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Estimating genetic parameters with molecular relatedness and pedigree reconstruction for growth traits in early mixed breeding of juvenile turbot

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Abstract

An introduced turbot population was used to establish families and to estimate genetic parameters of the offspring. However, there is a lack of pedigree information, and common environmental effects can be introduced when each full-sib family is raised in a single tank. Therefore, in the genetic evaluation, SSRs (simple sequence repeats) were used to reconstruct the pedigree and to calculate molecular relatedness between individuals, and the early mixed-family culture model was used to remove the impact of the common environmental effects. After 100 d of early mixed culture, twenty SSRs were used to cluster 20 families and to calculate paired molecular relationships (n=880). Additive genetic matrices were constructed using molecular relatedness (MR) and pedigree reconstruction (PR) and were then applied to the same animal model to estimate genetic parameters. Based on PR, the heritabilities for body weight and body length were 0.214±0.124 and 0.117±0.141, and based on MR they were 0.101±0.031 and 0.102±0.034, respectively. Cross validation showed that the accuracies of the estimated breeding values based on MR (body weight and body length of 0.717±0.045 and 0.629±0.141, respectively) were higher than those of PR (body weight and body length of 0.692±0.052 and 0.615±0.060, respectively). The MR method ensure availability of all genotyped selection candidates, thereby improving the accuracy of the breeding value estimation.

Key words: turbot, SSR, genetic parameter, mixed breeding

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1 Introduction

Turbot (*Scophthalmus maximus*, Linnaeus, 1758) is one of the most economically important seawater fish species in northern China. This species has the advantages of rapid growth, strong adaptability, and delicious flavor. The turbot is a marine fish native to Europe (Blanquer et al., 1992; Lei and Liu, 1995) and was introduced to China from Britain in 1992 (Lei and Liu, 1995). In 1999, technology for raising productive seedlings was developed, and the "greenhouse+deep well seawater" production model was created (Men, 2002; Lei et al., 2002). This led to the rapid development of the turbot breeding industry along the northern coast of China. In 2005 and 2009, the production of turbot in China exceeded 50 000 t and 68 890 t, respectively (Lei, 2006; Wang, 2010). As an introduced fish species, the parent population of turbot has fewer sources, and their relationships are not clear. Owing to the only lay focused on production and a neglect of breeding scheme, the inbreeding rate has increased and genetic diversity has decreased after culturing for many generations (Shen et al., 2005; Lei, 2002). For the sustainable development of the turbot industry, it is particularly important to improve the economic traits of turbot (Lyu et al., 2017).

The evaluation of breeding value based on the best linear unbiased prediction (BLUP) genetic evaluation system combined with inbreeding control has led to significant progress in aquatic animal genetic breeding in recent years: these methods were first used in *Oncorhynchus mykiss* (Gall et al., 1993) and *Salmo salar* (Gjerde and Korsvoll, 1999), and in China they were first applied in the breeding of *Fenneropenaeus chinensis* (Luan et al., 2008). Accurate genetic relationships between individuals are necessary for estimating the genetic parameters (Hu et al., 2016). Traditionally, genetic parameter evaluation is generally based on pedigree inference (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Wright, 1922). However, a reliable additive genetic correlation matrix cannot be obtained for the genetic evaluation of turbot because turbot breeding started relatively recently in China, the stock has an unclear genetic background, and the species has a long generation cycle (male turbots generally are sexually mature for 1.5–2 a, and females are sexually mature for 3–4 a) (Wang, 2010; Hu et al., 2016). However, an additive genetic correlation matrix can be attained through pedigree reconstruction

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(PR) and molecular relatedness (MR) using molecular markers such as simple sequence repeat (SSR) and single nucleotide polymorphisms (SNPs) when the pedigree is completely unknown (Nguyen et al., 2014; Gheyas et al., 2009; Mas-Muñoz et al., 2013). So far, many new methods that use molecular markers to directly calculate paired molecular relatedness have been developed (Wang, 2007). Blonk et al. (2010) used 10 SSR loci to estimate the genetic parameters and breeding values of the common sole using MR and PR. Guan et al. (2016) evaluated the breeding value of turbot using average molecular correlations between families. Hu et al. (2016) evaluated genetic parameters using parental molecular correlation rather than pedigree. Lyu et al. (2017) evaluated breeding values by applying SSRs and pedigree and proved that MR is superior to pedigree in genetic evaluation. Li (2016) estimated heritability by calculating MR using SSRs and found that the accuracy based on MR was similar to that of pedigree.

Compared with other aquatic species, the reproductive cycle of turbot is longer, and the BLUP genetic evaluation system was initiated more recently. Additionally, the background of the first introduced population is unknown, which affects the accuracy of genetic parameter estimations based on pedigree (Lyu et al., 2017; Shen et al., 2004). Another problem related to breeding is that it is difficult to individually identify aquatic animals in the early stages of development. Breeding different families in separate tanks may be a solution to this; however, the effect of breeding tanks might cause differences in growth among families and bias of the parameter estimations (Karaket and Poompuang, 2012). It is important to reduce the impact of environmental effects on genetic evaluation to improve the accuracy of breeding and to accelerate the breeding process. Mixed culture of different families is an effective method to reduce the effect of the environment on the genetic evaluation.

This study compared the accuracy of PR and MR to estimate genetic parameters using an introduced turbot population as the primary generation (G0) in an early family selection process through family construction and breeding. Moreover, the study explored the effect of the environment on the accuracy of the genetic parameter assessment by assessing early mixed cultures of all full-sib families. The purpose was to provide a reference for the introduction of populations and the genetic evolution of populations without reliable pedigrees and also to explore the accuracy of the genetic evaluation of turbot growth traits using PR and MR. In addition, this study investigated whether mixed breeding conditions can effectively remove the common environmental effects to make the genetic evaluation more accurate.

2 Materials and methods

2.1 *Experimental materials*

The parental generation (G0) consisted of three-year-old individuals introduced from a farm in Rushan City, Shandong Province. The research population was the first generation (G1), which was established by artificial insemination at the Yellow Sea Fisheries Company in Haiyang, Shandong Province in 2018. A total of 33 families (9 half-sib families) were established, involving 33 females and 26 males. Before artificial insemination, the parental turbots were tagged with radio frequency identification, and the dorsal fin was clipped from each parental turbot and stored at –20°C for genomic DNA extraction. Some fin samples were not obtained from parental turbots due to human factors; in total, 31 parents including 16 females and 15 males were sampled. The artificial breeding was implemented successively for 28 d, and fertilized eggs were hatched in 14–17.5°C water. The offspring were divided into 7 tanks (6 m×6 m×0.5 m) according to day-age to create the mixed cultures, and the number of fertilized eggs in each tank was equal. During the rearing period, the water was exchanged approximately 5 times over the course of one day. The turbots were fed with formulated diets six times every day, and the total daily supply accounted for 3% body weight and was adjusted daily. After 100 d, 150 fishes were randomly sampled from each tank. The body length (BL) and body weight (BW) of each fish were measured, and dorsal fin tissues were sampled and stored at –20°C until genomic DNA preparation. During the entire breeding process, the breeding density, water temperature, feeding amount, and dissolved oxygen content in the 7 tanks were artificially controlled to maintain a consistent breeding environment.

2.2 *SSR genotyping*

Genotyping of candidate parent and offspring by SSR was performed. Sixteen SSR loci with repeated core sequences of 3–4 bases were selected for family traceability analysis and molecular relatedness calculation. These SSR loci were selected from loci previously developed in this laboratory and had a high level of polymorphisms and few genotyping errors (Ruan et al., 2010). Genomic DNA was extracted from fin ray 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 using an ABI 3730 automatic genetic analyzer (Applied Biosystems, USA). SSR alleles were sized with a GeneScanTM-500 LIZ size standard (Applied Biosystems, USA) and scored using GeneMapper™ V4.1 (Applied Biosystems, USA). The forward primer for each primer pair was labeled with one of four fluorescent dyes (Sangon Biotech, Shanghai): 6-FAM, HEX, ROX, and TAMRA (Lyu et al., 2017). Detailed information of the SSR loci primer sequences is listed in Table 1.

2.3 *Pedigree reconstruction*

Cervus 3.0 (Kalinowski et al., 2007) was used to obtain the allele frequency, heterozygosity, polymorphic information content (PIC) and exclusion probability for each locus, and the relationship between the number of SSR loci and the exclusion probability was simulated and analyzed. According to the PIC value of the loci, the cumulative identification ability of the loci was calculated by increasing the number of alleles from high to low. Colony 1.0 (Jones and Wang, 2010) was used to reconstruct the pedigree of the 7 batches of turbot and evaluate its accuracy. The real identification efficiency was then calculated according to the experimental results (Wang, 2007).

2.4 *Molecular relatedness*

This study used the method described by Wang (2007) to calculate pairwise MR (r_{XY}) using marker genotype data. The computational process of r_{XY} from SSR genotype data was executed in Coancestry 1.0 software (Wang, 2011). Pearson correlations were calculated between MR and pedigree relatedness based on PR using R (R Core Team, 2013).

2.5 *Genetic analysis*

The single-trait animal model and the restricted maximum likelihood method (using ASReml software) were used to estimate the components of variance (Gilmour et al., 2009). Both the PR and MR methods were used to construct an additive genetic relation matrix and applied in the same model. The model is as follows:

Table 1. Characteristics of SSR primers (Ruan et al., 2010)

Loci	Primer sequence (5′-3′)	Repeats	Size/bp	Fluorescent dye	Temperature/°C
YSKr61	F: TCAGTGGGCAGTGAGGTG R: AAGTCAGAGAAACATCCAGA	GTCT	164–173	HEX	62.0
YSKr71	F: TGGGATACATACACATTC R: AGTGAGTTGACAGACAGAG	ACGC	172–178	TAMRA	53.4
YSKr72	F: CCAGACAGATAACTACACA R: GTAAGGCTCGTTAGTCAC	ACGC	132–168	FAM	58.0
YSKr85	F: TACTTACACTGTGTATGTGC R: GAGAACCGAAGAAATGAGA	GTGC	252–290	ROX	56.0
YSKr92	F: CCACGCTGTGTATTTCCTCAT R: GGTCAACATTCAAACCCAACT	GTGC	188–208	HEX	60.0
YSKr101	F: CGGATAGTTAGTACCTCAT R: GAAAACTGAAGCTGAATG	ACGC	112–133	TAMRA	56.0
YSKr111	F: AACTGGGACTGGAGTGGAC R: CTCATTAGAGCCGCTGTAT	TGCG	340–366	FAM	62.0
YSKr119	F: GCTCTTCCAAGTGCCA R: TGTAGTGTACCAAATGC	ACGC	242–271	ROX	54.6
YSKr121	F: CAGAGGACAGCGACGAAGAC R: AGCATTGCATTGGGTTGAGT	ACGC	183–188	HEX	62.0
YSKr124	F: CAGCCGTTCTGACCTCGTAG R: ACCCTCCACTGCTTGTCCTTG	GTGC	178–187	TAMRA	62.0
YSKr125	F: ACTTATTTGCCTATGGAGAG R: TTCATTCACATCACTGGTC	CGTG	138–151	FAM	56.0
YSKr6	F: CTAACAAACAACGCAGTCG R: AGAAACAGGGTAGCATCAC	CTT	299–313	ROX	62.0
YSKr141	F: TTCTGCTCCCTTCTTCGTGT R: TCGGTGCTTGTGGAAATCG	GCG	171–189	FAM	61.0
YSKr169	F: TAATCTCCTGTTGCCTAATG R: AACGGACGAGTTCGGTGC	AAC	179–185	ROX	62.0
YSKr170	F: GCTACAGTGATGTCGCA R: ATTTATCCAGTGTTTCG	AAC	276–304	HEX	54.6
YSKr173	F: CTGGATTTGCCACGTCAGTAC R: TCTCGCTAACGCTTCACCTC	AAG	323–474	TAMRA	59.0

$$y_{ijk} = \mu + t_j + a_i + d_k + e_{ijk}, \tag{1}$$

where y_{ijk} is the observation of BW or BL; μ is the mean; t_j is the fixed effect of tank j, and the value of j ranges from 1 to 7; a_i is the random additive genetic effect of animal i; d_k is family effect; and e_{ijk} is the random error.

Assuming that the distribution of random effects a_i and e_{ijk} is normal, and their mean is zero, the variance-covariance matrix is expressed as:

$$v\begin{bmatrix} a \\ d \\ e \end{bmatrix} = \begin{bmatrix} \boldsymbol{A}\sigma_a^2 & 0 & 0 \\ 0 & \boldsymbol{I}_d\sigma_d^2 & 0 \\ 0 & 0 & \boldsymbol{I}_e\sigma_e^2 \end{bmatrix}, \tag{2}$$

where σ_a^2, σ_d^2 and σ_e^2 are the variances of the random effects a, d and e, respectively; $\boldsymbol{A}$ is the additive genetic relation matrix; and $\boldsymbol{I}_e$ is the identity matrix.

The formula for calculating phenotypic variance was as follows:

$$\sigma_p^2 = \sigma_a^2 + \sigma_d^2 + \sigma_e^2. \tag{3}$$

The heritability was computed as follows:

$$h^2 = \sigma_a^2/\sigma_p^2. \tag{4}$$

2.6 *Cross validation*

Ten times cross validation (CV) was used to compare the predictive power of the model. The full data set (n=880) was randomly split equally into ten subsets. Among them, nine subsets were treated as the training data sets and the remaining one was treated as the validation data set. The predictive values of the validation data set were generated based on models constructed based on the training data set. The Pearson correlations between the predictive values and the observed phenotypic values of the validation set were considered to evaluate the predictive ability of the model. The CV was repeated for 500 times, and the average Pearson correlation was calculated. The prediction of the unobserved phenotypic data was performed by ASReml software (Gilmour et al., 2009), and other operations of cross validation were performed by R (R Core Team, 2013).

3 Results

3.1 *SSR genotyping*

The genotype data of 16 SSR loci were collected. The parameter information of these loci was obtained used Cervus 3.0 and is shown in Table 2. The analysis results from Cervus 3.0 showed that the 16 markers shared 158 alleles, with an average allele number of 9.875 and a maximum of 25. The average observed heterozygosity was 0.695 8, and the average expected heterozygosity was 0.760 8. The average PIC was 0.654 4. The cumulative exclusion probability was 0.997 4 when the parent sex was unknown, 0.999 5 when one parent sex was known, and 0.999 9 when both parent genotype sexes were known.

3.2 *Pedigree reconstruction*

One thousand and fifty offspring and 31 parents were analyzed. In total, 880 offspring were assigned to 20 full-sib families produced by 27 parents (14 females and 13 males). Four parents were not assigned to offspring, while 170 offspring could not be traced to their parents. The family sizes are shown in Fig.1. The pedigree information identified by the 16 SSR molecular markers

Table 2. Genetic diversity information of 16 SSR loci

Loci	*K*	*n*	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F (Null)
YSKr61	3	909	0.359	0.498	0.437	0.876	0.746	0.606	0.313	0.580	***	0.175 0
YSKr71	9	715	0.815	0.762	0.727	0.625	0.447	0.258	0.092	0.392	***	0.042 9
YSKr72	10	909	0.792	0.831	0.813	0.495	0.325	0.146	0.047	0.346	***	0.026 3
YSKr85	14	904	0.885	0.848	0.831	0.462	0.299	0.128	0.040	0.336	***	0.021 4
YSKr92	9	907	0.882	0.801	0.774	0.566	0.388	0.205	0.067	0.366	***	–0.050 0
YSKr101	10	901	0.685	0.708	0.674	0.683	0.502	0.302	0.119	0.426	***	0.018 5
YSKr111	14	901	0.921	0.861	0.848	0.428	0.271	0.106	0.033	0.328	***	0.035 7
YSKr119	11	899	0.871	0.843	0.823	0.483	0.315	0.144	0.044	0.340	***	0.014 7
YSKr121	8	906	0.925	0.824	0.801	0.523	0.349	0.172	0.054	0.352	***	0.060 3
YSKr124	10	905	0.783	0.753	0.731	0.614	0.428	0.223	0.083	0.394	***	0.023 1
YSKr125	6	909	0.674	0.706	0.654	0.715	0.546	0.369	0.139	0.432	***	0.029 9
YSKr6	6	903	0.858	0.762	0.720	0.646	0.468	0.290	0.098	0.394	***	0.061 9
YSKr141	6	902	0.627	0.619	0.571	0.788	0.622	0.442	0.193	0.489	***	0.008 8
YSKr169	4	901	0.615	0.627	0.554	0.793	0.649	0.489	0.212	0.490	***	0.013 2
YSKr170	13	888	0.555	0.826	0.805	0.510	0.338	0.158	0.051	0.350	***	0.197 1
YSKr173	25	867	0.780	0.902	0.894	0.331	0.198	0.063	0.018	0.304	***	0.072 8

Note: Number of individuals, 931; number of loci, 16; mean number of alleles per locus, 9.875 0; mean proportion of loci typed, 0.955 0; mean expected heterozygosity, 0.760 8; mean polymorphic information content (PIC), 0.728 5; combined non-exclusion probability (first parent), 0.000 157; combined non-exclusion probability (second parent), 0.000 000 55; combined non-exclusion probability (parent pair), 2.396×10^{-11}; combined non-exclusion probability (identity), 1.366×10^{-18}; combined non-exclusion probability (sib identity), 0.000 000 28. *K*, number of alleles at the locus; *n*, number of individuals typed at the locus; HObs, observed heterozygosity; HExp, expected heterozygosity; PIC, polymorphic information content; NE-1P, average non-exclusion probability for one candidate parent; NE-2P, average non-exclusion probability for one candidate parent given the genotype of a known parent of the opposite sex; NE-PP, average non-exclusion probability for a candidate parent pair; NE-I, average non-exclusion probability for identity of two unrelated individuals; NE-SI, average non-exclusion probability for identity of two siblings; HW, significance of deviation from Hardy-Weinberg equilibrium; F (Null), estimated null allele frequency. ***, significant at the 0.1% level. The significance level includes a Bonferroni correction if the Bonferroni correction option was selected.

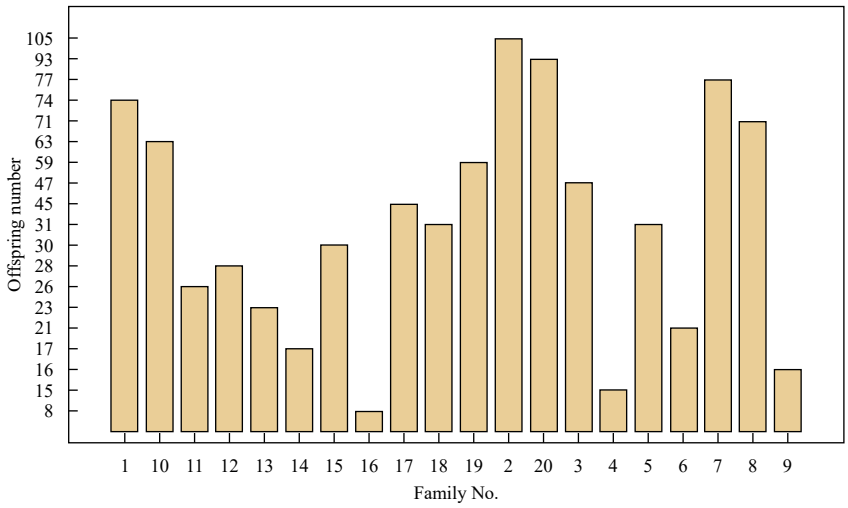

Fig. 1. Number of offspring in each family.

was used as pedigree data to calculate individual relationships.

3.3 *Molecular relatedness*

The MR calculated based on the 16 SSR molecular markers were continuously distributed from 0 to 1 (0.998 4), with an average value of 0.061 6±0.120 4. Because the parents of the families were from an introduced population, this study assumed that the genetic relationship between the parents was 0. Hence, there are only three levels of pedigree relationships between individuals, that is, 0 for uncorrelated, 0.25 for half-sib and 0.5 for the full-sib.

3.4 *Genetic analysis*

Analysis of variance showed that the fixed effect of the tank was significant ($p<0.05$). The additive genetic variances of BW and BL based on pedigree reconstruction were (0.326±0.172) g^2 and (0.038±0.046) cm^2, respectively, and the additive genetic variances based on molecular genetic relationships were (0.139±0.036) g^2 (BW) and (0.031±0.001) cm^2 (BL). The maternal effects of weight estimated by the two methods were (0.705±0.303) g^2 (PR and BW) and (0.631±0.251) g^2 (MR and BW), and the maternal effects of BL were (0.106±0.045) cm^2 (PR and BL) and (0.126±0.052) cm^2 (PR and BL). The obtained BW phenotypic variances estimated by the two methods were (1.519±0.298) g^2 (PR) and (1.379±0.253) g^2 (MR), and the BL phenotypic variances were (0.306±0.045) cm^2 (MR) and (0.327±0.053) cm^2 (PR). Based on PR, the BW heritability was 0.214±0.121 and the BL heritabil-

Table 3. Variance component of body weight

	σ_a^2	σ_d^2	σ_e^2	σ_p^2	h^2±SE
PR	0.326±0.172	0.705±0.303	0.488±0.124	1.519±0.298	0.214±0.121
MR	0.139±0.036	0.631±0.251	0.608±0.033	1.379±0.253	0.101±0.031

Note: σ_a^2, additive genetic variance (g^2); σ_d^2, maternal common environmental variance (g^2); σ_e^2, residual variance (g^2); σ_p^2, phenotypic variance (g^2); h^2, heritability; SE, stardard error.

Table 4. Variance component of body length

	σ_a^2	σ_d^2	σ_e^2	σ_p^2	h^2±SE
PR	0.038±0.046	0.126±0.052	0.162±0.034	0.327±0.053	0.117±0.141
MR	0.031±0.001	0.106±0.045	0.169±0.001	0.306±0.045	0.102±0.034

Note: σ_a^2, additive genetic variance (cm^2); σ_d^2, maternal common environmental variance (cm^2); σ_e^2, residual variance (cm^2); σ_p^2, phenotypic variance (cm^2); h^2, heritability; SE, stardard error.

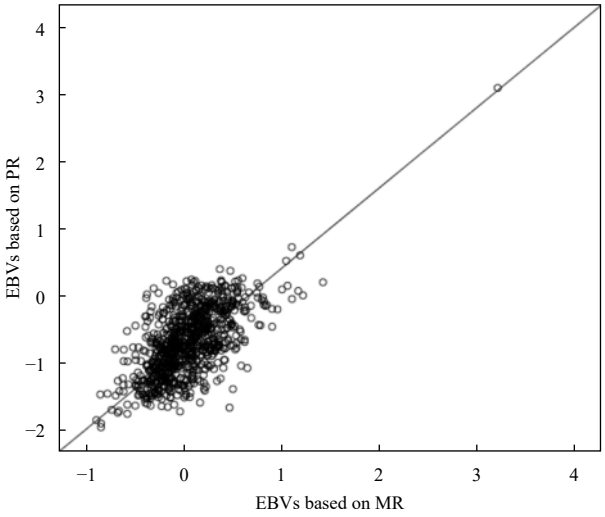

Fig. 2. Relationship between estimated breeding values (EBVs) from molecular relatedness (MR) and pedigree reconstruction (PR).

ity was 0.117±0.141. Based on MR, the BW heritability was 0.101± 0.031 and the BL heritability was 0.102±0.034 (Tables 3 and 4). The estimated breeding values (EBVs) based on PR and MR were positively correlated with the EBV by MR. The Pearson correlation coefficients of BW and BL were 0.624 and 0.856, respectively (Fig.2). Genetic and phenotypic correlations were also calculated by both methods (Table 5).

3.5 *Cross validation*

The average Pearson correlation coefficients of the observed and predicted values by MR and PR after 500 times of 10 fold cross validation were 0.692±0.052 (PR, BW), 0.615± 0.060 (PR, BL), 0.717±0.045 (MR, BW), and 0.629±0.058 (MR, BL). As shown in Table 6, the *t*-test showed a significant difference in accuracy between the two methods (BW and BL) (p<0.01). The relationships between all of the observed and predicted values based on the two methods are shown in Fig. 3 and Fig. 4. The distribution of the Person correlation coefficients for 500 times cross validations is shown in Fig. 5.

4 Discussion

4.1 *Model and methods*

The individual animal model is considered to be the ideal genetic evaluation model because it can reflect the dynamics of breeding populations (Luan et al., 2008) while simultaneously achieving the best linear unbiased estimate of the fixed effect breeding value and the BLUP of the random effect breeding value (Wang, 2007). It is possible to improve the accuracy of EBVs by building a genetic matrix and its inverse matrix using all known relatedness data between offspring and parents when estimating the breeding value (Luan et al., 2008). In the current study, the animal model containing maternal common environmental effects was proved to be the most suitable model for evaluating the genetic parameters of turbot (Guan et al., 2016; Ma et al., 2009; Lyu et al., 2017). The age covariates could be omitted from the model because all individuals were the same age.

Table 5. Genetic correlation and phenotypic correlation of body weight (BW) and body length (BL) based on pedigree reconstruction (PR) and molecular relatedness (MR)

Correlation coefficient	Genetic correlation	Phenotypic correlation
MR	0.856	0.668
PR	0.723	0.624

In this study, SSR genotyping data were used to calculate both PR and MR. Genetic evaluations based on PR from the pedigree reconstruction using SSRs have been often reported for aquatic animals (e.g., Lucas et al., 2006; Vandeputte et al., 2004; Lyu et al., 2017; Hu et al., 2016; Guan et al., 2016; Li, 2016). In the absence of ideal pedigree information, genetic evaluation using a small number of SSRs for pedigree reconstruction is possible. Genetic evaluation has also been developed in aquatic animals through MR based on SSR typing data (Wang, 2007).

4.2 *Genetic analysis*

In this study, the genetic parameters of growth traits of a breeding population were evaluated using PR and MR. The estimated heritability of turbot by PR (the heritability of BW and BL were 0.214±0.121 and 0.117±0.141, respectively) were higher than those estimated by MR (the heritability of BW and BL were 0.101±0.031 and 0.102±0.034, respectively). The estimated heritability of BW based on MR showed medium heritability. Other parameters showed low heritability 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). The low heritability probably arose because molecular relatedness might underestimate genetic variance and standard error (Bink et al., 2008). The residual of the PR method was lower than that of the MR method, and more factors affected the accuracy of the PR method. The standard error of MR was lower because the

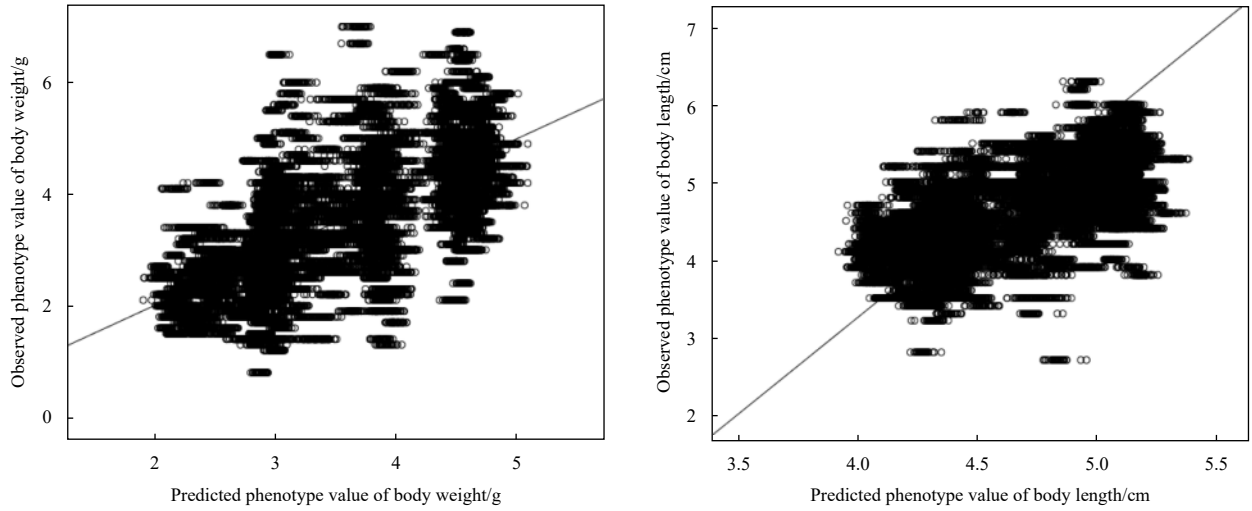

Fig. 3. Relationship between MR-based observed and predicted values.

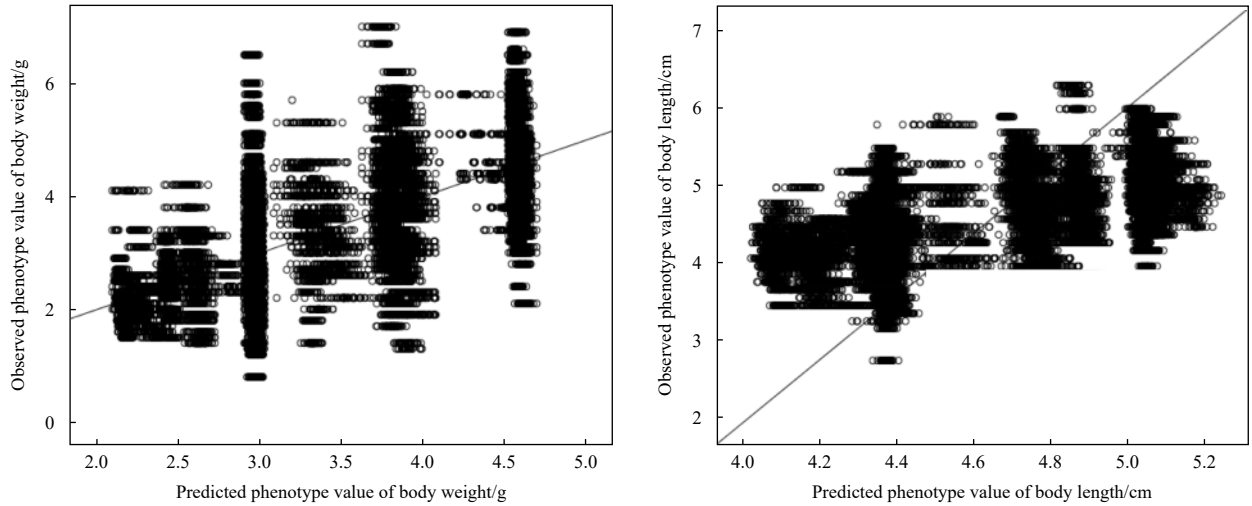

Fig. 4. Relationship between the observed and predicted values based on PR.

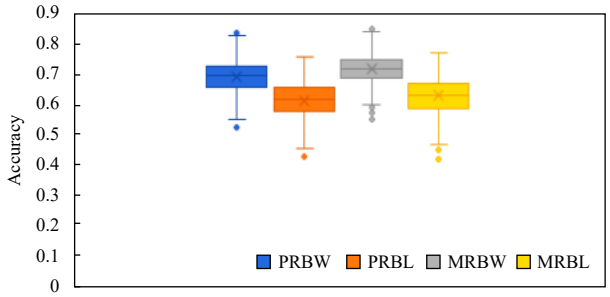

Fig. 5. Distribution of Person correlation coefficients for 500 times cross validations. PR, pedigree reconstruction; MR, molecular relatedness; BW, body weight; BL, body length.

genetic relationships between individuals obtained by molecular markers were continuously distributed, and heritability estimates were more accurate (Lyu et al., 2017).

Lower heritability can be observed in both the MR and PR methods. Some studies of the genetic evaluation of juvenile turbot growth traits have been reported. Liu et al. (2011) and Guan et al. (2016) assessed weight heritability of 100 d old turbot (0.22 and 0.19, respectively). Hu et al. (2016) evaluated the weight heritability of turbot using PR (0.55) and MR (0.52). Moreover, Lyu et al. (2017) evaluated the heritability in turbot of BW and BL by using MR and PR, finding that heritability 0.33 (PR) and 0.23 (MR) for BW and 0.24 (PR) and 0.23 (MR) for BL, respectively. In this study, the results of the two methods were similar to those of earlier studies. Here, the heritability estimated by the two methods using the same model was relatively similar, which is consistent with previous studies (Lyu et al., 2017; Hu et al., 2016). The Pearson correlation coefficient of the relationship obtained using PR and MR was 0.54 (BW), which was slightly lower than 0.55 of Lyu et al. (2017) and lower than 0.8 of Blonk et al. (2010). The

Table 6. Results of cross validation between observations and predicted values

	Pearson correlation coefficient of body weight	Pearson correlation coefficient of body length
Molecular relatedness	0.717±0.045	0.629±0.058
Pedigree reconstruction	0.692±0.052	0.615±0.060

lower Pearson correlation coefficient in the current study may have been due to the relatively small number of SSRs and only one generation included in the calculation.

4.3 *Common environmental effects*

In the breeding process of aquatic animals, separated breeding is used because the animal bodies cannot be physically labeled in the early growth stage, which introduces common environmental effects and causes difficulties in accurately calculating the variance components. For an accurate estimation of heritability, it is important to remove non-hereditary variance from the total phenotypic variance, which would otherwise confound the analysis of variance components (Guan et al., 2016).

Therefore, to accurately estimate the genetic parameters of individuals, the early mixed family culture mode was adopted to remove common environmental effects and effectively remove non-genetic variance from the total phenotypic variance. In this study, the significant fixed effect of the tank could have led to underestimating the additive genetic variance, which may have been caused by the large environmental differences between the tanks, finally affecting the genetic evaluation process. The results indicated that family breeding separately may be more conducive to the genetic assessment process, or all families could be mixed in a tank large enough to avoid common environmental effects, perhaps making the assessment results more accurate.

4.4 *Cross validation*

In this study, the results of the cross validation were credible based on 500 times cross validation and the prediction of each individual phenotypic value multiple times. The cross validation results showed that the Pearson correlation coefficient of PR (0.692) for the predicted BW and the observed value was lower than that of MR (0.717). The Pearson correlation coefficient of the predicted BL and observed value based on PR (0.615) was also lower than that of MR (0.629). The *t*-test showed that the accuracy of the two methods was significantly different ($p<0.01$), which further proved that estimating the breeding value using MR was more accurate than using PR when the physical pedigree was shallow. The Pearson correlation coefficients obtained by MR were higher than those of PR, indicating that MR was more accurate for estimating the genetic parameters and breeding values. Compared with the BL data, the results showed a stronger predictive ability for cross validation for the weight data, which was not consistent with the results of Lyu et al. (2017). This difference likely appeared because the population used in the current study consisted of juveniles, and BW data is more credible compared with BL in the early stage.

This study cannot determine which method estimated the genetic relationship closer to the true value due to the lack of pedigree data for comparison, but the predictive ability of phenotypic data through cross validation can reflect the accuracy of the breeding value estimation. Therefore, it is feasible to estimate the breeding value using MR when the genetic background of the parent population is unknown, and this method can be highly useful when introducing a population with an unknown foreign genetic background to construct a breeding population.

References

Bink M C A M, Anderson A D, van de Weg W E, et al. 2008. Comparison of marker-based pairwise relatedness estimators on a pedigreed plant population. Theoretical and Applied Genetics, 117(6): 843–855, doi: 10.1007/s00122-008-0824-1

Blanquer A, Alayse J P, Berrada-Rkhami O, et al. 1992. Allozyme variation in turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) (Osteichthyes, Pleuronectoformes, Scophthalmidae) throughout their range in Europe. Journal of Fish Biology, 41(5): 725–736, doi: 10.1111/j.1095-8649.1992.tb02702.x

Blonk R J W, Komen H, Kamstra A, et al. 2010. Estimating breeding values with molecular relatedness and reconstructed pedigrees in natural mating populations of common sole, *Solea solea*. Genetics, 184(1): 213–219, doi: 10.1534/genetics.109.110536

Cardellino R, Rovira J. 1987. Mejoramiento Genético Animal. Buenos Aires, Argentina: Hemisferio Sur

Falconer D S, Mackay T F C. 1996. Introduction to Quantitative Genetics. 4th ed. Longman, Harlow: Benjamin Cummings

Gall G A E, Bakar Y, Famula T. 1993. Estimating genetic change from selection. Aquaculture, 111(1–4): 75–88, doi: 10.1016/0044-8486(93)90026-U

Gheyas A A, Woolliams J A, Taggart J B, et al. 2009. Heritability estimation of silver carp (*Hypophthalmichthys molitrix*) harvest traits using microsatellite based parentage assignment. Aquaculture, 294(3–4): 187–193, doi: 10.1016/j.aquaculture.2009.06.013

Gilmour A R, Gogel B J, Cullis B R, et al. 2009. ASReml User Guide Release 3.0. Hemel Hempstead, UK: VSN International Ltd

Gjerde B, Korsvoll S A. 1999. Realized Selection Differentials for Growth Rate and Early Sexual Maturity in Atlantic Salmon. Oostende, Belgium: Aquaculture Europe, 99: 73–74

Guan Jiantao, Wang Weiji, Luan Sheng, et al. 2016. Estimation of genetic parameters for early growth trait of turbot (*Scophthalmus maximus* L.) using molecular relatedness. Aquaculture Research, 47(7): 2205–2214, doi: 10.1111/are.12673

Hu Yulong, Guan Jiantao, Ma Yu, et al. 2016. An estimation of genetic parameters of growth traits in juvenile turbot (*Scophthalmus maximus* L.) using parental molecular relatedness. Acta Oceanologica Sinica, 35(2): 126–130, doi: 10.1007/s13131-015-0643-6

Jones O R, Wang Jinliang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10(3): 551–555, doi: 10.1111/j.1755-0998.2009.02787.x

Kalinowski S T, Taper M L, Marshall T C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16(5): 1099–1106, doi: 10.1111/j.1365-294X.2007.03089.x

Karaket T, Poompuang S. 2012. CERVUS vs. COLONY for successful parentage and sibship determinations in freshwater prawn *Macrobrachium rosenbergii* de Man. Aquaculture, 324–325: 307–311, doi: 10.1016/j.aquaculture.2011.10.045

Lei Jilin. 2002. Problem and suggestion of introducting species for marine culture. China Fisheries, (2): 63–65

Lei Jilin. 2006. Outlook of the marine fish culture industry in China. Marine Fisheries Research (in Chinese), 27(2): 1–9

Lei Jilin, Liu Xinfu. 1995. A primary study on culture of turbot, *Scophthalmus maeoticus* L. Modern Fisheries Information (in Chinese), 10(11): 1–3

Lei Jilin, Men Qiang, Wang Yingeng, et al. 2002. Review of "Green House+Deep Well Seawater" industrialized culture pattern of turbot (*Scophthalmus maximus*). Marine Fisheries Research (in Chinese), 23(4): 1–7

Li Dongyu. 2016. Genetic parameter estimation for growth and meat rate traits of Pacific white shrimp (*Litopenaeus vannamei*) in low temperature condition by microsatellite markers (in Chinese)[dissertation]. Nanjing: Nanjing Agricultural University

Liu Baosuo, Zhang Tianshi, Kong Jie, et al. 2011. Estimation of genetic parameters for growth and upper thermal tolerance traits in turbot *Scophthalmus maximus*. Journal of Fisheries of China (in Chinese), 35(11): 1601–1606

Luan Sheng, Kong Jie, Wang Qingyin. 2008. Methods and application of aquatic animal breeding value estimation: a review. Marine Fisheries Research (in Chinese), 29(3): 101–107

Lucas T, Macbeth M, Degnan S M, et al. 2006. Heritability estimates for growth in the tropical abalone *Haliotis asinina* using microsatellites to assign parentage. Aquaculture, 259(1–4): 146–152, doi: 10.1016/j.aquaculture.2006.05.039

Lynch M, Walsh B. 1998. Genetics and Analysis of Quantitative Traits. Sunderland: Sinauer Associates, Inc: 360–361

Lyu Ding, Wang Weiji, Luan Sheng, et al. 2017. Estimating genetic parameters for growth traits with molecular relatedness in turbot (*Scophthalmus maximus*, Linnaeus). Aquaculture, 468: 149–155, doi: 10.1016/j.aquaculture.2016.09.049

Ma Aijun, Wang Xinan, Lei Jilin. 2009. Genetic parameterization for turbot *Scophthalmus maximus*: implication to breeding strategy. Oceanologia et Limnologia Sinica (in Chinese), 40(2): 187–194

Mas-Muñoz J, Blonk R, Schrama J W, et al. 2013. Genotype by environment interaction for growth of sole (*Solea solea*) reared in an intensive aquaculture system and in a semi-natural environment. Aquaculture, 410–411: 230–235, doi: 10.1016/j.aquaculture.2013.06.012

Men Qiang. 2002. Overview on turbot, *Scophthalmus maximus* (Linnaeus) introduced to China for ten years. Modern Fisheries Information (in Chinese), 17(9): 14–17

Nguyen T T T, Hayes B J, Ingram B A. 2014. Genetic parameters and response to selection in blue mussel (*Mytilus galloprovincialis*) using a SNP-based pedigree. Aquaculture, 420–421: 295–301, doi: 10.1016/j.aquaculture.2013.11.021

R Core Team. 2013. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/ [2018-01-07]

Ruan Xiaohong, Wang Weiji, Kong Jie, et al. 2010. Genetic linkage mapping of turbot (*Scophthalmus maximus* L.) using microsatellite markers and its application in QTL analysis. Aquaculture, 308: 89–100, doi: 10.1016/j.aquaculture.2010.08.010

Sambrook J, Fristch E F, Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. New York: Cold Spring Harbor Laboratory

Shen Xueyan, Gong Qingli, Lei Jinlin, et al. 2004. Population genetic structure analysis of the imported turbot seedlings *Scophthalmus maximus* L. using RAPD and microsatellite technique. Oceanologia et Limnologia Sinica (in Chinese), 35(4): 332–341

Shen Xueyan, Kong Jie, Gong Qingli, et al. 2005. The investigation and exploition of turbot (S*cophthalmus maximus* L.) genetic resources. Marine Fisheries Research (in Chinese), 26(6): 94–100

Vandeputte M, Kocour M, Mauger S, et al. 2004. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture, 235(1–4): 223–236, doi: 10.1016/j.aquaculture.2003.12.019

Wang Gang. 2010. Turbot industry transformation period has arrived. Ocean And Fishery (in Chinese), (1): 14–16, 20

Wang Jinliang. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetics Research, 89(3): 135–153, doi: 10.1017/S0016672307008798

Wang Jinliang. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources, 11(1): 141–145, doi: 10.1111/j.1755-0998.2010.02885.x

Wright S. 1922. Coefficients of inbreeding and relationship. The American Naturalist, 56(645): 330–338, doi: 10.1086/279872