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Estimating genetic parameters for growth traits with molecular relatedness in turbot (*Scophthalmus maximus*, Linnaeus)

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ABSTRACT

Turbot (*Scophthalmus maximus*, Linnaeus, 1758) is an important aquaculture species in China, however, selective turbot breeding is restricted because there is no reliable pedigree data. The objective of this study was to evaluate the performance of a molecular relatedness method to estimate genetic parameters for growth traits in turbot. The experimental population consisted of 843 15-month-old turbot from 79 full-sib families produced via fertilization involving 50 sires and 34 dams. Twenty unlinked microsatellite loci in this population were genotyped to calculate their molecular relatedness. Both molecular relatedness and pedigree were used to construct an additive genetic matrix to apply to the same animal model and estimate genetic parameters and breeding value. Thereafter, we compared the accuracy of two estimators by cross validation. Heritability for body weight and length were 0.33 ± 0.15 and 0.24 ± 0.14, respectively, based on pedigree and both were 0.23 ± 0.04 based on molecular relatedness. Genetic correlation and phenotypic correlation were 0.96 ± 0.02 and 0.87 ± 0.01, respectively, based on molecular relatedness and 0.99 ± 0.02 and 0.89 ± 0.01, respectively, based on pedigree. Cross validation revealed that the accuracy of estimated breeding values was 0.85 with pedigree and 0.92 with molecular relatedness. These results suggest that molecular relatedness is a feasible approach to genetic parameter estimation when pedigree information is either inaccurate or absent.
Statement of relevance: The authors have declared that no commercial aquaculture exists.

1. Introduction

Turbot (*Scophthalmus maximus*, Linnaeus, 1758) is a marine fish distributed in the Atlantic coast of Europe, containing Baltic, Black, and Mediterranean seas (Blanquer et al., 1992; Lei and Liu, 1995). With fast growth, strong tolerance to cold water, and delicious flavor turbot is the most widely cultured commercial flatfish in the world. From its introduction into China in 1992, turbot aquaculture has developed into an important mariculture industry (especially in northern China) with an annual production of >60,000 t in 2010 (Lei et al., 2012). However, as an introduced fish species, turbot farming is restricted by less genetic diversity and lack of provenance (Shen et al., 2004). Moreover, systematic artificial breeding has not received sufficient attention in the early years (Guan et al., 2012). For these reasons, turbot germplasm resources have degenerated, resulting in a decline in the main economic traits resulting in longer growth period, lower seedling production, and disease susceptibility (Shen et al., 2004; Guan et al., 2012). To continue developing the turbot industry, improving its economic traits through selective breeding and genetic improvement is particularly important. Growth traits are important economic traits, because the commercial value of a turbot depends primarily on its body weight.

The animal model BLUP (Best Linear Unbiased Prediction) method, involving mixed model equations (Henderson, 1975), has been widely used in the estimation of genetic parameters in aquatic species (Blonk et al., 2010; Gall and Bakar, 2002; Shikano, 2007). To apply BLUP one must first know the relationship coefficients between individuals. Traditionally, geneticists and breeders have inferred this as pedigree relatedness from certain pedigrees in studies of either laboratory or domesticated populations (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Wright, 1922). If pedigree information is lacking but all of the parents are known, genetic marker data can be used for parental allocation to obtain pedigree information (Duchesne and Bernatchez, 2002; Marshall et al., 1998). Over the last decade, many new methods that use molecular markers to directly calculate paired molecular relatedness have been developed (Wang, 2007). The biggest advantage of these methods is that pedigree and parent information are not necessary (Wang, 2007).

The molecular maker-based relatedness estimators can be divided into two types: method-of-moment estimators (MOM) (Queller and Goodnight, 1989; Wang, 2002; Li et al., 1993; Lynch and Ritland,

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1999; Ritland, 1996) and maximum-likelihood estimators (ML) (Milligan, 2003; Wang, 2007). Most MOM methods obtain relatedness by calculating shared codominant marker alleles between pairs, while ML methods mainly calculate the probability of a pair falling into each of a number of different relationship categories and then either obtaining the weighted average or adopting the most likely category (Mousseau et al., 1998; Thomas and Hill, 2000). Numerous studies have assessed the performance of different molecular relatedness estimators. One of the conclusions drawn was that their performance rank order depends on many factors, such as population structure and size, number of markers, and alleles, no single factor was universally superior to the others (Milligan, 2003; Casteele et al., 2001; Wang, 2007). Compared with moment estimators, ML methods have the advantage of automatically weighing information among alleles and among loci and showing smaller mean squared errors (Wang, 2007), but they may also overestimate relatedness, especially for low related dyads (Milligan, 2003). Besides, ML methods yield more biased estimates than MOM, because the values obtained by likelihood estimators are restricted within the theoretical bounds [0–1], but if the number of markers used in the analysis is large enough they tend to close (Milligan, 2003).

In turbot breeding programs in China, several adverse factors, such as comparatively long generation time compared to other aquatic species and late beginning of systemic selection restrict the applicability of the pedigree method. Furthermore, as a non-native species, the lack of information on imported breeding parents has plagued breeders for a long time (Shen et al., 2004). Therefore, a new method to estimate genetic parameters is urgently needed. Some studies have used molecular relatedness to estimate genetic parameters, but whether or not these methods can provide trustworthy results remains questionable. In this paper, we test the applicability of molecular relatedness in estimating adult turbot genetic parameters, to provide an alternative to, and perhaps better method than, pedigree. At first, paired molecular relatedness was calculated using 20 microsatellite markers. Both molecular relatedness and pedigree were then used to construct an additive genetic matrix to estimate heritability and breeding values of two growth traits, i.e., body weight (BW) and body length (BL), in a cultured adult turbot population. Finally, we compared the accuracy of both estimators by cross validation.

2. Materials and methods

2.1. Experimental materials

The experimental turbot population were bred at the Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences in 2013. The study population, which consisted of 79 full-sib families of G1, was produced via artificial fertilization involving 50 sires and 34 dams. Their parental generation (G0) was from eight introduced population, of which four were introduced from France, and of which the other four were introduced from Spain, Denmark, Britain and Chile respectively. Because some parent individuals were mated with more than one partner, there were also 17 maternal half-sib families and 17 paternal half-sib families in G1. Each full-sib family was raised in a fiberglass-reinforced plastic tank. At 35 and 70 days post hatching, 1000 larvae and 400 juvenile fish per family were randomly selected and reared in a new fiberglass-reinforced plastic tank. The rearing environments in the family tanks remained as similar as possible during this process. At 3 months post hatching, 40 individuals with the largest body weight from each family were selected and marked with a visible implant fluorescent elastomer tag, they were then distributed into three 5 × 5 × 0.6 m (L × W × H) test tanks randomly. At 15 months old, twenty individuals from each family were stored as parent fish and individuals without clear tag were discarded. The remainder of population composed experimental population and body weight and length were measured, sex was determined, and a piece of fin ray was taken for DNA extraction.

We selected 20 microsatellites with good polymorphism to genotype the experimental population. Seventeen of them are unlinked mutually and linkage groups of the other 3 are unknown (Ruan et al., 2010). The forward primer for each primer pair was labeled with one of four fluorescent dyes (Sangon Biotech, Shanghai): 6-FAM, TAMRA, ROX, and HEX. More details of genotyping microsatellite are shown in Table 1 (Ruan et al., 2010). Genomic DNA was extracted from fin ray in all individuals using standard phenol-chloroform procedures (Sambrook et al., 1989). PCR amplification was carried out according to Ruan et al. (2010). The PCR products were separated by an ABI 3130 automatic Genetic Analyzer (Applied Biosystems). Alleles from the microsatellite loci were sized with a GeneScanTM-500 LIZ Size Standard (Applied Biosystems) and scored using GeneMapper™ V4.1 (Applied Biosystems).

2.2. Molecular relatedness

In this study, we used the method described by Wang (2007) to calculate pairwise molecular relatedness using marker genotype data. Homologous genes are identical by descent (IBD) if they were copies descended from one gene of an ancestor. Coancestry ($\boldsymbol{\theta}$) is defined as the probability that any two alleles, sampled at random (one from each individual), are IBD genes (Malécot, 1948). Among the two alleles from individuals X and two alleles from individuals Y, there are exist 15 mutually exclusive and exhaustive IBD states (Jacquard, 1972). When paternal and maternal alleles are not distinguished, the 15 IBD states reduce to 9 condensed identity states (Harris, 1964; Jacquard, 1972; Lynch and Walsh, 1998), as shown in Table 2.

Because any two diploid individuals must fall into one of these nine states, we can get $\sum_{i=1}^{9} \boldsymbol{\Delta i} = 1$. The probability of the nine IBD states can be inferred from the genotypes of X and Y at a number of marker loci. This method was illustrated by Milligan (2003). Given the nine IBD coefficients, we can easily infer coancestry between individuals X and Y (Jacquard, 1972; Lynch and Walsh, 1998; Milligan, 2003):

$$\boldsymbol{\theta_{XY}} = \boldsymbol{\Delta}_1 + \frac{1}{2}(\boldsymbol{\Delta}_3 + \boldsymbol{\Delta}_5 + \boldsymbol{\Delta}_7) + \frac{1}{4}\boldsymbol{\Delta}_8.$$

Relatedness is calculated from coancestry as follows:

$$\boldsymbol{r_{XY}} = 2\,\boldsymbol{\theta_{XY}}.$$

The computational process of r_{XY} from microsatellite genotype data was executed in Coancestry software (Wang, 2011). Pearson correlations were calculated between molecular relatedness and pedigree relatedness using R (R Core Team, 2013).

2.3. Genetic analysis

To estimate the genetic parameters and breeding values, the restricted maximum-likelihood method was carried out in ASReml software (Gilmour et al., 2009) to analyze the following bivariate animal model:

$$\boldsymbol{y_{ijk}} = \boldsymbol{\mu} + \boldsymbol{cb_i} + \boldsymbol{t_i} + \boldsymbol{a_j} + \boldsymbol{d_k} + \boldsymbol{e_{ijk}},$$

where, $\boldsymbol{y_{ijk}}$ is the phenotypic observation for either BW or BL, $\boldsymbol{\mu}$ is the overall mean, $\boldsymbol{c}$ is the regression coefficient for the covariate for age of ith offspring $\boldsymbol{b_i}$, $\boldsymbol{t_i}$ is the fixed effect of test tank $\boldsymbol{i}$, the value of $\boldsymbol{i}$ ranges from 1 to 3, $\boldsymbol{a_j}$ is the random additive genetic effect of animal $\boldsymbol{j}$, $\boldsymbol{d_k}$ is the random effects of maternal and common environment to full-sib $\boldsymbol{k}$, and e is the random error term. The variance analysis revealed no significant fixed effects of sex, this factor was not analyzed further.

Table 1
Primer sequence, linkage group in female and male and fluorescent dyes of genotyping microsatellites. The asterisk (*) indicates linkage group is unknown (Ruan et al., 2010).

Locus	Primer sequence (5′-3′)	Linkage group in female	Linkage group in male	Fluorescent dyes
YSKr272	F:TGTGAGGGGAGACAAGATG R:CCACCTGTGAAATAGCG	F1	M1	ROX
YSKr263	F:ATCCTGGGTCTGGTGAAGT R:ACGACAGGTGATGACGGTG	*	*	6-FAM
YSKr80	F:TGAAGTGTGATGTCTCGGAT R:GAGGAATAAGGAGTGCTGTC	F5	M4	HEX
YSKr218	F:GTTTCGTGGAATCCGACCTC R:TCCTGCGTCCTCTCTACACTCT	F4	M5	TAMRA
YSKr96	F:TGGAATAGGCTACAAGGCT R:GGAGGAGGTGTCAGTCAGAT	F13	M13	ROX
YSKr277	F:ATGAGGCAGAAGAATGGAT R:ACAGAAACACGGGCACAG	F12	M11	6-FAM
YSKr17	F:GTGGGAATGAATCGGACAGG R:AACGCCTCCCCCTCATCTCT	F8	M18	HEX
YSKr119	F:GCTCTTCCAAGTGCCA R:TGTAGTGTACCAAATGC	F24	M17	TAMRA
YSKr231	F:TCACCTCCTGGTTTCCT R:TTGCTTTAGTGATGGACAG	F26	M3	ROX
YSKr54	F:GAACGAAGGGATGTAAGG R:ACTCAGCAACAGGGTCAC	F22	M15	6-FAM
YSKr260	F:ACAAGTCATCTGCTTCGTCAT R:ACCAACAGCCTCCAGACCAG	*	*	HEX
YSKr103	F:CTGTTGTCCCAAATCTGT R:CATTTCCTTGATAGTGC	F11	M20	TAMRA
YSKr197	F:AATAGAAGGGGAGAAAAGACC R:CCAATCAGATGCGAGAAAGT	F27	M10	ROX
YSKr94	F:CGCCGCACATTCATTCAC R:GGCTTGGCTGTCTCATAGTC	F10	M16	6-FAM
YSKr115	F:ACTGAGGGGAATGTTAG R:CTGAGTGGCATTAAGTCT	F6	M9	HEX
YSKr221	F:AGTGAGCACCAACACAAAGC R:ATCCCTCTCCACCCGTAG	F16	M8	TAMRA
YSKr124	F:CAGCCGTTCTGACCTCGTAG R:ACCCTCCACTGCTTGTCCTTG	F9	M19	ROX
YSKr204	F:CCTCTTCCGCTGCTGTCA R:CCCTGTCTCACCCCAAAC	F14	M21	6-FAM
YSKr245	F:ATGAAGCGTCACCAGAACT R:AGAAATCCTGCTGTCACTCG	*	*	HEX
YSKr108	F:TCTAAACTGGCTGTGATG R:TGTAACTTCTCCGATGTC	F3	M6	TAMRA

The distribution of the random effects $\boldsymbol{a_j}$, $\boldsymbol{d_k}$, and $\boldsymbol{e_{ijk}}$ were assumed to be normal, with means of zero. The variance-covariance matrix is:

$$V\begin{bmatrix} a \\ d \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I_d\sigma_d^2 & 0 \\ 0 & 0 & I_e\sigma_e^2 \end{bmatrix},$$

where, σ_a^2, σ_d^2, and σ_e^2 are the variance of the random effects *a*, *d*, and *e*, respectively. *A* is the n*n matrix of additive genetic relationship, and I_d and I_e are n*1 identity matrices. n is the number of individual.

In this study, both pedigree and molecular relatedness were used to construct an additive genetic relationship matrix. The relationship matrix based on molecular relatedness was constructed using three functions vec2sm, flipud and flrplr from two packages corpcor and pracma in R software (R Core Team, 2013). To obtain convergence in ASReml, the relationship matrix was made positive definite using the nearPD function in R matrix package. Last, it was transferred to ASReml as a "grm-file". File conversion was performed using write.relationshipMatrix function in R synbreed package.

Heritability was calculated using additive genetic variance, common environmental variance, and residual variance as:

$$\boldsymbol{h^2} = \boldsymbol{\sigma_a^2}/\left(\boldsymbol{\sigma_a^2} + \boldsymbol{\sigma_d^2} + \boldsymbol{\sigma_e^2}\right).$$

Genetic correlation was calculated as follows:

$$\gamma_{\boldsymbol{g}} = \boldsymbol{C_g}/\sqrt{\boldsymbol{\sigma_{a(BW)}^2} * \boldsymbol{\sigma_{a(BL)}^2}},$$

where, C_g is the genetic covariance, $\sigma_a{}^2{}_{(BW)}$ is the additive genetic variance of trait BW, and $\sigma_a{}^2{}_{(BL)}$ is the additive genetic variance of trait BL.

Phenotypic correlation was calculated as follows:

$$\gamma_{\boldsymbol{p}} = \boldsymbol{C_p}/\sqrt{\boldsymbol{\sigma_{p(BW)}^2} * \boldsymbol{\sigma_{p(BL)}^2}},$$

where, C_p is the phenotypic covariance, $\sigma_p{}^2{}_{(BW)}$ is the phenotypic variance of trait BW, and $\sigma_p{}^2{}_{(BL)}$ is the phenotypic variance of trait BL.

2.4. Cross validation

Ten-fold cross validation aimed at BW was used to compare the accuracy of both methods. For each ten-fold cross validation, the data set was randomly divided into ten equal sized subsets. The observed BW trait phenotypes of one subset were omitted and predicted by genetic model analysis using data from the other nine subsets. Pearson correlations between observed and predicted phenotypes of the omitted subset were calculated. Cross validations were repeated 5000 times. EBV accuracy was calculated as follows:

$$r_{a,\hat{a}} = r_{p,\hat{p}}/\sqrt{h^2},$$

where, $r(p,\hat{p})$ is the average Pearson correlation coefficient between observed and predicted phenotypes, and h^2 is the heritability estimated based on pedigree. Accuracy was divided by $\sqrt{h^2}$ based on pedigree to adjust for the upper limit of accuracy of a phenotype (Daetwyler et al., 2012). All of the cross validations were performed in R.

Table 2
Dyadic Identity By Descent (IBD) states and their probabilities. Homologous alleles a and b are from individual X, and c and d are from Y. Alleles that not specified are not IBD with any of those listed in column 2. Alleles that are IBD are in a set of parentheses. Multiple sets of parentheses for a given IBD state represent multiple equivalent identity configurations for one state (Wang, 2007).

IBD state	Genes IBD	Coancestry (θ)	Probability (S_i)
S_1	(abcd)	1	Δ_1
S_2	(ab,cd)	0	Δ_2
S_3	(abc),(abd)	0.5	Δ_3
S_4	(ab)	0	Δ_4
S_5	(acd),(bcd)	0.5	Δ_5
S_6	(cd)	0	Δ_6
S_7	(ac,bd),(ad,bc)	0.5	Δ_7
S_8	(ac),(ad),(bc),(bd)	0.25	Δ_8
S_9	None	0	Δ_9

3. Results

3.1. Molecular relatedness

At last, the number of individual extracted high quality genome DNA was 843. Distribution of the size of 79 families is shown in a Fig. 1. The number alleles in the 20 microsatellite loci ranged from 5 to 28, and the mean was 12.4. The molecular relatedness values were continuous and ranged from 0 to approximately 1 (0.9642). Average molecular relatedness was 0.0414 (σ_{MR} = 0.0813).

There was only one generation pedigree information available, so all parents were assumed to be unrelated (Falconer and Mackay, 1996). Therefore, the offspring pedigree relatedness values were 0 for unrelated, 0.25 for half-sib, and 0.5 for full-sib. Average pedigree relatedness was 0.0198 (σ_{PR} = 0.0808).

Pearson correlation coefficient between two sets of relatedness was 0.55. The average molecular relatedness (mean) and standard deviation (mean) for each pedigree relatedness class are shown in Table 3. For the unrelated pedigree relatedness class, the average molecular relatedness was slightly higher than 0. For half-sib and full-sib class, the average molecular relatedness was both less than pedigree relatedness. The standard deviation of molecular relatedness per class was relatively large.

3.2. Genetic analysis

The variance components, heritability, genetic correlation, and phenotypic correlation of two traits based on two types of relatedness are listed in Table 4. The additive genetic variance and heritability of BW estimated from pedigree (σ_A^2 = 1631.22, h^2 = 0.33 ± 0.15) were both higher than from molecular relatedness (σ_A^2 = 1041.67, h^2 = 0.23 ± 0.04). The variance components of common environmental effects and random error estimated by both methods were quite similar. The genetic analysis results of BL were similar to BW, but the difference in the heritability estimates from both methods was small. Genetic and phenotypic correlations were also calculated by both methods. All of them were very high ($\gamma g = 0.99 \pm 0.02$, $\gamma p = 0.89 \pm 0.01$ based on pedigree and $\gamma_g = 0.96 \pm 0.02$, $\gamma_p = 0.87 \pm 0.01$ based on molecular relatedness). Estimated breeding values (EBVs) of two traits from pedigree were both positively correlated with EBVs obtained from molecular relatedness (Fig. 2). The Pearson correlation coefficients were 0.75 for BW and 0.72 for BL.

Table 3
Mean and standard deviation (SD) of molecular relatedness (MR) between pairs per class of pedigree relatedness (PR).

PR class	MR	
	Mean	SD
Unrelated 0	0.0340	0.0656
Half-sib 0.25	0.1437	0.1255
Full-sib 0.5	0.3147	0.2185

3.3. Cross validation

Distribution of Pearson correlation coefficients between observed and predicted phenotypes in 5000 cross validations are shown in Fig. 3. The average Pearson correlation coefficient for pedigree was 0.58, and the accuracy of EBV was 0.85. For molecular relatedness, they were 0.63 and 0.92, respectively. The *t*-test suggested that the accuracy of both methods differed significantly ($P < 0.01$). For predictive ability and accuracy of breeding values, molecular relatedness based on 20 microsatellites markers performed better than pedigree. Because the phenotype of any one individual was predicted repeatedly after 5000 cross validations, average predicted phenotype was also calculated. Relationships between average predicted and observed BW phenotypes in both methods are shown in Fig. 4. Because the experimental population consisted of many full-sib families, the points in the pedigree method figure are stratified, and the points in the molecular relatedness method figure are a little more concentrated.

4. Discussion

4.1. Molecular relatedness

To apply BLUP to turbot breeding, investigating the additive genetic relationship between individuals of a breeding population is an important foundation (Henderson, 1975). In this study, we tested the

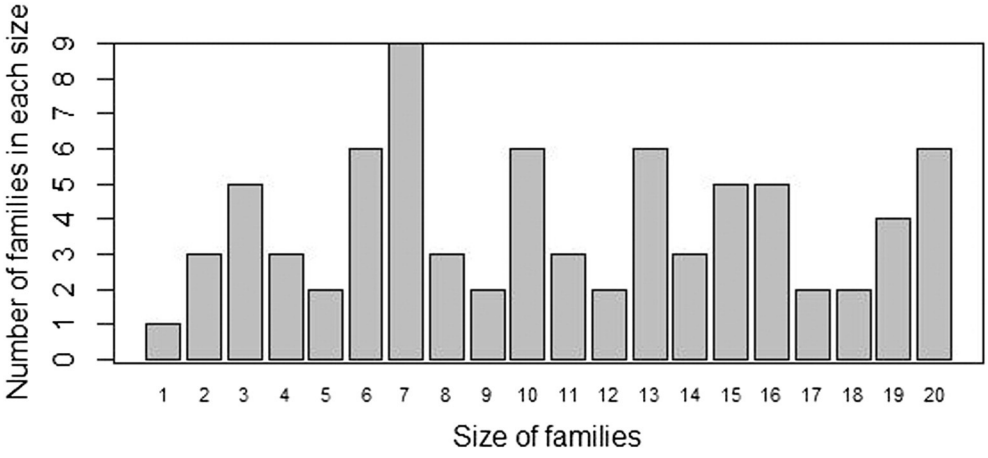

Fig. 1. Distribution of the size of 79 families.

Table 4
Variance components, heritability, genetic correlation, and phenotypic correlation of BW and BL based on pedigree relatedness (PR) and molecular relatedness (MR).

	BW				BL				$\gamma_g \pm$ se	$\gamma_p \pm$ se
	σ_A^2	σ_D^2	σ_e^2	$h^2 \pm$ se	σ_A^2	σ_D^2	σ_e^2	$h^2 \pm$ se		
PR	1631.22	880.73	2396.09	0.33 ± 0.15	0.70	0.48	1.70	0.24 ± 0.14	0.99 ± 0.02	0.89 ± 0.01
MR	1041.67	984.20	2449.85	0.23 ± 0.04	0.62	0.48	1.58	0.23 ± 0.04	0.96 ± 0.02	0.87 ± 0.01

suitability of molecular relatedness to estimate genetic parameters as a solution to not having reliable, accurate pedigree information. A population of 843 individuals were genotyped with 20 microsatellite markers. The estimator proposed by Wang (2007) was adopted to calculate molecular relatedness, because this is the most accurate method, having either the lowest root mean error or close to smallest one in different population structures and sizes, number of loci, and alleles (Wang, 2007).

Several studies have reported that the performance of molecular relatedness as an estimator was mainly effected by the number of loci and frequencies of alleles per locus. (Wang, 2002; Wang, 2007; Oliehoek et al., 2006). Oliehoek et al. (2006) reported that estimators that exhibit a correlation between molecular and pedigree relatedness continue to increase when going from 10 to 100 loci and proved that reasonable correlations (>0.7) are found when the number of loci reaches 50 markers in simulated experiment where each marker had four or five alleles. Nevertheless, the cost of marker genotyping must also be taken into account when increasing the number of markers. Blonk et al. (2010) obtained a correlation of 0.8 between molecular relatedness and relatedness from reconstructed pedigree using ten markers with 13.7 alleles per marker on average in *Solea solea*. Larger variation in relationship structures and highly skewed contribution of families to aquaculture populations were thought to be the reason for their success with relatively few markers (Blonk et al., 2010; Rodríguez-Ramilo et al., 2007; Vandeputte et al., 2004). In this study, a Pearson correlation coefficient of 0.55 was obtained using 20 unlinked markers with 12.4 alleles per marker on average; this moderate correlation is far less than the value obtained by Blonk et al. (2010). There are many potential reasons. Unlike simulated data, pedigree data are not perfect and may contain a certain error rate in real data. Similarly, markers can also suffer from a high genotyping error rate (Pompanon et al., 2005). However, marker errors have negligible effects if they occur randomly across individuals and loci (Wang, 2007). Besides, because of the lack of parental information, there are one generation pedigree and three pedigree relatedness classes, which may result in a low correlation. The average molecular relatedness was both less than pedigree relatedness for half-sib and full-sib class. Guan et al. (2016) using the same method to calculate molecular relatedness and found for five pedigree classes in seven, the average molecular relatedness was less than pedigree relatedness, but the gap is smaller. This resulted from nonspecific amplification in PCR and error in pedigree possibly. Besides, Wang (2007) reported that relatedness is underestimated when inbreeding is present but ignored by an estimate. Because we had no actual relatedness as a reference, it is not clear which estimate is more accurate. However, in this study, we tended to evaluate their performance in genetic analysis which provides better estimates of actual relationship.

4.2. Genetic analysis

The animal model containing common environmental effects used in this study proved to be an optimal model to estimate turbot genetic parameters (Guan et al., 2016; Ma et al., 2009). The fixed effect of sex was nonsignificant. This was in agreement with previous research. Although female turbot had higher body weight than males, the difference was not significant in the early stage (Wang et al., 2014). The day-age was the same in all individuals, therefore, the day-age covariant was omitted from the model.

Heritability estimates for BW and BL obtained from both methods were all medium in 15 month-old turbot, based on the following categorization: low (0.05–0.15), medium (0.20–0.40), high (0.45–0.60), and very high (>0.65) (Cardellino and Rovira, 1987). A limited number of studies have reported on the estimation of growth trait heritability in turbot. Ma et al. (2008, 2009) estimated heritability for growth traits in turbot, they reported 0.450–0.514 for BW and 0.251–0.425 for BL in 6-month-old fish, and 0.34 for BW in 15-month-old fish. Both Liu et al. (2011) and Guan et al. (2016) estimated heritability for BW in 100 juvenile turbot and obtained 0.22 and 0.19, respectively. The results from both methods in our study were close to those reported in earlier studies. What calls for attention is that selecting 40 largest body weigh individuals from each family at 3 months post hatching induced a slight bias to the estimation of heritability potentially, because it might increase resemblance of the family members. Heritability and standard error based on molecular relatedness were slightly lower than pedigree, because molecular relatedness estimated a smaller genetic variance. Several

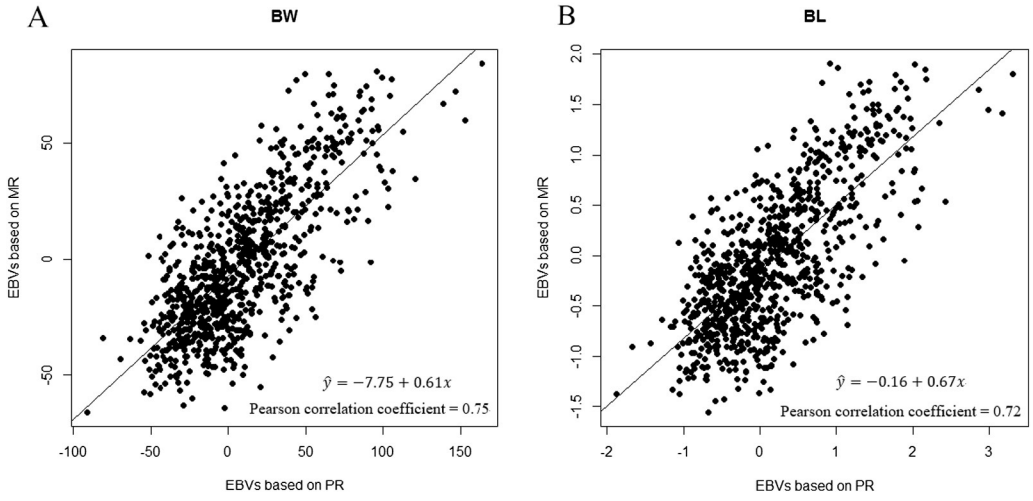

Fig. 2. Relationship between EBVs from molecular relatedness and pedigree. Panel A is the relationship between two sets of EBVs for BW, and panel B is BL.

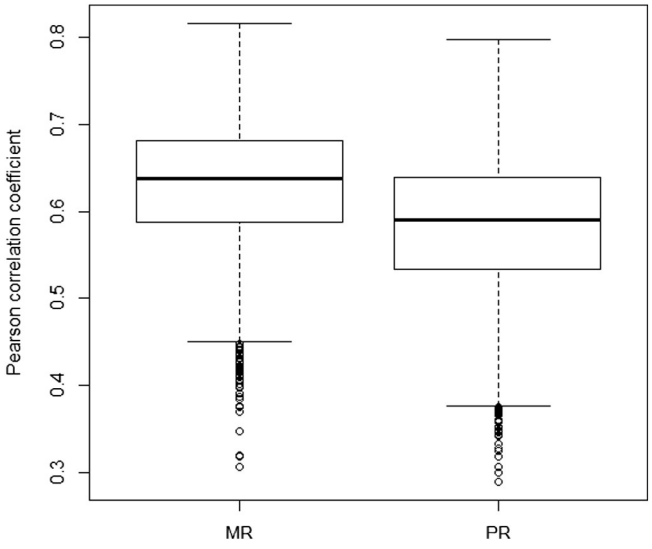

Fig. 3. Distribution of Pearson correlation coefficients between observed and predicted phenotypes in 5000 cross validations.

previous studies have also reported that the molecular relatedness method results in a decrease in genetic variance estimation, standard error, and heritability (Korecký et al., 2013; Van and Ritland, 2005). Moreover, Shikano (2007) showed that heritability estimates are more accurate when using a higher number of markers and approach values estimated by pedigree reconstruction. A similar conclusion has been drawn by other researchers (Bink et al., 2008; Hayes and Goddard, 2008; Lynch and Ritland, 1999). In this study, more marker loci and higher average relatedness in the population may explain the smaller gap in heritability between the two methods compared with those reported in previous studies.

In previous study, the genetic and phenotypic correlations estimated between BL and BW were 0.88 and 0.87, respectively, in 25-day-old juvenile turbot (Zhang et al., 2008), and 0.924 and 0.907, respectively, in 6-month-old turbot (Ma et al., 2008). The results from both methods in this study were consistent with the results above. The high genetic and phenotypic correlations between BW and BL indicated that selection for one trait will result in another positively correlated response. Compared with BL, BW fluctuated and was influenced by time and feeding status. Thus, selection in turbot breeding programs should be aimed at BL rather that BW.

4.3. Application of molecular relatedness in turbot breeding

In this paper, we estimated similar genetic parameters using molecular relatedness and pedigree. However, to successfully apply molecular relatedness, accurate EBV prediction is a key issue to consider (Goddard and Hayes, 2009). In this study, both in terms of predictive ability and breeding value accuracy, the performance of molecular relatedness is better compared with that of pedigree using 20 microsatellite markers. Therefore, using molecular relatedness from a few microsatellites to construct an additive genetic relationship matrix applied in mixed model equations is a feasible approach, especially in the absence of ideal pedigree information as we encountered in turbot breeding. Similar to microsatellite, single nucleotide polymorphism (SNP) is another available molecular marker to estimate relatedness (Ritland, 1996; Lynch and Ritland, 1999). The number of SNP is much larger than microsatellite and estimated at many millions (Halushka et al., 1999). Using whole-genome high-density SNPs to construct an additive genetic matrix to estimate genetic parameter and EBVs, termed as GBLUP, is one approach of genomic selection (Vanraden, 2008; Hayes et al., 2009). Odegård et al. (2014) applied GBLUP to Atlantic salmon (*Salmo salar*) breeding and found it outperformed the classical pedigree-based method. However, just as the other genomic selection approaches, this method depends on the genome information and DNA chip technology. The high cost restricts its wide usage in aquaculture species. When a small mount molecular markers were used to estimate relatedness, 10–20 microsatellites are roughly equivalent to 100–200 SNPs in terms of marker information content (Wang, 2015). Using microsatellite is likely to be a cheap and convenient method to estimate relatedness, although accuracy based on this method is lower than GBLUP.

It is unlikely that molecular markers will completely replace pedigree in breeding programs outright. A very significant reason is that more useful information can be obtained from pedigree. In genetic analysis, estimating variance of maternal and common environmental effects on full-sib families and the covariate day-age rely on details supplied by pedigree. In this paper, we acquired the day-age of individuals with the aid of pedigree information, while maternal and common environmental effects appear to be an important source of genetic variation. Because of the family structure in this study, we did not separate environmental from maternal effects. However, some studies have shown that maternal effects are high during early growth but have no obvious effect on adult fish (Liu et al., 2011; Furutsuka-Uozumi and Tabata, 1999; Shimada et al., 2007); we speculate that maternal and common environmental effects occurred during the separate rearing of full-sib families before tagging. To confirm this assumption further

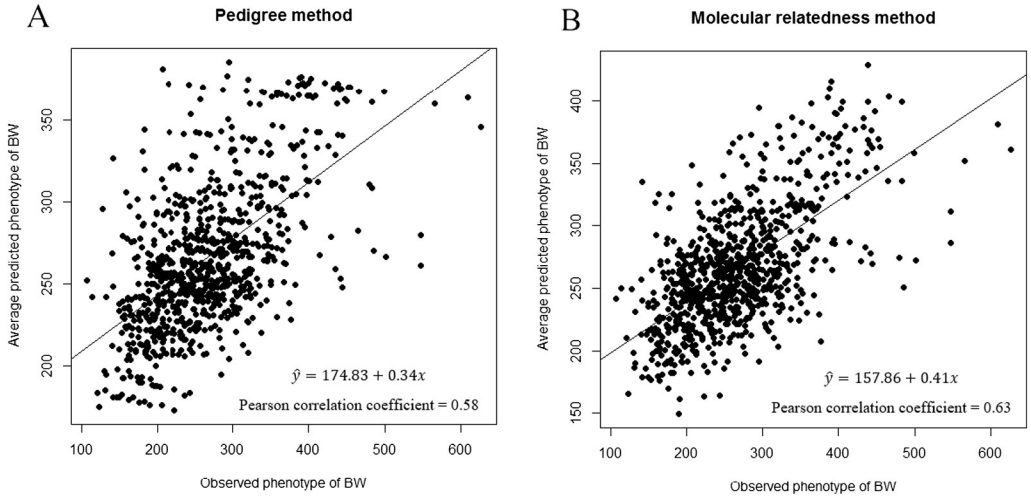

Fig. 4. Relationship between average predicted and observed BW phenotypes. The predicted phenotypes in panel A are based on the molecular relatedness method and the predicted phenotypes in panel B are based on the pedigree method.

investigation in turbot is necessary. In annual reproductive process of turbot, the day age gap between different families can be up to half a month to a month. The question of how to reduce or avoid the influence of the covariate of age in genetic analysis in the absence of a visible tag should be investigated in the future.

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