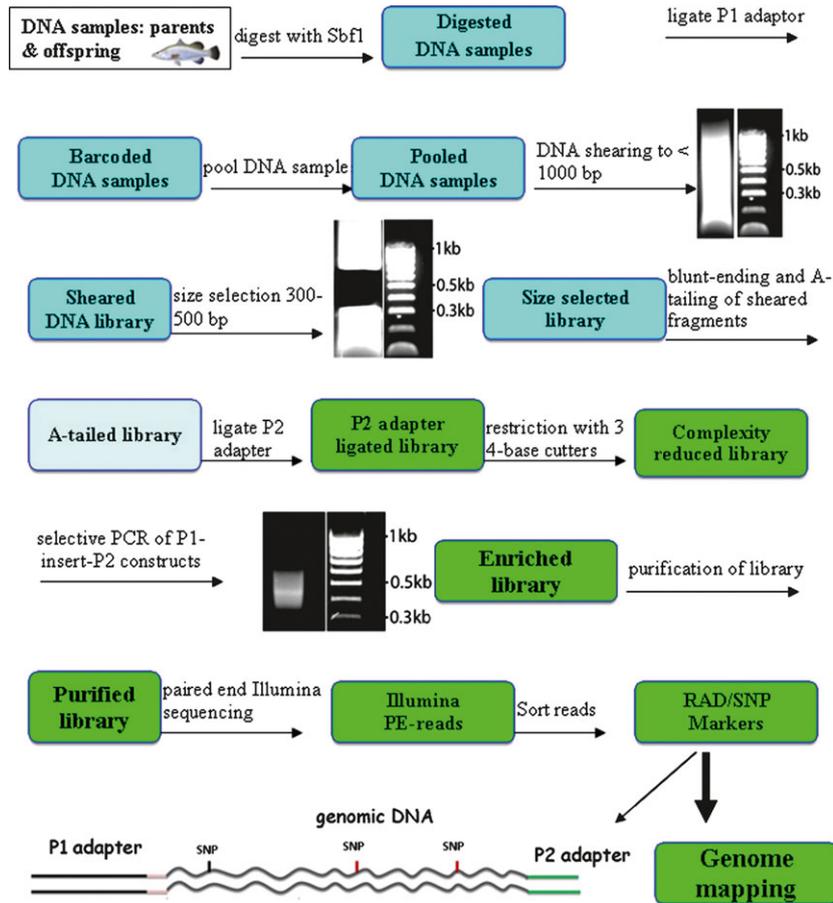


Figure 1 RAD-seq: discovery and genotyping of SNPs by next-generation sequencing for genome mapping. Details about the whole procedure can be seen in the paper of Miller *et al.* (2007).

linkage mapping (Wang *et al.* 2007). Using several reference populations, more markers can be mapped as markers could be informative in at least one of the populations (Wang *et al.* 2007; Xia *et al.* 2010). However, using more populations for mapping means that it is necessary to merge the maps from different populations. F_2 populations derived from F_1 hybrids, and backcross populations derived by crossing the F_1 hybrid to one of the parents, are the commonly used types of mapping populations.

Linkage analysis of markers

After genotyping all individuals in the reference populations with markers, the next step is to conduct pairwise and multipoint linkage analysis for constructing a linkage map using computer programs. To determine whether two markers are linked or not, LOD (a logarithm of odds) score is

used (Risch and Giuffra 1992). Traditionally, a LOD score > 3 is used as the threshold for determining significant linkage between two markers. To detect a greater level of linkage, LOD values may be lowered, whereas to place additional markers within constructed maps, higher LOD values may be applied. There are a number of softwares available for linkage mapping. Details of these softwares can be found in several websites, such as <http://www.stat.wisc.edu/~yandell/stat-gen/software/biosci/linkage.html> and <http://linkage.rockefeller.edu/soft/>. Readers have a wide choice of programs. Some programs (e.g. Crimap) are free, while others (e.g. Joinmap) are commercially available. The type of software used is largely dependent on the experimental design, including family structure and the number of families. Joinmap (Stam 1993), Mapchart (Voorrips 2002), Mapmaker (Lincoln *et al.* 1992) and Crimap (Green *et al.* 1990) are commonly used for

linkage mapping in aquaculture species. For constructing high-density linkage maps, Crimap and Joinmap may perform better, as they can handle large data sets easily.

Status of linkage mapping

Although linkage mapping for aquaculture species was initiated later as compared with other agricultural animals, it has progressed substantially over the last 25 years (Kocher and Kole 2010). In Table S1, I have summarized the status of linkage maps in aquaculture species. Linkage maps have been constructed in over 40 aquaculture species. Currently, linkage maps are being constructed for other species, such as freshwater pearl mussel (*Hyriopsis cumingii*, Unionidae; J.L. Li, personal communication). In several species, such as tilapia (Lee *et al.* 2005; Guyon *et al.* 2012), catfish (Kucuktas *et al.* 2009; Ninwichiana *et al.* 2012), Asian seabass (Wang *et al.* 2011b), Japanese flounder (Castano-Sanchez *et al.* 2010; Song *et al.* 2012), European sea bass (Volckaert *et al.* 2007), salmon (Lien *et al.* 2011) and rainbow trout (Rexroad *et al.* 2008; Palti *et al.* 2011, 2012; Guyomard *et al.* 2012), second generation linkage maps are already available. Mapping of more codominant markers onto the linkage maps is ongoing for a number of species. Although linkage mapping has progressed rapidly in aquaculture species, several problems still exist. Firstly, as shown in Table S1, most linkage maps were constructed using both dominant (e.g. AFLP & RAPD) and codominant markers (mainly microsatellites). The maps based on dominant markers are not as useful as those constructed with codominant markers, as dominant markers are not easily transferable between different laboratories. Secondly, the reference families used for linkage mapping are rather small, ranging from 41 to 192 individuals. The small reference family could make the ordering of DNA markers difficult. Thirdly, the density of most linkage maps is still very low, although it is already smaller than 20 cM and enough for a preliminary QTL analysis. Fourthly, in some species (e.g. common carp, half-smooth tongue sole), different researcher groups constructed several linkage maps using different markers and different reference families, and only very few markers were common among these linkage maps, making the merge of these maps an uphill task. Some sort of coordination

among different researcher groups working on linkage mapping of the same species is required to avoid repeated work and wasting resources. It is also of note that in shrimp, prawns and crabs, and clams, oysters and mussels, the construction of linkage maps using microsatellites is problematic because microsatellites in these species are usually very difficult to genotype (Scarborough *et al.* 2002). Genotyping difficulties include non-amplification of one of the two alleles at a given locus and large slippage effect. These difficulties are probably due to the very long repeats of microsatellites, and null alleles resulting from high mutation rates in primer binding sites in the flanking regions of microsatellites in these species (Scarborough *et al.* 2002). Other codominant markers (e.g. SNPs) may be the better markers for constructing linkage maps in these species.

Mapping of quantitative trait loci

Most economically important traits such as growth, flesh quality and disease resistance are controlled by a number of genes, environmental factors and their interactions. The underlying single genes usually have small effects. QTL are chromosomal regions (single gene or gene clusters) determining a quantitative character (Geldermann 1975). The purpose of mapping QTL is to understand the numbers and effects of genes that determine a trait and to assist in selective breeding to accelerate genetic improvement of important traits (Naish and Hard 2008). The mechanisms of QTL mapping can be found in the paper of Doerge (2002).

Important traits in QTL analyses in aquaculture species

Growth traits including growth rate, body weight and length are the most important traits in aquaculture species (Gjedrem and Baranski 2009). They can be easily measured. Due to their high heritability in most aquaculture species, they can be improved using traditional selection methods. QTL analysis for growth traits has been conducted in almost all species, in which QTL analyses have been performed (see details in the section 'Status of QTL mapping in aquaculture species'), such as Asian seabass (Wang *et al.* 2006, 2008a), rainbow trout (Wringe *et al.* 2010) and tilapia (Cnaani *et al.* 2003).

In most aquaculture species, feed accounts for about 65–75% of the total production cost (Gjedrem and Baranski 2009). Even in salmon industry, feed accounts for about 50% of the total cost (Marineharvest 2012). Therefore, FCR is another important trait. Feed intake of each individual is generally difficult to measure in aquaculture species due to unequal feed intake over days and the requirement of a single tank to raise each fish in each of the reference families. No QTL analysis on FCR has been conducted in aquaculture species so far.

Meat quality traits include fat percentage and distribution, fatty acid profiling, colour, texture and dressing percentage (Gjedrem and Baranski 2009). In most cases, accurate measurements of these traits can only be conducted in slaughtered individuals (Derayat *et al.* 2007; Baranski *et al.* 2010). QTL for fat percentage and distribution (Derayat *et al.* 2007) as well as flesh colour (Baranski *et al.* 2010) were mapped on the genome of salmon.

Sexual maturation is also an important trait as it leads to reduced growth, low FCR and decreased fillet quality in several aquaculture species (Martyniuk *et al.* 2003; Moghadam *et al.* 2007; Kuttner *et al.* 2011). Therefore, selection for later maturation has been carried out in some species. As phenotypic selection of this trait is difficult and time-consuming, MAS in fingerling stage is preferred. QTL for sexual maturation have been analysed in rainbow trout (Martyniuk *et al.* 2003), salmon (Araneda *et al.* 2009) and Arctic charr (Moghadam *et al.* 2007; Kuttner *et al.* 2011).

Sex determination (SD) is controlled by one or more genetic factors, environment and their interactions. SD factors are located on sex chromosomes and/or on autosomes. In some species, sex is significantly related to growth. For example, in tilapia, males grow much quicker than females (Eshel *et al.* 2012), while in common carp, females are much bigger than males at the same age (Sun and Liang 2004). Therefore, sex is regarded as an important trait in aquaculture species. QTL analysis for SD has been conducted in some fish species, such as tilapia (Lee *et al.* 2004), salmon (Davidson *et al.* 2009) and rainbow trout (Alfaqih *et al.* 2009).

Disease resistance is one of the most frequently researched traits in QTL studies as diseases represent one of the major challenges and bottlenecks in aquaculture (Ozaki *et al.* 2001; Khoo *et al.*

2004; Moen *et al.* 2004; Fuji *et al.* 2006; Gilbey *et al.* 2006; Houston *et al.* 2008). To quantify the resistance of each individual to pathogens, challenge experiments were often conducted. Usually, survival, death and the time of survival are recorded. QTL for disease resistance have been mapped in several aquaculture species, such as salmon (Houston *et al.* 2008), trout species (Khoo *et al.* 2004), Japanese flounder (Fuji *et al.* 2006) and oysters (Yu and Guo 2006; Lallias *et al.* 2009; Sauvage *et al.* 2010).

Salinity tolerance and temperature tolerance are two traits of interest for some fish species, such as tilapia (Rengmark *et al.* 2007; Yan and Wang 2010), rainbow trout (Le Bras *et al.* 2011), Arctic charr (Norman *et al.* 2011) and Asian seabass (Bai *et al.* 2012). The purpose of selecting for salinity and temperature tolerance is to develop fishes that could reproduce and grow in areas of higher salinity and lower/higher temperature. Tolerance to salinity and low/high temperature is usually measured by a challenge under high salinity and high/low temperature, respectively (Danzmann *et al.* 1999; Somorjai *et al.* 2003; Rengmark *et al.* 2007; Yan and Wang 2010; Norman *et al.* 2011). QTL for salinity tolerance and temperature tolerance have been identified in some species, such as rainbow trout (Danzmann *et al.* 1999; Le Bras *et al.* 2011).

Gene expression levels can be analysed as quantitative traits (Hubner *et al.* 2005). To dissect the transcriptional regulation of the entire transcriptome, QTL (eQTL) for gene expressions have been conducted in model organisms (Gilad *et al.* 2008). To date, this analysis relies upon the use of segregating populations. No such study has been reported in aquaculture species.

Families for mapping QTL

In many cultured fish species, large numbers of offspring can be generated during spawning, thus any kinds of families can be easily obtained. Although F_2 families are commonly used for QTL mapping, in marine fishes such as Pacific oyster, Asian seabass and Japanese flounder, F_1 families have been used in QTL mapping (Wang *et al.* 2006; Ozakil *et al.* 2007; Guo *et al.* 2012). This is mainly because in these species, the parents used for constructing the reference families segregate at both DNA markers and phenotypic values. To increase the power for QTL mapping, parents can

be selected based on genotypes at marker loci (Wang *et al.* 2006). This strategy has been used in setting up reference families for QTL mapping in Asian seabass (Wang *et al.* 2006). Another issue related to the power of the QTL mapping is the family size. The optimum number of family size in the QTL mapping population depends on the intrinsic power of the experiment. Usually, large family sizes (>300 individuals) are needed for detecting QTL of small effects.

Methods to detect QTL

Basically, three methods are frequently used for mapping QTL and estimating their effects, namely single-marker association analysis (SMAA), simple interval mapping (SIM) and composite interval mapping (CIM) (Crosses 2001; Flint and Mott 2001; Doerge 2002). The SMAA is the simplest approach for identifying QTL associated with single markers based on linkage disequilibrium (LD) (Edwards *et al.* 1987). The major advantage of the SMAA is that it does not require reference families or a complete linkage map and can be applied in any population raised under the same conditions. However, the major disadvantage of this method is its low power for detecting QTL, allowing only QTL closely linked to the marker to be identified. The SIM uses a large number (usually > 100) of informative DNA markers evenly covering the entire genome (<20 cM/marker space) and analyses intervals between neighbouring pairs of linked markers along each LG simultaneously, thus increasing the power of QTL mapping in comparison with the SMAA (Lander and Botstein 1989; Haley and Knott 1992). In the CIM, the interval mapping was combined with linear regression. The CIM also includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Jansen 1993). The main advantage of the CIM is its higher precision and power at mapping QTL as compared to the SMAA and SIM, especially when linked QTL are involved. The interaction among detected QTL can also be examined (Korol *et al.* 2012). Details of methods of QTL mapping can be found in published papers (e.g. Edwards *et al.* 1987) and reviews (e.g. Doerge 2002). More new methods for QTL mapping are being developed (Korol *et al.* 2012) to improve the accuracy of QTL mapping. In aquaculture species, the CIM has often been used to map QTL for important traits

(Robison *et al.* 2001; Zimmerman *et al.* 2005; Drew *et al.* 2007; Jin *et al.* 2012), due to its higher precision and power at mapping QTL.

Software for QTL mapping

A large number of softwares are now available for QTL analysis using different methods. A complete list of the programs can be found on the following websites <http://linkage.rockefeller.edu/soft> and <http://www.stat.wisc.edu/~yandell/stat-gen/software/biosci/linkage.html>. Most of the programs were developed as stand-alone software packages. Researchers can easily select one suitable for their experimental designs for QTL mapping. The software such as MapMaker/QTL (Lincoln *et al.* 1992), Map Manager (Manly *et al.* 2001), QTL Express (Seaton *et al.* 2002) and MapQTL (Ooien *et al.* 1996) have been frequently used in QTL analysis in aquaculture species. In our hand, MapQTL is easy to use and it is very fast to generate results in the format of table and figures. However, the software is not free software, and a licence fee must be paid. GridQTL (<http://www.gridqtl.org.uk/>) is the enhanced version of QTL Express and is web-based free software, which can perform QTL mapping on a variety of population types. Furthermore, GridQTL enables higher-resolution detection of QTL by adding a linkage disequilibrium–linkage analysis (LDLA) tool in tandem with a haplotyping analysis. An epistasis option for two-QTL determination in F₂ populations is also implemented. This software has been extensively used in QTL mapping in livestock since 2006 (e.g. Siwek *et al.* 2010), while in aquaculture species, it just has been used recently (Gutierrez *et al.* 2012). Therefore, it is expected that its use in QTL mapping in aquaculture species will substantially increase. PROC QTL procedure in the SAS package can also be used for mapping binary trait loci (Hu and Xu 2009). This software package can perform QTL mapping in almost all lines of crossing experiments. However, this software is not a free-ware. MapManager and QTX are commonly used to perform single-marker analysis (Manly *et al.* 2001; Joehanes and Nelson 2008). MapMaker/QTL (Lincoln *et al.* 1992) and QGene (Joehanes and Nelson 2008) have been used to conduct SIM, whereas MapQTL (Ooien *et al.* 1996), Cartographer (Basten *et al.* 2003), MapManager QTX (Basten *et al.* 2003) and PROC QTL (Hu and Xu

2009) have been used to perform CIM in aquaculture species. Other programs developed using the R package [e.g. R/qtl (Broman *et al.* 2003) and R/qtlbim (Yandell *et al.* 2007)] may also be used in QTL mapping in aquaculture species. As these above-mentioned programs were developed by different researchers, they usually require specific formats of data. Users have to prepare their data using different formats before they can switch between these different programs, especially when both continuous and categorical traits are involved. Therefore, it could be a good idea to develop some software to convert data formats for QTL mapping using different softwares. It is not uncommon that QTL detected using different softwares are slightly different, especially when the effects of QTL are small. Therefore, it is advisable to use at least two softwares for QTL mapping to ensure the accuracy and reliability of QTL analyses.

Factors affecting QTL analysis

The power of mapping QTL can be influenced by a number of factors, such as genetic properties of QTL, experiment design, environmental effects, marker density and informativeness, genotyping errors and precision of trait measurement. Details about how these factors influence the power of QTL mapping can be found in some very good reviews (e.g. Crosses 2001; Flint and Mott 2001; Doerge 2002). In mapping QTL, the main errors are genotyping errors and mistakes in phenotypic evaluation, which can lead to a wrong estimation of the order and distance between markers on the whole genome (Hackett and Broadfoot 2003). The accuracy of the measurement of phenotypic values of traits is of great importance for the accurate locating of QTL and estimating of QTL effects. In model organisms such as mouse (Gates *et al.* 2011) and agronomic plant species (Iyer-Pascuzzi *et al.* 2010), tools have been developed to automatically measure traits (e.g. weight and length). However, in aquaculture species, such tools are still not available. Researchers in the field of molecular breeding may cooperate with mechanic engineers to develop such tools to measure traits of aquaculture species precisely and efficiently. In addition, QTL mapping studies should be verified in different populations (Wang *et al.* 2008a; Moen *et al.* 2009). Such verifications may involve independent populations

constructed from the same parental genotypes or closely related genotypes used in the primary QTL mapping study. Sometimes, larger population sizes may be required.

Status of QTL mapping in aquaculture species

QTL analyses for important traits have been conducted for more than 20 aquaculture species including finfish, mussels and crustaceans (see details in Table S2). Among these over 20 species, tilapia is one of the most important freshwater food fish species, whereas salmon and rainbow trout are the major cultured marine food fish species (Gjedrem and Baranski 2009). Pacific oyster is the most popular shellfish species and has been cultured for hundreds of years (Plough and Hedgecock 2011). Asian seabass (Wang *et al.* 2006), Japanese flounder (Fuji *et al.* 2006) and Kuruma prawn (Li *et al.* 2006) represent relatively new species for marine aquaculture. In tilapia, Atlantic salmon, rainbow trout, Pacific oyster, Asian seabass, Japanese flounder and Kuruma prawn, QTL for some important traits have been mapped, and some QTL were verified in different populations. Therefore, I have summarized the advances of QTL mapping in these seven representative species to give readers a brief overview on the status of QTL mapping in aquaculture species. More information about QTL mapping in other aquaculture species can be found in Table S2.

QTL mapping in tilapia

QTL have been identified for cold tolerance and body size (Cnaani *et al.* 2004), temperature tolerance (Cnaani *et al.* 2003), general disease resistance and immune response (Cnaani *et al.* 2004), body colour (Lee *et al.* 2005), SD (Lee *et al.* 2004; Shirak *et al.* 2008; Eshel *et al.* 2012) and salinity tolerance (Rengmark *et al.* 2007). QTL for innate immunity, response to stress, biochemical blood parameters (Cnaani *et al.* 2004) and body size were confirmed in additional experiments (Cnaani *et al.* 2004). In the following two paragraphs, I have summarized details about QTL for SD and salinity tolerance as these two traits are very important to ensure the profitability and sustainability of tilapia production.

The SD loci have been extensively searched for in tilapia species. In the Nile and Mozambique tilapia (*Oreochromis Spp.*), sex is determined by XX/XV (male heterogametic) (Carrasco *et al.*

1999), while in the species blue tilapia (*Oreochromis aureus*),

the SD system is a WZ/ZZ (female heterogametic). Several sex-linked markers have been identified in *O. niloticus* and *O. aureus* and mapped to different LGs (Lee *et al.* 2003, 2004; Shirak *et al.* 2006; Eshel *et al.* 2010, 2012). In purebred *O. niloticus*, the QTL for SD were detected on LG 1 and LG 23 (Lee *et al.* 2003; Eshel *et al.* 2010, 2012), while in *O. niloticus* × *O. aureus* hybrids, a QTL for SD was mapped on LG 3 (Lee *et al.* 2005). In Nile tilapia, Lee *et al.* (2003) reported that two microsatellites UNH995 and UNH104 on LG 1 were a few centimorgans away from a major SD locus. More recently, Eshel *et al.* (2012) reported that a major QTL for SD was located between SSR markers ARO172 and ARO177 on LG 23. Twelve adjacent markers located near the QTL were homozygous in females and either homozygous for the alternative allele or heterozygous in males. This segment was defined as the sex region. It seems that at least two major sex determining loci on different LG 1 and LG 23 determine the sex in Nile tilapia. However, it is not known whether and how these two loci interact with each other. Further study on their interactions is required. Recently, Shirak *et al.* (2008) found that the CNV of lipocalins was associated with SD. They found that females with the high male-specific protein copy (MSPC) number were more frequent by more than twofold compared with males. In other species blue tilapia (*Oreochromis aureus*, Cichlidae), SD is controlled by a major QTL on LG 3. Eleven microsatellite markers located near the major QTL were associated with phenotypic sex (Lee *et al.* 2004). Three markers UNH168, GM271 and UNH131 on LG 3 were located a few centimorgans away from the SD locus. The putative W chromosome haplotype was able to predict the sex of 97% of male and 85% of female individuals. Markers on LG 1 were also strongly associated with sex. Analysis of epistatic interactions among QTL for SD on LG1 and LG 3 revealed that the QTL (the W haplotype) on LG 3 may act as a dominant male repressor, whereas the QTL (the Y haplotype) on LG 1 may function as dominant male determiner. Although it is known that in tilapia species, sex is determined by several major loci, other loci with small effects and environmental factors may also be involved. It is also possible that the sex of

tilapia species is determined by a network of genes and environmental effects. The interactions among genes and environmental factors may be very complicated, and warrant further study.

QTL for salinity tolerance were mapped on two chromosomes using a pure *O. niloticus* F₂ full-sib family with 292 individuals (Lee 2003). However, markers linked to the salt tolerance have not been released. Recently, candidate genes for salt tolerance, such as genes beta haemoglobin, Ca²⁺-transporting plasma membrane ATPase, pro-opiomelanocortin and beta-actin, were mapped in Nile tilapia (Rengmark *et al.* 2007) to an existing linkage map (Lee *et al.* 2005). Further study on QTL for salinity tolerance is required to develop new salinity-tolerant tilapia varieties.

QTL mapping in Atlantic salmon

QTL analyses have been conducted for resistance to the infectious pancreatic necrosis (IPN) virus (Houston *et al.* 2007, 2008; Storset *et al.* 2007; Moen *et al.* 2009), *Cryptobia salmositica* (Ozaki *et al.* 2005), *Gyrodactylus salaries* infection (Gilbey *et al.* 2006), susceptibility to *Lepeophtheirus* (Gharbi *et al.* 2009) and infectious salmon anaemia (Moen *et al.* 2005, 2007), growth and harvest traits (Reid *et al.* 2005; Houston *et al.* 2009a; Baranski *et al.* 2010; Vasemagi *et al.* 2010), sex (Davidson *et al.* 2009), body lipid percentage (Derayat *et al.* 2007) and flesh colour (Baranski *et al.* 2010). In the following paragraphs, we have focused on some details about QTL for resistance to IPN, flesh colour and body lipid percentage because disease resistance and meat quality are economically important in breeding salmon and other fish species.

A major QTL affecting resistance to IPN was identified on LG 21 by several independent experiments (Ozaki *et al.* 2001; Houston *et al.* 2007, 2008, 2009b; Moen *et al.* 2009; Gheyas *et al.* 2010a,b). These studies showed the QTL for IPN explained over 45% of the phenotypic variance (PV). Three DNA markers (SSa0285BSFU, Alu333 and SSa0374BSFU/II) on LG 21 were closely linked to the QTL for resistance to IPN. In a family where both parents were segregating for the QTL, and in offspring homozygous for resistant QTL alleles, there was no mortality, whereas in offspring homozygous for susceptible QTL alleles, the mortality was 100%. Other studies also confirmed the major QTL for resistance to IPN on LG 21 (e.g. Gheyas *et al.* 2010a). These studies indicate that

the resistance to IPN is determined by a major locus and some other loci with small effects.

Three significant QTL for flesh colour were mapped on Chr 6, Chr 26 and Chr 4 in an F₂ population. Two QTL for flesh colour on Chr 6 and 26 accounted for 24% of the PV. In addition, 32 suggestive QTL with smaller effects were also identified (Baranski *et al.* 2010). This study suggests that the trait of flesh colour was determined by many QTL.

For body lipid percentage, a QTL scan on the whole genome was carried out in five large full-sib families including 10 parents and 153 offspring (Derayat *et al.* 2007). The study found evidence of a significant QTL for fat percentage on linkage groups LNS 16. The microsatellite marker Ssa0016NVH (at position of 1.3 cM) was found to be tightly linked to QTL affecting fat percentage. However, the study did not report the effect of the QTL on the PV of lipid percentage. After the publication of Derayat *et al.* (2007), more than 5 years have passed, yet no other paper on QTL for this trait were published, suggesting that study on body lipid percentage is a difficult task. It is also possible because measuring of body fat percentage and composition is expensive. As the body lipid percentage and composition are important quality traits, further QTL analysis for these traits using more DNA markers in other mapping families is required.

QTL mapping in rainbow trout

QTL mapping was conducted for a number of traits, such as thermal tolerance (Jackson *et al.* 1998; Danzmann *et al.* 1999; Perry *et al.* 2001, 2005; Somorjai *et al.* 2003), spawning time (SPT) (Sakamoto *et al.* 1999; O'Malley *et al.* 2003; Colihueque *et al.* 2010), embryonic development (Robison *et al.* 2001; Sundin *et al.* 2005; Easton *et al.* 2011; Xu *et al.* 2011), growth traits (Martyniuk *et al.* 2003; O'Malley *et al.* 2003; Martinez *et al.* 2005; Moghadam *et al.* 2007; Wringe *et al.* 2010), stress (Drew *et al.* 2007; Vallejo *et al.* 2009), salinity tolerance (Le Bras *et al.* 2011), resistance to IPN (Rodriguez *et al.* 2004; Zimmerman *et al.* 2004; Ozakil *et al.* 2007; Barroso *et al.* 2008; Baerwald *et al.* 2011), infectious hematopoietic necrosis (IHN) (Khoo *et al.* 2004), bacterial cold-water disease (Vallejo *et al.* 2010) and natural killer cell-like activity (Zimmerman *et al.* 2004). The QTL for thermal tolerance, SPT and stress resistance are quite interesting.

In 1998, QTL for thermal tolerance were first identified (Jackson *et al.* 1998). Two QTL for upper temperature tolerance were mapped near the microsatellite loci Omy325UoG and Ssa14DU (Jackson *et al.* 1998). The two QTL were located on different LGs and accounted for 13 and 9% of the overall additive genetic variance in upper temperature tolerance. A marker allele associated with a QTL enhanced thermal resistance in one genomic background showed the opposite association in the other genomic background (Danzmann *et al.* 1999). Potential candidate genes (e.g. small heat shock proteins, HSP-90 genes and COUP-TFII) associated with heat tolerance have been recently identified using a positional candidate gene method (Quinn *et al.* 2011).

In an early attempt to map QTL for SPT (Sakamoto *et al.* 1999), thirteen QTL markers for SPT were mapped on seven LGs. A recent study (Colihueque *et al.* 2010) using microsatellites linked and unlinked to QTL for SPT showed that QTL for SPT also influenced the double annual reproductive cycle (DARC) trait. In two case-control comparisons, three linked markers (OmyFGT12TUF, One3ASC and One19ASC) had significant levels of allelic frequency differentiation and marker-character association. Furthermore, alleles of One3ASC and One19ASC had significantly higher frequencies in populations carrying the DARC trait. These results suggest that QTL for SPT and DARC are somewhat related.

A major QTL affecting stress response was detected using Bayesian methods of complex segregation analysis (Vallejo *et al.* 2009). The QTL explained 22–39% of the PV. However, this QTL has not been confirmed in other populations. Interval and composite interval mapping were used to map QTL for post-stress cortisol levels in doubled haploid offspring of an OSU × Arlee hybrid (Drew *et al.* 2007). Two significant QTL with opposing additive effects on cortisol levels were detected, explaining 43% of the PV. It seems that the stress response is determined by a few major QTL and some other QTL with smaller effects. However, in the study of Drew *et al.* (2007), the QTL were mapped in large chromosomal regions (>5 cM). Therefore, it is essential to narrow down the QTL in smaller chromosomal regions by genotyping more DNA markers near the detected QTL to make marker-assisted selection for stress response possible.

QTL mapping in Asian seabass

In Asian seabass, QTL analyses have been mainly conducted for growth traits (Wang *et al.* 2006, 2008a, 2011b), adaptive traits (Wang *et al.* 2011a), cold tolerance (Bai *et al.* 2012) and fatty acid profiling (Xia *et al.* unpublished data).

For growth traits at the age of 3 months post-hatch, interval mapping and multiple QTL model mapping detected five significant and 27 suggestive QTL on ten LGs in an F₁ family comprising 380 individuals (Wang *et al.* 2006). Among the five significant QTL, three QTL for body weight, total body length and standard body length, respectively, were located on the same position near the microsatellite Lca287 on LG 2, accounting for 28.8, 58.9 and 59.7% of the PV. Two additional QTL affecting body weight were mapped on LG 2 (near the marker Lca371) and 3, explaining 6.4 and 8.8% of the PV, respectively. On LG 23, some suggestive QTL for growth traits were also detected. In another study (Wang *et al.* 2008a), the previously detected QTL for body weight and length linked to marker Lca371 on LG 2 were confirmed in two different populations (i.e. the MAC and THAI families), whereas other QTL previously identified on LG2 (near the marker Lca287), LG 3 and 23 were only detected in one of the two families. QTL for body weight and length were detected in a region on LG 10 where the IGF2 and tyrosine hydroxylase 1 (TH1) genes are located in the MAC family, but not in the THAI family. Significant epistatic interactions were identified between markers Lca287 on LG 2 and IGF2 on LG 10 for growth traits QTL in the F₁ family (i.e. MAC family) generated using brooders from Singapore, but not in the F₁ family (i.e. THAI family) produced using brooders from Thailand. Effects of the IGF2, TH1 and parvalbumin 1 candidate genes were family specific. Recently, by genotyping more DNA markers in the significant QTL regions, Wang *et al.* fine-mapped some QTL for growth traits in chromosomal regions between 0.3 and 6 cM (Wang *et al.* 2011b). These results indicate that most QTL are family specific in Asian seabass.

Recently, Xia *et al.* (unpublished data) conducted a whole-genome scan for growth traits and fatty acid profiles at the age of 6 and 9 months post-hatch in an F₂ family consisting of 359 F₂ individuals (unpublished data). They found nine significant QTL for body weight measured at 6 months post-hatch and nine other QTL for body

weight measured at 9 months post-hatch, respectively. Five of these QTL were located at similar intervals on LG 5, 7, 15, 21 and 24, explaining 2.8–10% of the PV, respectively. However, QTL on LG 2, 3, 6, 9, 11, 13, 18 and 23 were detected only at one of the two stages. These data suggest that QTL for growth in different developing stages may differ although the growth traits were highly correlated at the age of 3 and 9 months post-hatch (Wang *et al.* 2008b).

Four QTL for total omega-3 polyunsaturated fatty acid content in flesh of 314 fishes (at the age of 9 months) from an F₂ family were recently mapped on LG 6 and 23, explaining 3.8–6.3% of the PV. Two QTL with a large PV ranging from 17.2 to 36.4% were detected for DPA3 (C22:5 n-3) on LG 2 and LG 6 (Xia *et al.* unpublished data). These results suggest the percentages of different omega-3 polyunsaturated fatty acids were determined by several chromosomal regions with relatively small effects.

In addition to the whole-genome scans, analyses of associations between SNPs in candidate genes and traits including growth (Xu *et al.* 2006; He *et al.* 2012) and cold tolerance (Bai *et al.* 2012) have been also performed. Polymorphisms in prolactin and parvalbumin genes were associated with growth traits (Xu *et al.* 2006; He *et al.* 2012). The polymorphisms in the calreticulin gene were associated with cold tolerance (Bai *et al.* 2012). However, these significant associations must be examined in different families to ascertain whether these associations are family specific or universal.

QTL mapping in Japanese flounder

Whole-genome scans for QTL focused on resistance to lymphocystis disease (Fuji *et al.* 2006, 2007; He *et al.* 2008a), *Streptococcus iniae* infection (Ozaki *et al.* 2010) and reproductive traits (He *et al.* 2008a,b). Candidate gene method was used in the analysis of associations between SNPs in genes and reproductive traits (He *et al.* 2008a, b). Using a segregating population with 136 individuals, a significant QTL for the resistance to lymphocystis disease was mapped on LG 15. A microsatellite marker (Poli.9-8TUF) located in the QTL explained 50% of the total PV in the experimental population (Fuji *et al.* 2006). An allele of the microsatellite Poli9-8TUF that had a dominant effect and was associated with resistance to lymphocystis disease has been used in selection

of fish resistant to lymphocystis disease (Fuji *et al.* 2007).

QTL mapping in Kuruma prawn

QTL analysis was focused on growth traits in Kuruma prawn. Li *et al.* (2006) used AFLP markers to map QTL for body weight, total length (TL) and carapace length (CL) in one full-sib *Penaeus japonicus* family with 102 F₂ individuals. No significant marker–trait association was detected in the female map, whereas one QTL for CL and TL was identified on LG 1 of the male map using interval mapping. The same region and one other suggestive QTL on LG 25 of the male map were detected by composite interval mapping. The two QTL were located on the end of LGs. One AFLP marker was located in the QTL region in LG 1 and one in the middle of LG 25. The AFLP markers located in the major QTL region, accounting for 16% of the PV, were further characterized (Lyons *et al.* 2007). Flanking sequences were analysed, and allelic variants responsible for segregation patterns of these markers were identified. The genomic sequence surrounding the AFLP marker 7.21a, located in the QTL, contained a gene called the elongation of very long chain fatty acids–like (ELOVL) protein. However, in recent years, no other papers on mapping QTL for other traits have been published, which may be due to the lack of a dense linkage map with codominant DNA markers in this species.

QTL mapping in Pacific oyster

QTL mapping has been recently conducted for growth traits (Guo *et al.* 2012), disease resistance (Sauvage *et al.* 2010) and inbreeding depression (Plough and Hedgecock 2011).

Sauvage *et al.* (2010) conducted a QTL analysis for survival of summer mortality and the load of *Ostreid Herpes virus type 1* using five F₂ full-sib families generated in a divergent selection experiment for resistance to summer mortality. Five significant QTL were mapped on four LGs (i.e. V, VI, VII and IX). They analysed QTL in single full-sib families and revealed differential QTL segregation between families. QTL for the two recorded traits were located in very similar locations. Guo *et al.* (2012) conducted a preliminary QTL analysis for growth traits and identified three significant QTL explaining 0.6–13.9% of the PV. They mapped one QTL for sex on LG 6 and found that the heritabilities of sex for paternal alleles and maternal alleles on that locus C15 were 39.8 and 0.01%, respectively.

Recently, Plough and Hedgecock (2011) carried out a quite interesting whole-genome search for QTL for inbreeding depression in two F₂ hybrid families. They located the causative deleterious mutations and characterized their mode of gene action. They found 14–15 viability QTL (vQTL) in the two families. In general, genotypic frequencies at vQTL suggested selection against recessive or partially recessive alleles. Their results support the dominance theory of inbreeding depression. However, they did not detect epistasis among vQTL, suggesting unlinked vQTL presumably had independent effects on survival. They, for the first time, tracked segregation ratios of vQTL-linked markers through the life cycle, to determine their stage-specific expression. In the earliest life stages, almost all vQTL did not exist, confirming zygotic viability selection. Over 90% of vQTL were expressed before the juvenile stage, mostly (50%) at metamorphosis. They estimated that, altogether, selection on vQTL caused 96% mortality in these families, accounting for nearly all the actual mortality. Their study suggests that it is essential to further study the genetic load caused substantial mortality in inbred Pacific oysters, particularly during a critical developmental transition stage (i.e. metamorphosis).

Although QTL mapping for important traits progressed quickly in aquaculture species, there are still some issues that need to be addressed. The majority of QTL analyses were conducted with very small sample size (<200 individuals) and a few markers (<150) covering only part of the genome, which may lead to some important QTL for the traits of interest being missed. Furthermore, some recent studies have proposed that QTL positions and effects should be evaluated/confirmed in independent populations, because QTL mapping based on typical population sizes results in a low power of QTL detection and a large bias of QTL effects (Crosses 2001; Doerge 2002). Unfortunately, due to constraints, such as lack of research funding and time, and possibly a lack of understanding of the need to confirm the results of QTL mapping, QTL mapping studies are rarely confirmed. Some notable exceptions are the confirmation of QTL associated with growth traits in Asian seabass (Wang *et al.* 2008a) and rainbow trout (Fuji *et al.* 2007; Wringe *et al.* 2010), and disease resistance in salmon (Moen *et al.* 2009) and Japanese flounder (Fuji *et al.* 2007).

Marker-assisted selection

The advantages of MAS are obvious as compared with the conventional selective breeding (Lande and Thompson 1990). MAS is especially useful for traits that are difficult to measure, exhibit low heritability and/or are expressed late in development. Implementation of MAS requires DNA markers that are tightly linked to QTL for traits of interest based on QTL mapping or association studies (Lande and Thompson 1990). Ideally, the DNA markers should be the causative mutation underlying the phenotypic variation. QTL studies in aquaculture species covered a wide range of traits including growth, meat quality, egg production, disease resistance, stress resistance, reproduction and other traits. The results of these studies provide a good starting point to search for QTL within breeding populations. Of the QTL from experimental crosses, only a small number of them have been followed up by confirmation and fine-mapping. The responsible mutations in genes have not been described for detected QTL. However, there are already a few applications of MAS in commercial breeding programmes in aquaculture species (Ozaki *et al.* 2012).

In Japanese flounder, an allele of the microsatellite Poli9-8TUF located in the major QTL for resistance to LD showed a significant and dominant effect on resistance to the disease (Fuji *et al.* 2007). Fuji *et al.* (2007) developed a new population of Japanese flounder using MAS with the marker Poli9-8TUF. They selected a female homozygous for the favourable allele (B-favourable) and a male with a higher growth rate and good body shape, but without the resistant allele as parents. In the females, the marker Poli9-8TUF is tightly linked to the QTL for resistance to LD; therefore, a female was selected as the LD-resistant parent. The B-favourable allele was transmitted from the mother to the progeny. All the progeny are heterozygotes with the LD-R allele, and field tests showed the progeny was really resistant to LD, while the control group without B-favourable alleles showed incidences of 4.5 and 6.3% of mortality due to lymphocystis disease. These results clearly demonstrate that MAS is an efficient strategy for breeding. MAS lymphocystis disease-resistant flounder had a market penetration rate of 35% in Japan in 2012 (Ozaki *et al.* 2012).

In salmon, IPN is a major problem in farming. Three markers (SSa0285BSFU, Alu333 and

SSa0374BSFU/II) on LG 21, significantly associated with resistance to IPN, are being applied in the selection for resistance to IPN in several companies producing salmon (e.g. Marine Harvest) (Moen *et al.* 2009). The GenoMar AS in cooperation with the Temasek Life Sciences Laboratory in Singapore is doing the genotyping service for salmon breeding companies to implant the MAS for resistance to IPN. However, the outcome of the MAS for resistance to IPN has not been released yet.

The application of MAS in breeding programmes means that brooders are now able to be selected according to both genotypes and performance records, rather than on performance alone. However, to date, little is known about the economic benefits gained from MAS in aquaculture species. Information of this nature is essential because the additional genetic gains depend on the magnitude of the allelic effects, and thus, the marginal increase should offset the costs of applying the technology (e.g. genotyping and manpower costs).

Conclusions and future directions

Genetic linkage maps, the necessary framework for any MAS programme, have been constructed for over 40 aquaculture species and are being constructed for other species. However, the density of the maps varies considerably among species. The majority of the linkage maps were constructed by using a combination of dominant (e.g. RAPD and AFLP) and codominant markers (e.g. microsatellites and SNPs). In the future, linkage maps should be constructed using only codominant markers, as they are easily transferable between different laboratories, and identification of codominant markers is no longer expensive due to the rapid development of sequencing technologies. In shrimp, prawns and crabs, and clams, oysters and mussels, SNPs may be the better markers for constructing linkage maps because microsatellites are very difficult to genotype in these species (Scarborough *et al.* 2002). It is necessary to integrate different linkage maps of a species into a consensus map. Ideally, a reference linkage map for a species should be constructed using the same reference families by different research groups.

Although QTL analyses have been carried out for some economically important traits in over 20 aquaculture species, most QTL were only mapped in large spaces between markers, and only QTL

with moderate-to-large effects were detected with the current experimental design. Only a few QTL have been confirmed and fine-mapped in different genetic backgrounds. Further confirmation and fine-mapping of identified QTL for traits of interest in different populations are essential for future MAS. For FCR, which is one of the most important traits in aquaculture industry, no QTL analysis has been conducted due to the difficulty in measuring FCR. It is essential to establish effective methods to measure FCR for mapping QTL for this trait.

Marker-assisted selection has only been applied in selection of resistance to lymphocystis disease in Japanese flounders and resistance to IPN in salmon. Currently, MAS has not played a major role in genetic improvement programmes in the majority of aquaculture species (Sonesson 2007; Ozaki *et al.* 2012). However, the rapid advances in sequencing technologies and statistical analysis of large data will soon create a lot of information that can be exploited for the genetic improvement of aquaculture species. The cost for genotyping a large number of DNA markers will substantially decrease in the near future. The eventual application of MAS in practical breeding programmes will be largely dependent on the economic benefits. For the time being, the application of MAS will be limited to QTL of moderate-to-large effect. Observable phenotypes will remain an important component of genetic improvement programmes because it takes into account the collective effect of all genes. Integration of MAS with the conventional selective breeding may be the most effective way for genetic improvement in aquaculture species. It is expected that more breeding companies will be integrating MAS strategies into conventional breeding programmes to improve breeding stocks.

Because the QTL approaches only detect QTL with moderate-to-large effects for MAS, the QTL with small effects may be missed. Therefore, researchers are now working towards identifying huge numbers of DNA variations associated with traits in the whole-genome sequences in humans, model organisms and agronomic species, that is, genome-wide association studies (GWAS) (Hirschhorn and Daly 2005; McCarthy *et al.* 2008). GWAS, also called as whole-genome association study, is an examination of a large number of variations (e.g. >500 000 SNPs) in the whole genome of a large number (e.g. >2000) of individuals of a particular species to see how many variations

differ from individual to individual (Hirschhorn and Daly 2005; McCarthy *et al.* 2008). Different variations are then associated with different traits, such as diseases and growth. In humans, this technique has led to the discovery of associations of particular genes with a number of common diseases (Hirschhorn and Daly 2005; McCarthy *et al.* 2008). The main advantage of GWAS is their high ability to detect very small effects of marker–trait associations, as they are based on linkage disequilibrium. Because natural populations are usually used in GWAS, recombination that accumulates over many generations of the population may break any long-range associations between markers and traits leaving short stretches of the genome associated with the trait. If alleles at marker loci are significantly associated with superior phenotypes, they can be used for selection across breeding populations. The MAS using genetic markers associated with traits of interest, which are identified in GWAS, is called genomic selection (GS). Simulation results and limited experimental results suggest that breeding values can be predicted with high accuracy using SNPs along the whole genomes (Goddard and Hayes 2007; Sonesson 2007; Sonesson and Meuwissen 2009). GS is better suited to breeding programmes of aquaculture species, which aims to maintain a large genetic variation to maintain the sustainability of breeding programmes, that is, brooder stocks with a large number of families. In contrast, the QTL approach is ideal for within-family selection because most QTL detected are family specific, and only a few are universal (Sonesson 2007). A major concern for GWAS and GS in aquaculture species is the high cost. Even now in humans, a GWAS with 2000 individuals genotyped with 1 million SNPs costs over 1 million USD (McCarthy *et al.* 2008), which is still too expensive for aquaculture industry. The recent development of rapid, high-throughput and cost-effective genotyping techniques by direct sequencing has drastically reduced the cost of genotyping SNPs, which makes GWAS and GS feasible. It is expected that in the next few years, the cost of genotyping by sequencing will be reduced substantially (at least 10 folds). To our best knowledge, GWAS are being started in a few aquaculture species. In Singapore, the National Research Foundation has funded 10 million Singapore dollars to the Temasek Life Science Laboratory for genome sequencing, GWAS and GS in Asian seabass. On the other hand, QTL

mapping remains largely a research tool to improve our understanding of the number, distribution and mode of action of QTL controlling economically important traits in aquaculture species. QTL can also play a role in GWAS as a vehicle for validating and confirming significant SNP correlations identified in association populations (Thumma *et al.* 2005). In the near future, GWAS promise to yield numerous SNP markers that could be used in GS for early selection of superior alleles associated with a wide range of traits. As the efficiency of techniques for DNA sequencing, SNP discovery, genotyping and other molecular procedures improves further and experimental costs decrease, the opportunities to incorporate GS into breeding programmes for aquaculture species will surely increase.

Acknowledgements

I would like to thank my colleagues Drs Jun Hong Xia, Wang Le and Feng Liu, as well as Ms Grace Lin for critical comments on previous versions of this manuscript, and Ms May Lee and Grace Lin for English correction. This research is supported by the National Research Foundation Singapore under its Competitive Research Program (CRP Award No. NRF-CRP002-001).

References

- Alfaqih, M.A., Brunelli, J.P., Drew, R.E. and Thorgaard, G.H. (2009) Mapping of five candidate sex-determining loci in rainbow trout (*Oncorhynchus mykiss*). *BMC Genetics* **10**, 2.
- Araneda, C., Lam, N., Diaz, N.F. *et al.* (2009) Identification, development, and characterization of three molecular markers associated to spawning date in Coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **296**, 21–26.
- Baerwald, M.R., Petersen, J.L., Hedrick, R.P., Schisler, G.J. and May, B. (2011) A major effect quantitative trait locus for whirling disease resistance identified in rainbow trout (*Oncorhynchus mykiss*). *Heredity* **106**, 920–926.
- Bai, Z.Y., Yuan, Y., Yue, G.H. and Li, J.L. (2011) Molecular cloning and copy number variation of a ferritin subunit (Fth1) and its association with growth in freshwater pearl mussel *Hyriopsis cumingii*. *PLoS ONE* **6**, e22886.
- Bai, Z.Y., Zhu, Z.Y., Wang, C.M., Xia, J.H., He, X.P. and Yue, G.H. (2012) Cloning and characterization of the calreticulin gene in Asian seabass (*Lates calcarifer*). *Animal* **6**, 683–687.
- Baranski, M., Moen, T. and Vage, D.I. (2010) Mapping of quantitative trait loci for flesh colour and growth traits in Atlantic salmon (*Salmo salar*). *Genetics, Selection, Evolution* **42**, 17.
- Barroso, R.M., Wheeler, P.A., LaPatra, S.E., Drew, R.E. and Thorgaard, G.H. (2008) QTL for IHNV resistance and growth identified in a rainbow (*Oncorhynchus mykiss*) x Yellowstone cutthroat (*Oncorhynchus clarki bouvieri*) trout cross. *Aquaculture* **277**, 156–163.
- Basten, C.J., Weir, B.S. and Zeng, Z.B. (2003) *QTL Cartographer, version 1.17*. A reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, NC.
- Broman, K.W., Wu, H., Sen, S. and Churchill, G.A. (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**, 889–890.
- Carrasco, L.A.P., Penman, D.J. and Bromage, N. (1999) Evidence for the presence of sex chromosomes in the Nile tilapia (*Oreochromis niloticus*) from synaptonemal complex analysis of XX, XY and YY genotypes. *Aquaculture* **173**, 207–218.
- Castano-Sanchez, C., Fuji, K., Ozaki, A. *et al.* (2010) A second generation genetic linkage map of Japanese flounder (*Paralichthys olivaceus*). *BMC Genomics* **11**, 554.
- Cnaani, A., Hallerman, E.M., Ron, M. *et al.* (2003) Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F-2 tilapia hybrid. *Aquaculture* **223**, 117–128.
- Cnaani, A., Zilberman, N., Tinman, S., Hulata, G. and Ron, M. (2004) Genome-scan analysis for quantitative trait loci in an F2 tilapia hybrid. *Molecular Genetics and Genomics* **272**, 162–172.
- Colihueque, N., Cardenas, R., Ramirez, L., Estay, F. and Araneda, C. (2010) Analysis of the association between spawning time QTL markers and the biannual spawning behavior in rainbow trout (*Oncorhynchus mykiss*). *Genetics and Molecular Biology* **33**, 578–582.
- Crosses, E. (2001) Review of statistical methods for QTL mapping in experimental crosses. *Lab Animal* **30**, 44–52.
- Danzmann, R.G., Jackson, T.R. and Ferguson, M.M. (1999) Epistasis in allelic expression at upper temperature tolerance QTL in rainbow trout. *Aquaculture* **173**, 45–58.
- Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M. and Blaxter, M.L. (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* **12**, 499–510.
- Davidson, W.S., Huang, T.K., Fujiki, K., von Schalburg, K.R. and Koop, B.F. (2009) The sex determining loci and sex chromosomes in the family salmonidae. *Sexual Development* **3**, 78–87.
- Derayat, A., Houston, R.D., Guy, D.R. *et al.* (2007) Mapping QTL affecting body lipid percentage in Atlantic salmon (*Salmo salar*). *Aquaculture* **272**, S250–S251.

- Doerge, R.W. (2002) Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews Genetics* **3**, 43–52.
- Drew, R.E., Schwabl, H., Wheeler, P.A. and Thorgaard, G.H. (2007) Detection of QTL influencing cortisol levels in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **272**, 183–194.
- Du, Z.Q., Ciobanu, D.C., Onteru, S.K. *et al.* (2010) A gene-based SNP linkage map for pacific white shrimp, *Litopenaeus vannamei*. *Animal Genetics* **41**, 286–294.
- Easton, A.A., Moghadam, H.K., Danzmann, R.G. and Ferguson, M.M. (2011) The genetic architecture of embryonic developmental rate and genetic covariation with age at maturation in rainbow trout *Oncorhynchus mykiss*. *Journal of Fish Biology* **78**, 602–623.
- Edwards, M., Stuber, C. and Wendel, J. (1987) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics* **116**, 113–125.
- Eshel, O., Shirak, A., Weller, J. *et al.* (2010) Fine mapping of a locus on linkage group 23 for sex determination in Nile tilapia (*Oreochromis niloticus*). *Animal Genetics* **42**, 222–224.
- Eshel, O., Shirak, A., Weller, J., Hulata, G. and Ron, M. (2012) Linkage and physical mapping of sex region on LG23 of Nile tilapia (*Oreochromis niloticus*). *G3: Genes - Genomes - Genetics* **2**, 35–42.
- Flint, J. and Mott, R. (2001) Finding the molecular basis of quantitative traits: successes and pitfalls. *Nature Reviews Genetics* **2**, 437–445.
- Fuji, K., Kobayashi, K., Hasegawa, O., Coimbra, M.R.M., Sakamoto, T. and Okamoto, N. (2006) Identification of a single major genetic locus controlling the resistance to lymphocystis disease in Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **254**, 203–210.
- Fuji, K., Hasegawa, O., Honda, K., Kumasaka, K., Sakamoto, T. and Okamoto, N. (2007) Marker-assisted breeding of a lymphocystis disease-resistant Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **272**, 291–295.
- Gates, H., Mallon, A.M. and Brown, S.D.M. (2011) High-throughput mouse phenotyping. *Methods* **53**, 394–404.
- Geldermann, H. (1975) Investigations on inheritance of quantitative characters in animals by gene markers I. Methods. *Theoretical and Applied Genetics* **46**, 319–330.
- Gharbi, K., Glover, K.A., Stone, L.C. *et al.* (2009) Genetic dissection of MHC-associated susceptibility to *Lepeophtheirus salmonis* in Atlantic salmon. *BMC Genetics* **10**, 20.
- Gheyas, A.A., Haley, C.S., Guy, D.R. *et al.* (2010a) Effect of a major QTL affecting IPN resistance on production traits in Atlantic salmon. *Animal Genetics* **41**, 666–668.
- Gheyas, A.A., Houston, R.D., Mota-Velasco, J.C. *et al.* (2010b) Segregation of infectious pancreatic necrosis resistance QTL in the early life cycle of Atlantic salmon (*Salmo salar*). *Animal Genetics* **41**, 531–536.
- Gilad, Y., Rifkin, S.A. and Pritchard, J.K. (2008) Revealing the architecture of gene regulation: the promise of eQTL studies. *Trends in Genetics* **24**, 408–415.
- Gilbey, J., Verspoor, E., Mo, T.A. *et al.* (2006) Identification of genetic markers associated with *Gyrodactylus salaris* resistance in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **71**, 119–129.
- Gjedrem, T. and Baranski, M. (2009) *Selective Breeding in Aquaculture: An Introduction*. Springer, New York.
- Goddard, M.E. and Hayes, B.J. (2007) Genomic selection. *Journal of Animal Breeding and Genetics* **124**, 323–330.
- Graubert, T.A., Cahan, P., Edwin, D. *et al.* (2007) A high-resolution map of segmental DNA copy number variation in the mouse genome. *PLoS Genetics* **3**, e3.
- Green, P., Falls, K. and Crooks, S. (1990) *Documentation for CRI-MAP, version 2.4*. Washington University School of Medicine, St. Louis, MO.
- Guichoux, E., Lagache, L., Wagner, S. *et al.* (2011) Current trends in microsatellite genotyping. *Molecular Ecology Resources* **11**, 591–611.
- Guo, X., Li, Q., Wang, Q.Z. and Kong, L.F. (2012) Genetic mapping and QTL analysis of growth-related traits in the Pacific oyster. *Marine Biotechnology* **14**, 218–226.
- Gutierrez, A.P., Lubieniecki, K.P., Davidson, E.A. *et al.* (2012) Genetic mapping of quantitative trait loci (QTL) for body-weight in Atlantic salmon (*Salmo salar*) using a 6.5 K SNP array. *Aquaculture* **358–359**, 61–70.
- Guyomard, R., Boussaha, M., Krieg, F., Hervet, C. and Quillet, E. (2012) A synthetic rainbow trout linkage map provides new insights into the salmonid whole genome duplication and the conservation of synteny among teleosts. *BMC Genetics* **13**, 15.
- Guyon, R., Rakotomanga, M., Azzouzi, N. *et al.* (2012) A high-resolution map of the Nile tilapia genome: a resource for studying cichlids and other percomorphs. *BMC Genomics* **13**, 222.
- Hackett, C. and Broadfoot, L. (2003) Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. *Heredity* **90**, 33–38.
- Haley, C.S. and Knott, S.A. (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315–324.
- He, F., Wen, H.S., Dong, S.L. *et al.* (2008a) Identification of single nucleotide polymorphism cytochrome P450-c19a and its relation to reproductive traits in Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **279**, 177–181.
- He, F., Wen, H.S., Dong, S.L. *et al.* (2008b) Identification of estrogen receptor [alpha] gene polymorphisms by SSCP and its effect on reproductive traits in Japanese flounder (*Paralichthys olivaceus*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **150**, 278–283.

- He, X.P., Xia, J.H., Wang, C.M., Pang, H.Y. and Yue, G.H. (2012) Significant associations of polymorphisms in the prolactin gene with growth traits in Asian seabass (*Lates calcarifer*). *Animal Genetics* **43**, 233–236.
- Hirschhorn, J.N. and Daly, M.J. (2005) Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics* **6**, 95–108.
- Houston, R.D., Guy, D.R., Hamilton, A. *et al.* (2007) Mapping QTL affecting resistance to infectious pancreatic necrosis (IPN) in Atlantic salmon (*Salmo salar*). *Aquaculture* **272**, S269.
- Houston, R.D., Haley, C.S., Hamilton, A. *et al.* (2008) Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). *Genetics* **178**, 1109–1115.
- Houston, R.D., Bishop, S.C., Hamilton, A. *et al.* (2009a) Detection of QTL affecting harvest traits in a commercial Atlantic salmon population. *Animal Genetics* **40**, 753–755.
- Houston, R.D., Haley, C.S., Hamilton, A. *et al.* (2009b) The susceptibility of Atlantic salmon fry to freshwater infectious pancreatic necrosis is largely explained by a major QTL. *Heredity* **105**, 318–327.
- Houston, R., Davey, J., Bishop, S. *et al.* (2012) Characterisation of QTL-linked and genome-wide restriction site-associated DNA (RAD) markers in farmed Atlantic salmon. *BMC Genomics* **13**, 244.
- Hu, Z. and Xu, S. (2009) PROC QTL- A SAS procedure for mapping quantitative trait loci. *International Journal of Plant Genomics* **2009**, 141234.
- Hubner, N., Wallace, C.A., Zimdahl, H. *et al.* (2005) Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nature Genetics* **37**, 243–253.
- Hulata, G. (2001) Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. *Genetica* **111**, 155–173.
- Iyer-Pascuzzi, A.S., Symonova, O., Mileyko, Y. *et al.* (2010) Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. *Plant Physiology* **152**, 1148–1157.
- Jackson, T.R., Ferguson, M.M., Danzmann, R.G. *et al.* (1998) Identification of two QTL influencing upper temperature tolerance in three rainbow trout (*Oncorhynchus mykiss*) half-sib families. *Heredity* **80**, 143–151.
- Jansen, R.C. (1993) Interval mapping of multiple quantitative trait loci. *Genetics* **135**, 205–211.
- Jin, S., Zhang, X., Jia, Z., Fu, H., Zheng, X. and Sun, X. (2012) Genetic linkage mapping and genetic analysis of QTL related to eye cross and eye diameter in common carp (*Cyprinus carpio* L.) using microsatellites and SNPs. *Aquaculture* **259**, 176–182.
- Joehanes, R. and Nelson, J.C. (2008) QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics* **24**, 2788–2789.
- Khoo, S.K., Ozaki, A., Nakamura, F. *et al.* (2004) Identification of a novel chromosomal region associated with infectious hematopoietic necrosis (IHN) resistance in rainbow trout *Oncorhynchus mykiss*. *Fish Pathology* **39**, 95–101.
- Kocher, T.D. and Kole, C. (2010) *Genome Mapping and Genomics in Fishes and Aquatic Animals*. Springer, New York.
- Kocher, T.D., Lee, W.J., Sobolewska, H., Penman, D. and McAndrew, B. (1998) A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). *Genetics* **148**, 1225–1232.
- Kofler, R., Schlotterer, C. and Lelley, T. (2007) SciRoKo: a new tool for whole genome microsatellite search and investigation. *Bioinformatics* **23**, 1683–1685.
- Komura, D., Shen, F., Ishikawa, S. *et al.* (2006) Genome-wide detection of human copy number variations using high-density DNA oligonucleotide arrays. *Genome Research* **16**, 1575–1584.
- Korol, A., Frenkel, Z., Orion, O. and Ronin, Y. (2012) Some ways to improve QTL mapping accuracy. *Animal Genetics* **43**, 36–44.
- Kucuktas, H., Wang, S.L., Li, P. *et al.* (2009) Construction of genetic linkage maps and comparative genome analysis of catfish using gene-associated markers. *Genetics* **181**, 1649–1660.
- Kuttner, E., Moghadam, H.K., Skulason, S., Danzmann, R.G. and Ferguson, M.M. (2011) Genetic architecture of body weight, condition factor and age of sexual maturation in Icelandic Arctic charr (*Salvelinus alpinus*). *Molecular Genetics and Genomics* **286**, 67–79.
- Lallias, D., Gomez-Raya, L., Haley, C.S. *et al.* (2009) Combining two-stage testing and interval mapping strategies to detect QTL for resistance to bonamiosis in the European flat oyster *Ostrea edulis*. *Marine Biotechnology* **11**, 570–584.
- Lande, R. and Thompson, R. (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* **124**, 743–756.
- Lander, E.S. and Botstein, D. (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.
- Le Bras, Y., Dechamp, N., Krieg, F. *et al.* (2011) Detection of QTL with effects on osmoregulation capacities in the rainbow trout (*Oncorhynchus mykiss*). *BMC Genetics* **12**, 46.
- Lee, W.J. (2003) Detection of QTL for salinity tolerance in tilapia. *Plant and Animal Genomes XI Conference* **W24**.
- Lee, B.Y., Penman, D. and Kocher, T. (2003) Identification of a sex-determining region in Nile tilapia (*Oreochromis niloticus*) using bulked segregant analysis. *Animal Genetics* **34**, 379–383.
- Lee, B.Y., Hulata, G. and Kocher, T.D. (2004) Two unlinked loci controlling the sex of blue tilapia (*Oreochromis aureus*). *Heredity* **92**, 543–549.

- Lee, B.Y., Lee, W.J., Streebman, J.T. *et al.* (2005) A second-generation genetic linkage map of tilapia (*Oreochromis spp.*). *Genetics* **170**, 237–244.
- Li, Y.T., Dierens, L., Byrne, K. *et al.* (2006) QTL detection of production traits for the Kuruma prawn *Penaeus japonicus* (Bate) using AFLP markers. *Aquaculture* **258**, 198–210.
- Lien, S., Gidskehaug, L., Moen, T. *et al.* (2011) A dense SNP-based linkage map for Atlantic salmon (*Salmo salar*) reveals extended chromosome homeologies and striking differences in sex-specific recombination patterns. *BMC Genomics* **12**, 615.
- Lincoln, S., Daly, M. and Lander, E. (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. *Whitehead Institute Technical Report*. Whitehead Institute, Cambridge, MA.
- Liu, Z.J. (2011) *Next Generation Sequencing and Whole Genome Selection in Aquaculture*. Wiley-Blackwell, New York.
- Liu, Z. and Cordes, J. (2004) DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* **238**, 1–37.
- Lyons, R.E., Dierens, L.M., Tan, S.H., Preston, N.P. and Li, Y. (2007) Characterization of AFLP markers associated with growth in the kuruma prawn, *Marsupenaeus japonicus*, and identification of a candidate gene. *Marine Biotechnology* **9**, 712–721.
- Manly, K.F., Cudmore, R.H. Jr and Meer, J.M. (2001) Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* **12**, 930–932.
- Marineharvest. (2012) *The Marineharvest Salmon Industry Handbook 2012*. Marineharvest, Bergen, pp. 41.
- Martinez, V., Thorgaard, G., Robison, B. and Sillanpaa, M.J. (2005) Posterior evidence of multiple QTL influencing early development in double haploid lines of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **247**, 25–25.
- Martyniuk, C.J., Perry, G.M.L., Mogahadam, H.K., Ferguson, M.M. and Danzmann, R.G. (2003) The genetic architecture of correlations among growth-related traits and male age at maturation in rainbow trout. *Journal of Fish Biology* **63**, 746–764.
- McAndrew, B. and Napier, J. (2010) Application of genetics and genomics to aquaculture development: current and future directions. *Journal of Agricultural Science* **149**, 143–151.
- McCarthy, M.I., Abecasis, G.R., Cardon, L.R. *et al.* (2008) Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature Reviews Genetics* **9**, 356–369.
- Metzker, M.L. (2009) Sequencing technologies the next generation. *Nature Reviews Genetics* **11**, 31–46.
- Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A. and Johnson, E.A. (2007) Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Research* **17**, 240–248.
- Miller, M.R., Brunelli, J.P., Wheeler, P.A. *et al.* (2012) A conserved haplotype controls parallel adaptation in geographically distant salmonid populations. *Molecular Ecology* **21**, 237–249.
- Mills, R.E., Walter, K., Stewart, C. *et al.* (2011) Mapping copy number variation by population-scale genome sequencing. *Nature* **470**, 59–65.
- Moen, T., Fjalestad, K.T., Munck, H. and Gomez-Raya, L. (2004) A multistage testing strategy for detection of quantitative trait loci affecting disease resistance in Atlantic salmon. *Genetics* **167**, 851–858.
- Moen, T., Munck, H. and Raya, L.G. (2005) A genome scan reveals a QTL for resistance to infectious salmon anaemia in Atlantic salmon (*Salmo salar*). *Aquaculture* **247**, 25–26.
- Moen, T., Sonesson, A.K., Hayes, B., Lien, S., Munck, H. and Meuwissen, T.H.E. (2007) Mapping of a quantitative trait locus for resistance against infectious salmon anaemia in Atlantic salmon (*Salmo Salar*): comparing survival analysis with analysis on affected/resistant data. *BMC Genetics* **8**, 53.
- Moen, T., Baranski, M., Sonesson, A.K. and Kjøglum, S. (2009) Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics* **10**, 368.
- Moghadam, H.K., Poissant, J., Fotherby, H., Haidle, L., Ferguson, M.M. and Danzmann, R.G. (2007) Quantitative trait loci for body weight, condition factor and age at sexual maturation in Arctic charr (*Salvelinus alpinus*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Molecular Genetics and Genomics* **277**, 647–661.
- Naish, K.A. and Hard, J.J. (2008) Bridging the gap between the genotype and the phenotype: linking genetic variation, selection and adaptation in fishes. *Fish and Fisheries* **9**, 396–422.
- Ninwicheana, P., Peatman, E., Liu, H. *et al.* (2012) Second generation of catfish genetic linkage map and integration of catfish genetic linkage and physical maps using BAC-anchored microsatellites. *G3: Genes - Genomes - Genetics* **10**, 1233–1241.
- Norman, J.D., Danzmann, R.G., Glebe, B. and Ferguson, M.M. (2011) The genetic basis of salinity tolerance traits in Arctic charr (*Salvelinus alpinus*). *BMC Genetics* **12**, 81.
- O'Malley, K.G., Sakamoto, T., Danzmann, R.G. and Ferguson, M.M. (2003) Quantitative trait loci for spawning date and body weight in rainbow trout: testing for conserved effects across ancestrally duplicated chromosomes. *Journal of Heredity* **94**, 273–284.
- Ooien, J.W., Boer, M., Jansen, R. and Maliepaard, C. (1996) MapQTL 4.0. Software for the calculation of QTL positions on genetic maps. *Order* **501**, 6204.
- Ozaki, A., Sakamoto, T., Khoo, S. *et al.* (2001) Quantitative trait loci (QTLs) associated with resistance/susceptibility

- tility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*). *Molecular Genetics and Genomics* **265**, 23–31.
- Ozaki, A., de Leon, F.G., Glebe, B., Woo, P.T.K., Ferguson, M.M. and Danzmann, R.G. (2005) Identification of QTL for resistance to *Cryptobia salmositica* infection in Atlantic salmon (*Salmo salar*): a model for pathogen disease resistance QTL? *Aquaculture* **247**, 27–27.
- Ozaki, A., Okamoto, H., Yamada, T. *et al.* (2010) Linkage analysis of resistance to *Streptococcus iniae* infection in Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **308**, S62–S67.
- Ozaki, A., Araki, K., Okamoto, H. *et al.* (2012) Progress of DNA marker-assisted breeding in maricultured finfish. *Bulletin of Fisheries Research Agency* **35**, 31–37.
- Ozakil, A., Khoo, S.K., Yoshiura, Y. *et al.* (2007) Identification of additional quantitative trait loci (QTL) responsible for susceptibility to infectious pancreatic necrosis virus in rainbow trout. *Fish Pathology* **42**, 131–140.
- Palti, Y., Genet, C., Luo, M.C. *et al.* (2011) A first generation integrated map of the rainbow trout genome. *BMC Genomics* **12**, 180.
- Palti, Y., Genet, C., Gao, G.T. *et al.* (2012) A second generation integrated map of the rainbow trout (*Oncorhynchus mykiss*) genome: analysis of conserved synteny with model fish genomes. *Marine Biotechnology* **14**, 343–357.
- Perkel, J. (2008) SNP genotyping: six technologies that keyed a revolution. *Nature Methods* **5**, 447–454.
- Perry, G.M.L., Danzmann, R.G., Ferguson, M.M. and Gibson, J.P. (2001) Quantitative trait loci for upper thermal tolerance in outbred strains of rainbow trout (*Oncorhynchus mykiss*). *Heredity* **86**, 333–341.
- Perry, G.M.L., Ferguson, M.M., Sakamoto, T. and Danzmann, R.G. (2005) Sex-linked quantitative trait loci for thermotolerance and length in the rainbow trout. *Journal of Heredity* **96**, 97–107.
- Plough, L.V. and Hedgecock, D. (2011) Quantitative trait locus analysis of stage-specific inbreeding depression in the Pacific oyster *Crassostrea gigas*. *Genetics* **189**, 1473–1486.
- Quinn, N.L., McGowan, C.R., Cooper, G.A., Koop, B.F. and Davidson, W.S. (2011) Identification of genes associated with heat tolerance in Arctic charr exposed to acute thermal stress. *Physiological Genomics* **43**, 685–696.
- Reid, D.P., Szanto, A., Glebe, B., Danzmann, R.G. and Ferguson, M.M. (2005) QTL for body weight and condition factor in Atlantic salmon (*Salmo salar*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). *Heredity* **94**, 166–172.
- Rengmark, A.H., Slettan, A., Lee, W.J., Lie, O. and Lingaas, F. (2007) Identification and mapping of genes associated with salt tolerance in tilapia. *Journal of Fish Biology* **71**, 409–422.
- Rexroad, C.E., Palti, Y., Gahr, S.A. and Vallejo, R.L. (2008) A second generation genetic map for rainbow trout (*Oncorhynchus mykiss*). *BMC Genetics* **9**, 74.
- Risch, N. and Giuffra, L. (1992) Model misspecification and multipoint linkage analysis. *Human Heredity* **42**, 77–92.
- Robison, B.D., Wheeler, P.A., Sundin, K., Sikka, P. and Thorgaard, G.H. (2001) Composite interval mapping reveals a major locus influencing embryonic development rate in rainbow trout (*Oncorhynchus mykiss*). *Journal of Heredity* **92**, 16–22.
- Rodriguez, M.F., LaPatra, S., Williams, S., Famula, T. and May, B. (2004) Genetic markers associated with resistance to infectious hematopoietic necrosis in rainbow and steelhead trout (*Oncorhynchus mykiss*) backcrosses. *Aquaculture* **241**, 93–115.
- Rothschild, M.F. and Ruvinsky, A. (2007) Marker-Assisted Selection for Aquaculture Species. In: *Aquaculture Genome Technologies* (ed. Z.J. Liu). Blackwell Publishing, Iowa, pp. 199–214.
- Sachidanandam, R., Weissman, D., Schmidt, S.C. *et al.* (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* **409**, 928–933.
- Sakamoto, T., Danzmann, R.G., Okamoto, N., Ferguson, M.M. and Ihssen, P.E. (1999) Linkage analysis of quantitative trait loci associated with spawning time in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **173**, 33–43.
- Sauvage, C., Boudry, P., de Koning, D.J., Haley, C.S., Heurtebise, S. and Lapegue, S. (2010) QTL for resistance to summer mortality and OsHV-1 load in the Pacific oyster (*Crassostrea gigas*). *Animal Genetics* **41**, 390–399.
- Scarborough, J.R., Cowles, D.L. and Carter, R.L. (2002) Microsatellites in shrimp species. In: *Modern approaches to the study of Crustacea* (eds E. Escobar-Brones and F. Alvarez). Kluwer Academic Publisher, New York, pp. 291–299.
- Seaton, G., Haley, C.S., Knott, S.A., Kearsey, M. and Visscher, P.M. (2002) QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* **18**, 339–340.
- Sebat, J., Lakshmi, B., Troge, J. *et al.* (2004) Large-scale copy number polymorphism in the human genome. *Science* **305**, 525.
- Sharma, P.C., Grover, A. and Kahl, G. (2007) Mining microsatellites in eukaryotic genomes. *Trends in Biotechnology* **25**, 490–498.
- Shirak, A., Seroussi, E., Cnaani, A. *et al.* (2006) Amh and Dmrt2 genes map to tilapia (*Oreochromis* spp.) linkage group 23 within quantitative trait locus regions for sex determination. *Genetics* **174**, 1573–1581.
- Shirak, A., Golik, M., Lee, B.Y. *et al.* (2008) Copy number variation of lipocalin family genes for male-specific proteins in tilapia and its association with gender. *Heredity* **101**, 405–415.

- Stwek, M., Slawinska, A., Nieuwland, M. *et al.* (2010) A quantitative trait locus for a primary antibody response to keyhole limpet hemocyanin on chicken chromosome 14-Confirmation and candidate gene approach. *Poultry Science* **89**, 1850–1857.
- Somorjai, I.M.L., Danzmann, R.G. and Ferguson, M.M. (2003) Distribution of temperature tolerance quantitative trait loci in Arctic charr (*Salvelinus alpinus*) and inferred homologies in rainbow trout (*Oncorhynchus mykiss*). *Genetics* **165**, 1443–1456.
- Sonesson, A.K. (2007) Within-family marker-assisted selection for aquaculture species. *Genetics, Selection, Evolution* **39**, 301–317.
- Sonesson, A.K. and Meuwissen, T.H.E. (2009) Testing strategies for genomic selection in aquaculture breeding programs. *Genetics, Selection, Evolution* **41**, 37.
- Song, W., Pang, R., Niu, Y. *et al.* (2012) Construction of high-density genetic linkage maps and mapping of growth-related quantitative trait loci in the Japanese flounder (*Paralichthys olivaceus*). *PLoS ONE* **7**, e50404.
- Stam, P. (1993) Construction of integrated genetic linkage maps by means of a new computer package: Join-Map. *The Plant Journal* **3**, 739–744.
- Star, B., Nederbragt, A.J., Jentoft, S. *et al.* (2011) The genome sequence of Atlantic cod reveals a unique immune system. *Nature* **477**, 207–210.
- Storset, A., Strand, C., Wetten, M., Kjølglum, S. and Ramstad, A. (2007) Response to selection for resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **272**, S62–S68.
- Sun, X.W. and Liang, L.Q. (2004) A genetic linkage map of common carp (*Cyprinus carpio* L.) and mapping of a locus associated with cold tolerance. *Aquaculture* **238**, 165–172.
- Sundin, K., Brown, K.H., Drew, R.E., Nichols, K.M., Wheeler, P.A. and Thorgaard, G.H. (2005) Genetic analysis of a development rate QTL in backcrosses of clonal rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **247**, 75–83.
- Thumma, B.R., Nolan, M.F., Evans, R. and Moran, G.F. (2005) Polymorphisms in cinnamoyl CoA reductase (CCR) are associated with variation in microfibril angle in *Eucalyptus* spp. *Genetics* **171**, 1257–1265.
- Vallejo, R.L., Rexroad, C.E., Silverstein, J.T., Janss, L.L.G. and Weber, G.M. (2009) Evidence of major genes affecting stress response in rainbow trout using Bayesian methods of complex segregation analysis. *Journal of Animal Science* **87**, 3490–3505.
- Vallejo, R.L., Wiens, G.D., Rexroad, C.E. *et al.* (2010) Evidence of major genes affecting resistance to bacterial cold water disease in rainbow trout using Bayesian methods of segregation analysis. *Journal of Animal Science* **88**, 3814–3832.
- Vasemagi, A., Gross, R., Palm, D., Paaver, T. and Primer, C.R. (2010) Discovery and application of insertion-deletion (INDEL) polymorphisms for QTL mapping of early life-history traits in Atlantic salmon. *BMC Genomics* **11**, 156.
- Volckaert, F., Batargias, C., Chatziplis, D. *et al.* (2007) A second generation linkage map of European sea bass. *Aquaculture* **272**, S316–S316.
- Volfovsky, N., Haas, B.J. and Salzberg, S.L. (2001) A clustering method for repeat analysis in DNA sequences. *Genome Biology* **2**, 0027.1–0027.11.
- Voorrips, R. (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity* **93**, 77–78.
- Wang, K. and Bucan, M. (2010) Copy number variation detection via high-density SNP genotyping. *Cold Spring Harbor Protocols* **2008**, pdb. top46.
- Wang, D.G., Fan, J.B., Siao, C.J. *et al.* (1998) Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* **280**, 1077–1082.
- Wang, C.M., Lo, L.C., Zhu, Z.Y. and Yue, G.H. (2006) A genome scan for quantitative trait loci affecting growth-related traits in an F1 family of Asian seabass (*Lates calcarifer*). *BMC Genomics* **7**, 274.
- Wang, C.M., Zhu, Z.Y., Lo, L.C. *et al.* (2007) A microsatellite linkage map of Barramundi, *Lates calcarifer*. *Genetics* **175**, 907–915.
- Wang, C.M., Lo, L.C., Feng, F., Zhu, Z.Y. and Yue, G.H. (2008a) Identification and verification of QTL associated with growth traits in two genetic backgrounds of Barramundi (*Lates calcarifer*). *Animal Genetics* **39**, 34–39.
- Wang, C.M., Lo, L.C., Zhu, Z.Y. *et al.* (2008b) Estimating reproductive success of brooders and heritability of growth traits in Asian sea bass (*Lates calcarifer*) using microsatellites. *Aquaculture Research* **39**, 1612–1619.
- Wang, C.M., Lo, L.C., Zhu, Z.Y. *et al.* (2011a) Mapping QTL for an adaptive trait: the length of caudal fin in *Lates calcarifer*. *Marine Biotechnology* **13**, 74–82.
- Wang, C.M., Bai, Z.Y., He, X.P. *et al.* (2011b) A high-resolution linkage map for comparative genome analysis and QTL fine mapping in Asian seabass, *Lates calcarifer*. *BMC Genomics* **12**, 174.
- Weber, J.L. (1990) Informativeness of human (dC-dA) n.(dG-dT)n polymorphisms. *Genomics* **7**, 524–530.
- Wringe, B.F., Devlin, R.H., Ferguson, M.M., Moghadam, H.K., Sakhrani, D. and Danzmann, R.G. (2010) Growth-related quantitative trait loci in domestic and wild rainbow trout (*Oncorhynchus mykiss*). *BMC Genetics* **11**, 63.
- Xia, J.H., Liu, F., Zhu, Z.Y. *et al.* (2010) A consensus linkage map of the grass carp (*Ctenopharyngodon idella*) based on microsatellites and SNPs. *BMC Genomics* **11**, 135.
- Xu, Y., Zhu, Z., Lo, L. *et al.* (2006) Characterization of two parvalbumin genes and their association with growth traits in Asian seabass (*Lates calcarifer*). *Animal Genetics* **37**, 266–268.

- Xu, P., McIntyre, L.M., Scardina, J., Wheeler, P.A., Thorgaard, G.H. and Nichols, K.M. (2011) Transcriptome profiling of embryonic development rate in rainbow trout advanced backcross introgression lines. *Marine Biotechnology* **13**, 215–231.
- Yan, B.A. and Wang, Z.H. (2010) Growth, salinity tolerance and microsatellite analysis of the F-2 reciprocal hybrids of *Oreochromis niloticus* x *Sarotherodon galilaeus* at different salinities. *Aquaculture Research* **41**, 336–344.
- Yandell, B.S., Mehta, T., Banerjee, S. *et al.* (2007) R/qtl-bim: QTL with Bayesian interval mapping in experimental crosses. *Bioinformatics* **23**, 641–643.
- Yu, Z.N. and Guo, X.M. (2006) Identification and mapping of disease-resistance QTLs in the eastern oyster, *Crassostrea virginica* Gmelin. *Aquaculture* **254**, 160–170.
- Zhang, G.F., Fang, X.D., Guo, X.M. *et al.* (2012) The oyster genome reveals stress adaptation and complexity of shell formation. *Nature* **490**, 49–54.
- Zimmerman, A.M., Evenhuis, J.P., Thorgaard, G.H. and Ristow, S.S. (2004) A single major chromosomal region controls natural killer cell-like activity in rainbow trout. *Immunogenetics* **55**, 825–835.
- Zimmerman, A.M., Wheeler, P.A., Ristow, S.S. and Thorgaard, G.H. (2005) Composite interval mapping reveals three QTL associated with pyloric caeca number in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **247**, 85–95.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Linkage maps published for important aquaculture species.

Table S2. Quantitative trait loci reported for aquaculture species.