

## INVITED REVIEWS AND SYNTHESSES

# Fifteen years of quantitative trait loci studies in fish: challenges and future directions

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## Abstract

Understanding the genetic basis of phenotypic variation is a major challenge in biology. Here, we systematically evaluate 146 quantitative trait loci (QTL) studies on teleost fish over the last 15 years to investigate (i) temporal trends and (ii) factors affecting QTL detection and fine-mapping. The number of fish QTL studies per year increased over the review period and identified a cumulative number of 3632 putative QTLs. Most studies used linkage-based mapping approaches and were conducted on non-model species with limited genomic resources. A gradual and moderate increase in the size of the mapping population and a sharp increase in marker density from 2011 onwards were observed; however, the number of QTLs and variance explained by QTLs changed only minimally over the review period. Based on these findings, we discuss the causative factors and outline how larger sample sizes, phenomics, comparative genomics, epigenetics and software development could improve both the quantity and quality of QTLs in future genotype–phenotype studies. Given that the technical limitations on DNA sequencing have mostly been overcome in recent years, a renewed focus on these and other study design factors will likely lead to significant improvements in QTL studies in the future.

**Keywords:** association, fish, linkage mapping, phenomics, quantitative trait loci mapping, teleost

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## Introduction

A major challenge in biology is to understand the genetic basis of phenotypic variation. Most phenotypic variation is thought to be caused by quantitative genetic variation that results from the segregation of alleles at multiple quantitative trait loci (QTL) and is influenced by and sensitive to the environment (Mackay 2001). Insights into this complex genotype–phenotype map, including environmental effects, promises to yield important knowledge for predicting disease risks (Lehner 2013), supporting selective breeding programmes (Dekkers 2012) and understanding adaptive variation in natural populations (Savolainen *et al.* 2013). It is no surprise that numerous studies over recent years have

attempted to dissect the genotype–phenotype connections in a wide range of species.

Genotype–phenotype relationships can be mapped to identify the genomic regions controlling phenotypic traits, with the ultimate, yet often time-consuming, goal to locate the causal genes or nucleotide mutations underlying the trait in question (Rockman 2012). Linkage, association (linkage disequilibrium) and combined linkage and linkage disequilibrium (LDLA) methods have been developed to map areas containing quantitative trait loci. The general principle of these mapping methods is that they try to account for phenotypic trait variation by measuring its correlation with markers that segregate in a Mendelian fashion. Of these, linkage and association methods are most frequently used. These methods differ in several ways. The most noteworthy difference is that association studies are typically more precise at locating QTL regions but require many more

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markers to achieve the high level of precision (Ott *et al.* 2011). Another difference is that association studies can be conducted using samples from wild or captive populations with limited familial information, whereas linkage methods require some form of a relatedness matrix, which is usually obtained by the controlled breeding of a managed population.

Historically, the lack of abundant polymorphic markers has limited the application of these QTL mapping methods. However, rapid advances in DNA sequencing technologies and downstream analyses since the 1990s have enabled the discovery of a significantly larger number of markers. In particular, methods such as restriction site-associated DNA (RAD) sequencing (e.g. genotyping by sequencing) have made it possible to generate genomewide marker coverage in less time and cheaper than ever before (e.g. Elshire *et al.* 2011). The promise of the 'genomics era' is that it will bring a significant increase in the power and precision of QTL studies and an enhanced ability to finely map the genetic basis of phenotypic traits. While some have been critical of this claim (e.g. Ioannidis & Kavvoura 2006; Rockman 2012), others are optimistic about both what has been achieved so far and what the future of genotype–phenotype investigations will accomplish (e.g. McCarthy *et al.* 2008; Visscher *et al.* 2012). Indeed, over the last few years a vast number of new QTLs and associated genes have been identified across a wide range of invertebrate and vertebrate species (Stranger *et al.* 2011; Visscher *et al.* 2012). However, important QTL mapping issues have repeatedly been highlighted, including the lack of power to detect QTLs that have small effects on highly polygenic traits (missing heritability problem), problems around the biological relevance of QTL mapping studies (e.g. Beavis effect) and difficulties incorporating epistatic, environmental and complex phenotypic trait interactions into QTL mapping strategies (Mackay *et al.* 2009; Rockman 2012; Slate 2013; Wellenreuther & Hansson 2016).

Most work investigating genotype–phenotype relationships has been carried out on model species such as yeast (*Saccharomyces cerevisiae*), fruit flies (*Drosophila melanogaster*), mouse (*Mus musculus*) and zebrafish (*Danio rerio*) (see available information held in public database, Kahraman *et al.* 2005). The aforementioned advances in high-throughput DNA sequencing technologies are, however, now enabling an expansion of research on nonmodel species, which have previously been woefully lagging behind model species because of limited funding and resources (Elshire *et al.* 2011; Ellegren 2014). Teleost fish is one such group that contains many nonmodel organisms. They are a particularly interesting group for investigation because they (i) form the largest group of vertebrates (around 33 000 species, i.e. ~50%

of all vertebrates), (ii) exhibit high levels of morphological diversity and (iii) aquaculture is currently the fastest growing primary industry.

Despite several good reviews investigating some aspects of genotype–phenotype research in teleost fish (e.g. Hemmer-Hansen *et al.* 2014; Tong & Sun 2015), a general review that thoroughly investigates all teleost QTL studies over the last years has not been conducted. To address this gap, we conducted a systematic survey of the published QTL studies on teleost fish covering the last 15 years and extracted QTL information from each study, including factors concerning the experimental design and data analysis. Using these data, we first investigated temporal trends of QTL mapping studies, including changes over time to study design factors (e.g. number of markers and sample size) and outcomes of studies (e.g. number of QTLs found). Second, we identified a number of correlations between study design factors and outcomes of studies to investigate what factors might be influencing QTL mapping success. Finally, we discuss the findings and highlight the factors that will potentially improve the power of genotype–phenotype studies in the future.

## Methods

### Study selection

Fish QTL studies were selected using the Web of Science search engine with the 'Topic' search terms 'QTL' and 'fish' or 'Quantitative trait loci' and 'fish' for the period between 1 January 2000 and 31 December 2015. This search yielded a total of 715 individual published articles, of which 563 were removed because they were not available in English, did not represent QTL identification studies, were not conducted on teleost fish, or were based (fully or in part) on previously identified QTLs. Studies using previously identified QTLs were removed to reduce bias in QTL detection and mapping precision due to previous information. We only included studies that used *de novo* information. The final data set used in our review comprised 146 published studies. A second Web of Science search was carried out using the 'Topic' search terms 'GWAS' and 'fish' or 'Genome wide association' and 'fish' and restricted to the period between 1 January 2000 and 31 December 2015. This search yielded a total of 222 individual articles, of which there were an additional 11 genomewide association studies (GWAS) not found in the first search. These 11 studies were used to look at the number of studies using linkage and association methods, but were excluded from all other parts of the review.

For each newly described QTL in the first Web of Science search, we recorded publication date, species name, number of individuals sampled at each

generation in the pedigree, breeding design used for the pedigree (e.g. F2 backcross, intercross), genetic marker type and number of markers, QTL identification software, type of QTL detection method (linkage or association), individual QTL statistics (e.g. LOD, ANOVA, significance level, genomewide vs. linkage groupwide), target trait, trait distribution (discrete/quantitative), PVE, and peak, start and end location in centiMorgans (cM) of QTL regions. Additional information about the target species was also included in the data file, which included genome size (based on the C-value, Gregory 2016) and the species' primary habitat (fresh water, sea water or euryhaline).

#### *Temporal trends and basic study info*

All data analyses were carried out in the R statistical environment (version: 0.99.489) (R Core Team 2013). Information about experimental set-up of QTL studies was reported including the numbers of parents and progeny in the mapping populations, major influences in the construction of the mapping populations (e.g. backcrossing, interspecies crosses), the most common types of species investigated, and frequency of linkage and association study designs. We then investigated the temporal trends of studies, including how study design elements (software, sample size and marker density) and results of QTL studies have changed over time. Three measures of QTL study results were used, including the number of QTLs found per trait, percentage variance explained (PVE) for QTLs and QTL region width (cM). The *y*-axis for temporal graphs was placed on a log scale for all variables [except Studies (*n*)].

#### *Factors affecting QTL detection*

A correlation matrix was calculated using Spearman correlation coefficient for eight variables, namely number of QTLs (QTLs), PVE of QTLs (PVE), width of QTL regions (Width), number of traits investigated (Traits), number of genetic markers used (Markers), size of mapping population (Sample), size of genome based on C-values (Genome), and marker density (Markers/Genome) (Table 1). Correlations were calculated for all studies and for a subset containing only salmonid studies to reduce noise caused by the wide range of species. For all variables, the average value for each study was used. Sample size of the mapping population and PVE were graphed on a scatter plot. A regression line was placed using a general linear model (with 95% CI) and a log 10 transformation of the *x*- and *y*-axis. Significant difference of the slope from 0 was tested using bootstrapping (10 000×).

## Results

The initial Web of Science search identified 712 individual articles. Of these, studies were removed if they were not investigating new QTLs (411), conducted on teleost fish (67), based on previously identified QTLs (50), written in English (25), and other miscellaneous reasons (13) (Fig. S1, Supporting information). The remaining 145 studies included 128 linkage studies, 14 association studies and three studies using both linkage and association to identify QTLs and detected a total of 3627 putative QTLs. We removed one extreme outlier study from the majority of analyses because the number of markers was over 300 times higher than the next closest study (Ayllon *et al.* 2015). A second search identified 11 additional GWAS, which were only included in Fig. 2 of this review.

#### *Species and pedigree information*

Quantitative trait loci data from 49 fish species were reported in the studies. The three most commonly studied species were *Oncorhynchus mykiss* (15%), *Salmo salar* (10%) and *Cyprinus carpio* (8%). Considering all of the studies together, 71% were carried out on freshwater species (including anadromous species), 17% on marine species and 12% on euryhaline species.

The majority of studies (60%) produced their mapping population from a single set of parents (i.e. all progeny in the mapping population were full-siblings) (Fig. S2, Supporting information). The remaining studies ranged up to a maximum of 400 individuals. Of the different breeding designs, approximately half used interstrain or interspecies crosses with (~66%) or without (~33%) a secondary backcrossing step. Only five studies were conducted on wild (outbred) populations.

#### *Temporal trends*

A large increase in the number of QTL studies was observed over time (Fig. 1A). Prior to 2010, the number of studies per year averaged around five, but this increased from 2010 onwards to a maximum of 31 in 2015. Before 2015, simple sequence repeats (SSRs) were the most commonly used marker type (Fig. 1A). A noticeable shift to single nucleotide polymorphisms (SNPs) started to occur in 2011, and by 2015, almost all studies used SNPs. We found that a total of 24 different software packages were used for QTL identification. The most common packages were GRIDQTL, MAPQTL and various R packages (Fig. 1B). In particular, GRIDQTL came into use from 2009 onwards, MAPQTL was used across almost the entire survey period and different R packages were primarily used from 2011 onwards. A

**Table 1** Results for Spearman correlation between eight study design factors and study outcomes including number of QTLs found (QTLs), average PVE of QTLs (PVE), width of QTL regions (Width), number of traits investigated (Traits), number of markers (Markers), size of mapping population (Sample), genome size based on C-values (Genome) and marker density (Markers/Genome). Correlations were calculated for all studies (below diagonal) and only salmonid studies (above diagonal). The number of studies used for each correlation is shown in brackets next to the correlation coefficient. Correlations in bold are those with correlation coefficients  $>0.3$  in both total and salmonid only data sets

	QTLs	PVE	Width	Traits	Markers	Sample	Genome	Marker density
QTLs	–	–0.41 (36)	0.45 (10)	0.43 (51)	0.16 (51)	0.05 (51)	0.04 (51)	0.15 (51)
PVE	<b>–0.41 (145)</b>	–	0.38 (36)	–0.27 (36)	0.37 (36)	–0.41 (36)	–0.33 (36)	0.41 (36)
Width	0.11 (145)	–0.19 (120)	–	–0.46 (10)	0.84 (10)	–0.29 (10)	–0.71 (10)	0.83 (10)
Traits	<b>0.63 (145)</b>	–0.31 (120)	0.00 (57)	–	–0.15 (51)	0.22 (51)	0.17 (51)	–0.18 (51)
Markers	0.10 (145)	0.13 (120)	–0.01 (57)	–0.07 (145)	–	–0.20 (51)	–0.23 (51)	0.99 (51)
Sample	0.29 (145)	<b>–0.48 (120)</b>	0.45 (57)	0.34 (145)	–0.06 (145)	–	0.17 (51)	–0.22 (51)
Genome	0.21 (145)	–0.06 (120)	0.25 (57)	–0.08 (145)	0.00 (145)	0.04 (145)	–	–0.33 (51)
Marker density	0.04 (145)	0.10 (120)	–0.08 (57)	0.00 (145)	<b>0.85 (145)</b>	–0.04 (145)	<b>–0.44 (145)</b>	–

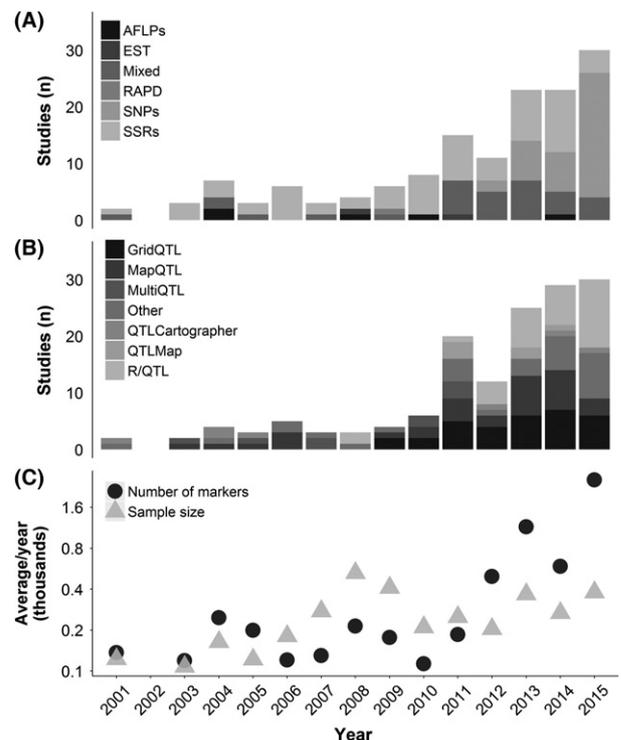
consequence of the shift to SNPs from 2011 and onwards (Fig. 1A) was a significant increase in the average number of markers used in each study. Prior to 2011, an average of 166 markers were reported in studies, but this increased to 1171 from 2011 onwards, and then to 2461 in 2015 (Fig. 1C). Unlike the rapid increase in marker density, the size of the mapping population increased more moderately. The mapping size increased from 122 in 2000 to 525 in 2008 and remained subsequently relatively stable from then on, with an average of 334 after 2008 (Fig. 1C). The average sample size of mapping populations across the survey period was 294.

Linkage studies were found to consistently outnumber association studies across all years (Fig. 2). This remained unchanged even after we included studies resulting from a second search that was specifically targeted at finding GWAS on teleost fish.

Minimal change over time was found when investigating the number of QTLs per trait, the PVE of identified QTLs or the width of QTL regions (Fig. 3).

#### Factors affecting QTL detection and fine-mapping

A Spearman correlation matrix was calculated for eight variables using the total data set and a subset of studies using only salmonid species, to reduce noise caused by the wide variety of species (Table 1). The correlation matrix indicated that the number of QTLs found per study and the number of traits measured were strongly positively correlated ( $r = 0.63$ ), suggesting that the studies with a higher number of phenotypic trait measurements found more QTLs compared with studies with fewer measurements. A moderate negative correlation ( $r = -0.48$ ) was observed between sample size and PVE of QTLs, indicating that with more samples the PVE of QTLs declined. Plotting the PVE of QTLs vs. sample



**Fig. 1** The distribution of genetic marker types (A), software used for QTL identification (B) and average number of markers and sample size (C) for studies over the review period. Average number of markers is a count of markers used for each study averaged for each year. Average sample size denotes the number of individuals in the mapping populations.

size with a linear regression and transformed axis indicated that the negative regression line was significantly different from zero (Fig. 4,  $P$ -value  $< 0.001$ ). The PVE of putative QTLs also declined as the number of QTLs increased ( $r = -0.41$ ). The strongest correlation for the width of QTL regions was sample size ( $r = 0.45$ ), which

suggested QTL width increased along with increasing sample size. However, this correlation was not supported in the salmonid only subset ( $r = -0.29$ ). The remaining correlations were mostly weak ( $<0.3$  in either the total data set or the salmonid subset).

**Discussion**

Insights into the genotype–phenotype map are of central interest in many fields of biology and also provide important information for applied research (e.g. Dekkers 2012; Lehner 2013; Savolainen *et al.* 2013). The ease with which polymorphic markers can now be discovered, even in nonmodel species, has led to significant advances in our ability to conduct QTL mapping studies. Contemporary studies now routinely use hundreds to thousands of markers. This new statistical power enables them to overcome the key limitation of previous work. Given the rise in our ability to search for QTLs in a true genomewide manner, it may be expected that both the detection and mapping precision of QTLs would have increased over time. Indeed, such an increase in mapping precision has been suggested in a previous study evaluating the future prospects of genotype–phenotype mapping efforts in the ‘genomics era’ (Mackay 2001). The aim of our review was to test these predictions and to assess the current state of QTL mapping in teleost fish by examining QTL studies from the last 15 years. We have concentrated on teleost QTLs because fish are the largest vertebrate group and they

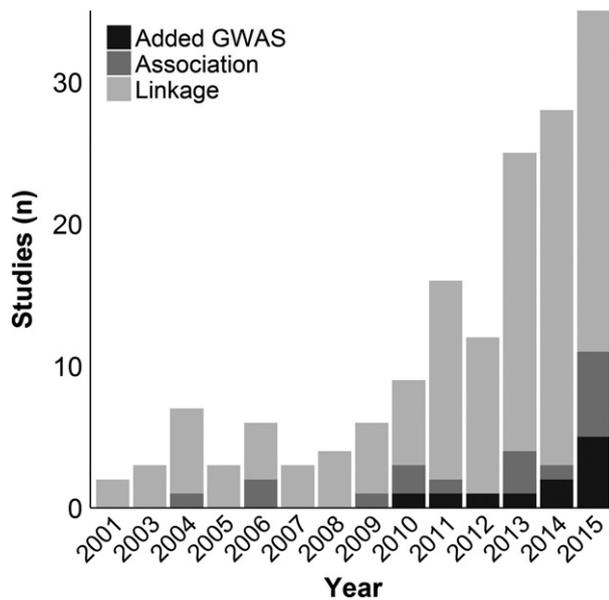


Fig. 2 Distribution over the review period of linkage and association studies. The results of an additional literature search for fish GWAS were also included.

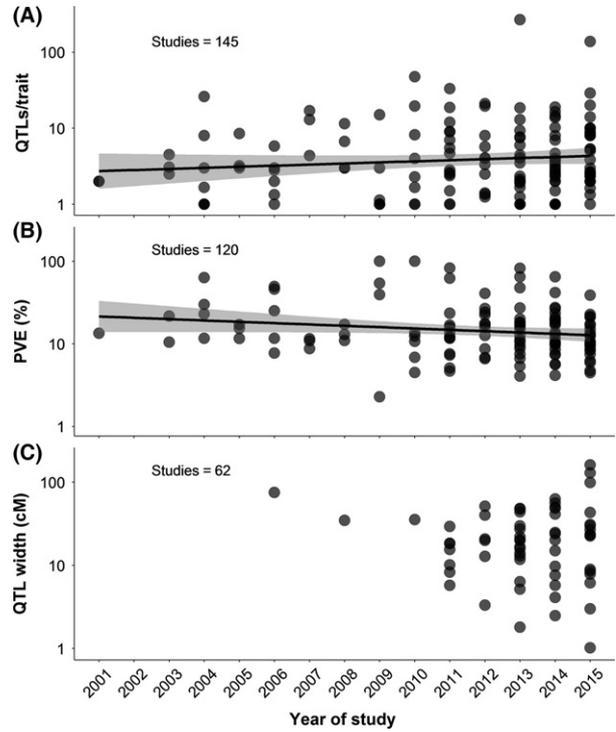


Fig. 3 Distribution of the number of QTLs found per trait (A), percentage variance explained (PVE) for identified QTLs (B) and QTL region width in centiMorgans (C) over the review period. Results are shown as average per study, with the number of studies in each plot also shown.

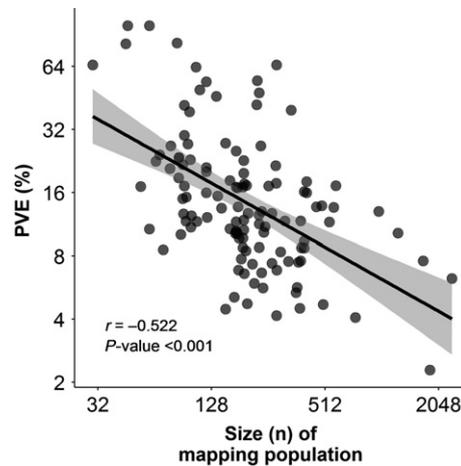


Fig. 4 A moderate negative correlation was observed between size (number of individuals) in the mapping population used by QTL studies and the average percentage variance explained (PVE) for QTLs in those studies. A regression line with 95% confidence intervals was placed using a generalized linear model. Pearson’s correlation coefficient ( $r$ ) with  $P$ -value for difference from 0 based on bootstrapping is also shown (see also Table 1 for Spearman correlation coefficient of this relationship).

are commonly subjected to QTL mapping studies and have a significant level of economic importance in a range of countries.

### Reviewed study designs

Our analysis revealed a steady increase in the number of QTL mapping studies over the past 15 years (Fig. 1A). Overall, the studies were diverse and included a wide range of target species ( $n = 48$ ), genetic markers (e.g. AFLPs, SNPs, SSRs) and study designs (e.g. two-stage linkage mapping, GWA). Interestingly, while a recent review by Ott *et al.* (2011) stated that linkage mapping has lost predominance in favour of association mapping, this trend was not obvious when evaluating fish studies (see Fig. 2). The absence of this trend is most likely the consequence of many studies being carried out on non-model species, which typically have fewer genetic markers and therefore favour linkage-based approaches. There was some variation in the types of mapping populations used by studies, although the majority of studies (60%) utilized a biparental cross and it was also common for studies (50%) to use interstrain or interspecies crosses (Fig. S2, Supporting information see Box 1 for some basic information about the advantages of different population designs). There has been an increase in marker density for fish studies over the last 15 years (Fig 1C), with a steep increase since 2011, the latter being primarily driven by the development of restriction site-associated DNA (RAD) sequencing approaches (e.g. genotyping by sequencing) coupled with decreasing sequencing costs (Elshire *et al.* 2011; Hilario *et al.* 2015). This increasing use of RAD sequencing is still working its way through the research community, with >50% of published articles in 2015 still not using the technology. One possible reason for this is the relatively high level of technical and bioinformatic skills that this method requires. However, given its singular advantage over other methods, it is expected that higher marker densities produced by RAD sequencing will become increasingly common for fish studies.

### Reviewed study results

In addition to reporting on the types of methods used by the reviewed studies, we attempted to evaluate the relative success of QTL studies. The ultimate goal of genotype–phenotype investigations, including QTL mapping, is to identify the genetic variants which collectively explain the heritable components of a given trait. However, a persistent problem is the inability to detect small-effect QTLs, which are implicated in the ‘missing heritability problem’ and ‘Beavis effect’ (Eichler *et al.* 2010; Slate 2013) and are thought to be

responsible for much, if not most, of the heritable variation (Rockman 2012). A wide array of potential factors contribute to this QTL detection weakness including a simple lack of statistical power (e.g. insufficient samples or markers) (Hong & Park 2012), choice of study design (e.g. population design), genetic architecture for a given trait, and potential epigenetic and uncontrolled environmental factors (Sham & Purcell 2014). A detailed quality assessment of the state of QTL mapping in teleosts was problematic because of the inherent variation in study designs, different genetic architectures among species and the other factors previously mentioned. However, the general expectation is that as the power of studies increases, a greater number of small-effect QTLs should become detectable. This in turn should improve the amount of explained heritability and the total number of QTLs detected (see Fig 2 by Visscher *et al.* 2012).

Using two basic measures (namely, number of QTLs found per trait and percentage variance explained), there appears to have only been minimal change to study results over the review period (see Fig 3). There may be a very small increase in number of QTLs detected (see Fig 3A) and a small reduction in percentage variance for identified QTLs (see Fig 3B), but these changes are not beyond contestation. It should be noted that all studies used a minimum QTL quality limit of 95% confidence with multiple sampling correction, which is important if the number of QTLs is used as a measure of QTL identification success. Higher sample sizes and marker densities are two of the most commonly discussed experimental design factors influencing QTL detection and mapping accuracy (Hu & Xu 2008; Massault *et al.* 2008; Hong & Park 2012). The results of our review suggest that there was only a weak relationship between these factors and the number of QTLs detected (sample size:  $r = 0.29$ , genetic markers:  $r = 0.10$ , Table 1). For sample size, the weakness of this correlation was most likely influenced by the relatively small range of sample sizes (min = 30, max = 3297) among the published studies. Visscher *et al.* (2012) plotted similar statistics for a number of complex traits from human studies and found strong correlations using a much larger range of sample sizes (min = 2000, max > 175K). Their finding suggests that a large increase in sample size (thousands of individuals) is needed to improve QTL mapping for teleosts. Larger sample sizes would also be beneficial as studies continue to use higher marker densities, which require more stringent multiple sampling corrections to reduce false positives. Another correlation of note was a moderate negative correlation between sample size and PVE (Fig 4, Table 1,  $r = -0.48$ ), which supports the idea that sample size in the current studies may be important for reducing the bias towards the detection of only large

effect alleles (see Box 1) (see also 'Beavis effect' discussion by Slate 2013). Although there was limited correlation between the number of genetic markers and QTLs found, higher marker densities are necessary for fine-mapping and GWA studies. Ultimately the highest level of genome coverage would be best, but this should always be balanced against the cost and the relative importance of other study design factors (e.g. sample size). Lastly, the width of QTL regions was also investigated as a potential measure of QTL mapping success, but the inconsistency of how this was reported made inferences from these data difficult (see next section). Overall, we observed limited improvement to the average width of QTL regions over time.

#### *Data reporting and software limitations*

The reviewed studies employed a wide diversity of methods in their search for QTL regions, particularly when reporting QTL regions for linkage studies. For

example, less than half of the studies (63 of 146) reported QTL regions in cMs (Fig 3C) and those that did record the width of the region employed a number of different methods. Methods used included the allele drop-off (Bras *et al.* 2011), bootstrapping (Kirschner *et al.* 2012), Bayesian credibility intervals (Sauvage *et al.* 2012), set LOD limit from the peak of the QTL (Gagnaire *et al.* 2013) or distance between markers above the significance level cut-off method (Jin *et al.* 2012). The finding that QTL regions are described using a wide array of methods is surprising, given that simulation studies have clearly indicated that some approaches are more accurate than others (Visscher *et al.* 1996; Manichaikul *et al.* 2006). In particular, simulations have shown that Bayesian credibility intervals are more accurate than bootstrapping, which itself is more accurate than the allele drop-off method (Visscher *et al.* 1996; Manichaikul *et al.* 2006). One possible reason for this wide diversity in reporting is variation among available software packages. For example, some, such as R/QTL,

#### **Box 1. Experimental design considerations**

Many factors are important when designing a QTL mapping study including, but not limited to, the number of genetic markers, type and size of the mapping population, type of statistics used and phenotyping and environmental factors.

With a significant increase in genotyping power over the recent years, due to lower cost and higher throughput sequencing, it may be timely to focus more attention on the limitations of other experimental factors. Reduced limitations on genotyping are likely to have an indirect effect of making larger sample sizes more achievable. As discussed by Hong & Park (2012), the sample size required for sufficient power in a QTL mapping study can be strongly influenced by the number of markers used, choice of linkage or association approaches, level of linkage disequilibrium and effect size of QTLs. Hong & Park (2012) indicated that testing a single marker required 248 cases (which would double to 496 cases when the controls are included) and testing 500 000 markers required 1206 cases (2412 cases when including controls) for 80% power when detecting weak-effect QTLs (5% disease prevalence, 5% minor allele frequency and complete linkage disequilibrium). Although these simulations were carried out for association methods, sample sizes for detection of weak-effect alleles with linkage methods should be at least as high as association tests with a single marker (496 based on Hong & Park (2012)). A sufficient sample size is important for being able to detect weak-effect alleles and avoid biasing our understanding of quantitative genetic variation by leading us to believe that most QTLs have large effects (Beavis effect) (Slate 2013).

Mapping population design is also an important consideration. The first decision is whether to use a natural population (unrelated group of individuals) or family groups. As discussed by Ott *et al.* (2011), one of the primary advantages of family designs is that they can be used to control for population stratification. However, a challenge presented by family-based designs is the level of resources required to construct and maintain them, which can be considerable. Investigations in natural populations have the advantage of providing insights into the fitness effects of traits under real-world circumstances (Slate 2005). A family-based design requires careful consideration for the type of pedigree and crosses used, because this influences the power for detecting QTLs and the complexity of the analytical approach. A biparental cross is the simplest design and compatible with most software packages and, indeed, is also the pedigree design that has been most often used in fish QTL studies (see Fig. S2). More sophisticated analyses are needed as the pedigrees become more complicated (e.g. mixed models, see Zhou & Stephens 2012). Some methods such as recombinant inbred lines or extreme phenotype selection can enhance QTL mapping power; however, if a diverse range of phenotypes (see Box 3) are of interest, these population designs, which are typically selected for a single trait, would be problematic (Risch & Zhang 1995; Rockman & Kruglyak 2008).

include Bayesian credibility interval functions, while others, such as GRIDQTL, do not. Additionally, software packages are often restricted to data that were obtained following a particular experimental design. For example, R/QTL requires single full-sib families, whereas GridQTL allows half-sib family designs. An ongoing focus on software development and implementation of best practices will be important as QTL studies continue to employ more complex methods and handle more complex genotyping, phenotyping, epigenetic and environmental data. Also, development of open-source software that can be updated, added to and has an active user/developer base would be of pronounced benefit.

### *Epigenetic markers*

While epigenetics was not investigated in any of the articles reviewed, the role that epigenetics plays in modulating gene expression and the genotype–phenotype process is increasingly being recognized as well as its potential application in selective breeding (Zhang & Hsieh 2013). Epigenetic mechanisms typically modify gene expression and these effects can vary depending on the genetic variation in and around regulatory elements. Locating epigenetic markers could help explain more precisely the role of genome modifications in producing a range of phenotypes. One good example of the power

of epigenetics in QTL mapping is Cortijo *et al.* (2014), which recently reported several methylated regions that acted as QTLs and accounted for 60–90% of the heritable variation for flowering time and primary root length in *Arabidopsis*. The important role that epigenetics can play in fish has been highlighted by Baerwald *et al.* (2016), who identified differential methylation patterns between rainbow trout individuals with different migratory behaviour. To facilitate the future use of epigenetics in research, high-throughput sequencing technology can be used to detect both DNA sequence variation and certain epigenetic markers and modifications (e.g. bisulphite sequencing), which will make information such as the level of DNA methylation and histone modification relatively straightforward to add to genomic-based QTL study (Li & Tollefsbol 2011).

### *Applications to selective breeding*

Selective breeding is one of the most important applications that can benefit from QTL mapping results. Traditional selection techniques use information from a known pedigree and known phenotypes to produce estimated breeding values (e.g. BLUP, see Henderson 1984). Moving from these traditional breeding approaches into programs applying marker-assisted selection (MAS) allows information from QTLs to be incorporated into

## **Box 2. Comparative genomics and data sharing**

Transferring knowledge (e.g. about QTLs, candidate genes and genetic markers) between studies and species provides a fruitful way for obtaining more widespread use of candidate genes and QTLs that have previously been identified. One of the more basic ways this might be done is by transferring information about candidate genes between species. One example of this was a study which compared growth hormone genes in a range of fish species while investigating a QTL in the promoter region which affected growth (Almuly *et al.* 2005). Another approach which can be used is meta-QTL analysis techniques, which can be carried out by collating the results from multiple studies which share a set of genetic markers (e.g. Lanaud *et al.* 2009). Two programs which have been developed to facilitate this are BioMercator (Arcade *et al.* 2004) and cMap (Fang *et al.* 2003). With the recent development of high-quality reference genome assemblies, a range of new comparative genomic techniques have become available (e.g. Sarropoulou *et al.* 2008; Sutherland *et al.* in press). Sarropoulou *et al.* (2008) describes a comparative genomics approach where genomewide EST markers from two species were aligned to the reference genome of a third species. This enabled orthologous linkage groups to be easily identified. To make this approach easier, the program MAPCOMP has been built to identify identical and proximal markers between linkage maps and use a reference genome of a related species as an intermediate. When a likely set of markers have been located, MAPCOMP allows transfer of QTLs between closely related species, even when their marker sets are quite different (Sutherland *et al.* in press). The application of comparative genomics in teleost fish is an important new development, because a wide diversity of nonmodel species are studied, which means that the overall research effort is spread much more thinly compared with land-based production species (e.g. cattle, corn and wheat). Therefore, open sharing of fish reference genomes and maps will be highly beneficial to researchers, because these will facilitate data integration and iterative approaches to QTL discovery. The growth of comparative-based research will be fuelled by more high-quality genome assemblies, the reconstruction of clear evolutionary and phylogenetic relationships and, perhaps most importantly, open and effective data sharing.

the selection process (see discussion by Rezende *et al.* 2012). Based on simulations, MAS has the potential to increase genetic gains by 8–38% over traditional methods (Meuwissen & Goddard 1996). Although the complete source of this gain was not clear, it was significantly increased if selection occurred before the trait was measured and if the trait had low heritability. A more recently developed method is genomic selection (GS) (Meuwissen *et al.* 2001), which simultaneously employs thousands of genetic markers that cover the entire genome (Goddard & Hayes 2007) and with this can improve the genetics-driven approach to selective breeding even further. One of the main features/advantages of GS (as noted by Desta & Ortiz 2014) is that it avoids the need for independent significance tests for each loci (used for QTL mapping and MAS) by accounting for all trait loci simultaneously while random effects from nontrait loci tend towards zero. As a result of this, GS can more effectively use high-density genomewide genotyping data sets than MAS and will likely become the gold standard of selective breeding in the near future. Currently, genomic selection

has been used extensively in animal breeding (see Samorè & Fontanesi 2016), to a minimal level in crop breeding (see Desta & Ortiz 2014), and little at all in teleosts. While epigenetics may add another important layer to QTL studies (see previous section), it has been suggested that epigenetics would only provide limited additional gains to existing GS methods (Goddard & Whitelaw 2014). The primary reason for this is that if epimutations are stable, then they will be in linkage disequilibrium with genetic markers, which means they will already be accounted for through existing GS methods (Goddard & Whitelaw 2014). Alternatively, if they are unstable, they will not be of much use for informing selective breeding decisions. As a final note, if genetic variation is being investigated for use in selective breeding, then a genomic selection approach is best, but if the interest is for more thorough characterization of a specific underlying variant, then a QTL mapping approach designed specifically to characterize causal QTNs might be best (e.g. Cohen-Zinder *et al.* 2005; Clop *et al.* 2006). For most studies in this review, the focus was on QTL

### Box 3. Improved phenotyping approaches

Whereas genotyping technologies have undergone a revolution in the last decades, advances in phenotyping have been slow. Indeed, despite awareness that the genotype–phenotype map is inaccessible without detailed phenotypic data our ability to characterize phenome lags greatly behind our ability to characterize genomes (see discussion by Houle *et al.* 2010). Improved high-dimensional phenotyping (many traits with known interactions) is important as it allows researchers to start to disentangle the interactions between phenotypes and between phenotypes and the environment, thereby reducing background variation and enhancing the power of QTL mapping studies (Benfey & Mitchell-Olds 2008; Houle *et al.* 2010). Mixed-model approaches looking at within and between trait variation are one example of the move towards a phenomics-based approach (Korte *et al.* 2012). Understanding the full range of phenotype and environmental interactions is also important for determining the biological relevance of genetic variants once they have been identified (Grigorenko 2005; Benfey & Mitchell-Olds 2008; Houle *et al.* 2010), for example understanding how those genetic variants produce a given phenotype under real-world circumstances and environments.

One of the main limitations to the use of more complex phenotyping is the lack of high-throughput phenotyping methods (Houle *et al.* 2010). Individual phenotypes typically require unique measurement processes, which make collection of the large amounts of data necessary for phenomics-based approaches difficult or even impossible. However, noteworthy progress is starting to be made towards high-throughput phenotyping in some fields, for example measuring wing traits in *Drosophila* through the construction of a wing machine (Houle *et al.* 2003). Likewise, in plants the program LEAFANALYSER was developed to simplify the measurement of leaf shape variation by placing a large number of evenly distributed landmarks along leaf margins and by recording the position of each automatically (Weight *et al.* 2008). In fish, some recent progress has also been made, with some procedures being developed that utilize image-based data input to automatically extract traits from still images or video footage (Silva *et al.* 2015; Viazzi *et al.* 2015; Navarro *et al.* 2016). However, these methods are often highly species-specific or require a specific technological set-up, as indicated by the absence of such methods in most if not all of the reviewed studies. Houle *et al.* (2010) noted three general areas that are critical for the development of phenomics, namely the development of technology (e.g. high-quality imaging), improved statistical and analytical capabilities and better methods for integrating study results (see also Box 2). We foresee that a lot of progress will come from methods that allow automated phenotyping technologies that are well integrated with databases, particularly from those technologies that can be applied to a suite of species and set-ups, rather than to a single scenario.

identification and the results would probably be best suited for use in MAS programs or further characterization with a second round of fine-mapping.

#### *Future outlook and conclusions*

The number of QTL mapping studies in fish has increased significantly over the review period. Most were carried out on nonmodel species and consequently many studies used fewer than 1000 markers and employed linkage-based mapping approaches. Accordingly, few of the studies were able to fine-map QTLs sufficiently to identify candidate genes or QTNs. However, the capability of studies has been increasing in recent times owing to the development of new approaches to variant discovery, such as RAD sequencing. Genotyping methods and the bioinformatics resources will continue to improve and these will be more widely adopted by the research community. Because the process of investigating genetic variation underlying traits is typically iterative, the studies conducted so far could be seen as representative of the first step towards identification of candidate genes and QTNs or as identifying QTLs suitable for informing MAS programs.

Future improvements to teleost genotype–phenotype investigations should involve a stronger move towards higher genome coverage of markers and a larger number of individuals in study groups (see Box 4). The ongoing construction of high-quality genome assemblies will help accelerate more widespread use of comparative genomics approaches, allowing knowledge about genes and QTLs to be transferred between species

informed by a phylogenetic framework. Fish studies would greatly benefit from these comparative genomics approaches, which could help make up for the lack of concentrated resources that has been a feature of land-based production species. Areas such as phenomics and epigenetics are currently not widely used and, however, are likely to take a more central role in future studies. An important aspect uniting these future areas is the ongoing need for better and more user-friendly data analysis software. This is particularly important for high-throughput genotyping, sequencing and phenotyping technologies because the volume of information produced can only be managed with automation and high-performance computing. For phenotyping, well-designed software can provide an effective method for reducing the burden of collecting large volumes of phenotypic data and thereby allow researchers to focus on the key biological questions that are of interest.

In conclusion, over the last 15 years there has been a large increase in the number of studies that managed to successfully identify QTLs in teleost fish. Many of these studies are underpowered compared with research that has been conducted on model species, but an ever-increasing level of genomic resources is also becoming available for many of the nonmodel species. Additionally, the development of high-quality genome assemblies offers many options for comparative genomics and promotes a data-sharing community of researchers. Future genotype–phenotype investigations have a very optimistic outlook with the rise of more sophisticated methods and affordable solutions that can be applied to fish.

#### **Box 4. Recommendations for future studies**

With genotyping limitations being largely overcome in recent years, other areas that are likely to be important for future studies carrying out genotype–phenotype mapping include:

- 1 **Increased sample size of the mapping populations** to help account for the ‘Beavis effect’, facilitate detection of weak-effect QTLs and provide power needed for GWAS and polygenic analyses.
- 2 **Polygenic analyses** to allow identification of the interaction among loci and better reflect the polygenic nature of variation underlying phenotypic variation.
- 3 **Advanced data analysis methods** (e.g. mixed models) to facilitate use of increasingly complex phenomic, epigenetic and environmental data in QTL investigations.
- 4 **Comparative genomics and data sharing** to facilitate more widespread use of known markers, QTLs and candidate genes in nonmodel species characterized by few genomic resources.
- 5 **Development of high-quality genome assemblies** to facilitate comparative genomic and meta-QTL approaches.
- 6 **Advanced phenotyping (phenomics)** to help disentangle the interactions between phenotypes, genotypes and the environment, thereby reducing background variation and increasing the power of QTL studies.
- 7 **Epigenetic approaches** to capture the interplay between environment and genes in affecting QTL expression.
- 8 **Improved software** to help researchers efficiently and accurately carry out a wide range of analyses while using best and most up-to-date methods. Advanced software and technology can also help with efficient phenotype data collection.

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## Supporting information

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

**Fig. S1.** Number of papers in each group from the initial Web of Science search after removal of duplicated studies.

**Fig. S2.** The proportion of studies using different numbers of parents and specific crossing strategies to produce mapping populations for QTL identification.

**Table S1** General info for 146 studies included in this review.