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An estimation of genetic parameters of growth traits in juvenile turbot (*Scophthalmus maximus* L.) using parental molecular relatedness

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Abstract

The estimation of genetic parameters has played an important role in animal selective breeding for growth traits. Recently studies show that molecular markers can be incorporated into genetic evaluations. In order to improve the performance of an incomplete pedigree (i.e, only parents are known) in the genetic evaluations, 12 microsatellite markers have been applied in the estimation of the genetic parameters for body weight in a farmed population (n=1 890) of juvenile turbot (*Scophthalmus maximus* L.). A new relatedness called parental molecular relatedness (PMR) is estimated based on results of genotyping of 48 parents (31 males, 17 females) with microsatellites markers. The feasibility of PMR in estimation of genetic parameters is verified by comparison with pedigree related (PR) which is obtained from a complete pedigree. The results demonstrate that a high correlation (0.872) between them is found. Heritabilities are estimated using the PMR (0.52±0.13) and PR (0.55±0.22) with the same animal model. A cross-validation shows that the predictive abilities of models using the PMR and the PR are identical (0.81). From that, a conclusion can be made that PMR and PR predicted genetic values equally well in a population of juvenile turbot. Therefore PMR can be applied as an alternative of the PR when only parents are known. However, for a better performance, more markers and more families should be included in a further study.

Key words: parental molecular relatedness, pedigree relatedness, turbot, genetic parameters

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1 Introduction

Turbot, *Scophthalmus maximus* L., is a marine finfish with a fast growth and strong tolerance to a cold water temperature and is the most widely cultivated commercial flatfish around the world with the highest annual aquaculture production (Lei et al., 2012). From its introduction into China in 1992 to now, its farming has developed into one of the dominant mariculture industries in China with an annual production of more than 60 kt (Lei et al., 2012). In aquaculture, the growth is an important trait with commercial interests. The faster growth can reduce the duration of the rearing cycle so to lower costs. However, the optimization of breeding programs is developed with the accurate and reliable estimation of genetic parameters of trait of interest (Henderson, 1984; Falconer and McKay, 1996). Some authors have relevant studies on the estimations of the genetic parameters for the growth traits of turbot (Zhang et al., 2008; Liu et al., 2011; Ma et al., 2009; Gjerde et al., 1997).

Knowledge of the accurate genetic relationship between individuals is the key for the estimation of the genetic parameters because it can provide the accurate relatedness which would be used in the partition of variance components. For the cultured population, the estimation of relatedness depends on the correctness, depth and completeness of a genealogy (Atkin et al., 2009). When the genealogy is completely unknown, the relatedness can be attained by a pedigree reconstruction and molecular relatedness using a molecular markers like simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) (Nguyen et al., 2013; Gheyas et al., 2009; Mas-Muñoz et al., 2013). However, for the pedigree reconstruction method, some studies like Rodríguez-Ramilo et al. (2007) show that there is not a satisfyingly high percentage (<80%) of correct assignments for full-sib and half-sib relationships. Besides that, the method usually cannot utilize all studied individuals though a high percentage (>90%) of individuals can be assigned to one single parental pair (Vandeputte et al., 2004). Moreover, there must be genotyping data of both parent candidates and offspring. For the molecular relatedness method, most studies focused on a genomic selection using thousands SNP markers (Daetwyler et al., 2012; Crossa

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et al., 2010; Hayes and Goddard, 2010) and these research has proved that the predictions of breeding values have been improved using dense SNP markers. Additionally, Blonk et al. (2010) has showed that the molecular relatedness can perform equally well in estimating breeding values compared with the pedigree reconstruction using only ten SSR markers. However, in practise, it is not always that the pedigree was completely unknown. When only parents were known, the pedigree information available can be used to estimate this relatedness along with the parental molecular markers due to that coancestry between parents from the molecular markers (Wang, 2007) can be translated to the relatedness between their offspring.

The aim of this study was to test the application of microsatellite markers of parents in the estimation genetic parameters of farmed population of juvenile turbot when only pedigree-offspring relationships were known for the pedigree. A complete pedigree of three generations was also used as a reference. Through comparison, the feasibility of parental SSR microsatellite markers was verified as a practicable method in the selective breeding of turbot.

2 Materials and methods

2.1 *Experimental data*

In May 2013, the study population was produced at the hatchery of the Yellow Sea Fisheries Company (Haiyang City, Shandong Province, China) from a cross between 31 males and 17 females, which was consisted of 1 890 progenies (G2) from 39 families. And the breeders (G1) were selected from the individuals reproduced at 2009 (17 families, 16 males, 4 females) and at 2010 (22 families, 15 males, 13 females). The parents of the breeders were from the base population which consisted of two introduced populations, one of which was from Denmark and France at 2005 and the other was also from Denmark and France at 2006. Among 39 families, there were nine maternal half-sib families groups and seven full-sib families. The artificial breeding was implemented successively during 22 d and fertilized eggs were hatched in water at 14–16°C. After incubation, all the families were reared separately in fiberglass-reinforced plastic (FRP) tanks (0.5 m^3), and the rearing environment of every tank was kept identical as possible. At weaning (about 35 d post-fertilization (dpf)), 1 000 weaned larvae per family [body mass (b.m.) is 250 mg] randomly selected, were split among 39 FRP tanks respectively and reared at a density of 2 000 larvae m^{-2}. After 70 dpf, 400 juveniles per family were randomly selected to fertilize in another 39 FRP tanks. Then all the fish were cultured in circular tanks which were supplied with 15 L/min of sea water with a salinity of 30.0±0.5, pH 7.8–8.0 and oxygen above 75% at all time. The rearing temperature of all the experimental groups was maintained between 15 and 24°C. At 100 dpf, approximate 50 individuals from each family were randomly selected and weighed. The fins of parents have been sampled after reproduction and stored at –20°C for a DNA extraction. The samples were genotyped by using 12 microsatellite markers: *YSKr271*, *YSKr262*, *YSKr108*, *YSKr231*, *YSKr80*, *YSKr244*, *YSKr197*, *YSKr115*, *YSKr221*, *YSKr124*, *YSKr218* and *YSKr259* (Ruan et al., 2010) which were selected from different linkage groups. A DNA isolation and a PCR amplification were performed as described by Ruan et al. (2010).

2.2 *Relatedness between offsprings*

To obtain the relatedness between offspring (i.e, PMR), firstly a coancestry (θ) between parents acquired using triadic IBD coefficients which were described by Wang (2007) was calculated. This step was realized by Coancestry 1.0.1.2 software (Wang, 2011). Next, if individuals P and Q were the progenies of A and B, and C and D respectively, then the coancestry of P and Q could be derived from the coancestries between A and B, and C and D as Eq. (1) (Plum, 1954). The coancestry (θ_{AA}) between A and itself can be calculated according to Eq. (2), where F_A is the inbreeding coefficient of individual A (Plum, 1954).

$$\theta_{PQ} = \frac{1}{4}\left(\theta_{AC} + \theta_{AD} + \theta_{BC} + \theta_{BD}\right) , \tag{1}$$

$$\theta_{AA} = \frac{1}{2}\left(1 + F_A\right) . \tag{2}$$

Finally the PMR between offsprings could be calculated as below (Crow and Kimura, 1970):

$$r_{PQ} = 2\theta_{PQ} . \tag{3}$$

All computations were completed by an R software (R Core Team, 2013).

Besides that, the other relatedness from the pedigree (i.e., pedigree relatedness, PR) was computed based on a complete pedigree of three generations. This was realized by an ASReml software (Gilmour et al., 2009).

2.3 *Statistical analysis*

Genetic parameters were estimated based on single trait animal models using a restricted maximum likelihood (REML) method with the ASReml software (Gilmour et al., 2009). The animal model was listed as follows:

$$y_{ijk} = \mu + cb_i + a_j + d_k + e_{ijk} , \tag{4}$$

where y is the phenotypic observations for the offspring of weight; μ is the mean; b_i is the covariate for age of ith offspring, c is the regression coefficient for b_i; a_j is the random additive effect of jth offspring; d_k is the maternal common environmental effect; and e is the random error. Though each family was reared in different tanks, the potential fixed effect of the tank was considered to be nonsignificant through multicomparison test.

The distribution of the random effects a_j and e were assumed to be normal, and the means of these values were all 0. The variance-covariance matrix is represented as

$$\boldsymbol{V}\begin{bmatrix} a \\ d \\ e \end{bmatrix} = \begin{bmatrix} \boldsymbol{A}\sigma_a^2 & 0 & 0 \\ 0 & \boldsymbol{I}_d\sigma_d^2 & 0 \\ 0 & 0 & \boldsymbol{I}_e\sigma_e^2 \end{bmatrix} , \tag{5}$$

where σ_a^2, σ_d^2 and σ_e^2 are the variances of the random effects a, d and e; $\boldsymbol{A}$ is the numerator relationship matrix; and I_e is the identity matrix.

For numerator relationship matrix A was constructed by two sorts of relatedness, PR and PMR. In details, for matrix A from the PR, there is only need to input a pedigree file into the ASReml software. However, for matrix A from the PMR, it had to be transformed to the GRM file according to the demand of the ASReml and then inputted it. Before its input, the matrix has been validated as a positive definite matrix by function "is.positive.definite" in the corpcor package of the R software (R Core Team, 2013).

The heritability was computed as follows:

$$h^2 = \sigma_a^2/\sigma_p^2 , \tag{6}$$

where σ_a^2 is the additive genetic variance; and σ_p^2 is the phenotypic variance.

The comparisons of the heritabilities estimates based on different relatednesses was implemented using two-tailed *t*-test.

2.4 *Cross-validation*

The prediction of the performance of families or individuals whose phenotypes to be observed is very important for selective breeding because such predictions could be beneficial to choose which targets to be selected. To compare the predictive ability of models, a ten-fold cross-validation (CV) was applied. The full data set (n=1 890) was randomly split equally into ten subsets. Among them, nine ones were treated as training data sets and the remaining one was treated as a validation data set. The predictive values of the validation data set were generated based on models constructed based on the training data set. Pearson correlation between the predictive values and the observed phenotypic values of the validation set was considered to evaluate the predictive ability of model. The CV was repeated 200 times and then calculated the average Pearson correlations.

3 Results

3.1 *Descriptive statistic*

The maximum, minimum and mean numbers of allele per locus were 12, 5 and 8.08, respectively. The PMR and the PR were both in a range of 0–1. For the PR between offsprings, there are seven classes (0, 0.062 5, 0.125 0, 0.250 0, 0.312 5, 0.375 0 and 0.500 0) corresponding to seven different relationships (e.g, unrelated, full-sib, half-sib). Compared with the PR, the PMR value was not a certain value but continuous for a specific relationship. A description statistic of the PMR has been completed on each PR class, which can be seen from Table 1. The distribution of the PMR on each PR class could be seen in Fig. 1. Pearson correlation coefficient between the PMR and the PR has been calculated as 0.872 (P<0.01).

Table 1. Description statistic [mean, standard deviation (SD), maximum (Max), minimum (Min)] of PMR on corresponding PR class

PR class	Mean	SD	Max	Min
0	0.050	0.061	0.281	0.000
0.062 5	0.107	0.063	0.242	0.003
0.125 0	0.115	0.060	0.267	0.017
0.250 0	0.287	0.126	0.473	0.037
0.312 5	0.329	0.034	0.358	0.294
0.375 0	0.356	0.052	0.483	0.251
0.500 0	0.586	0.072	0.808	0.501

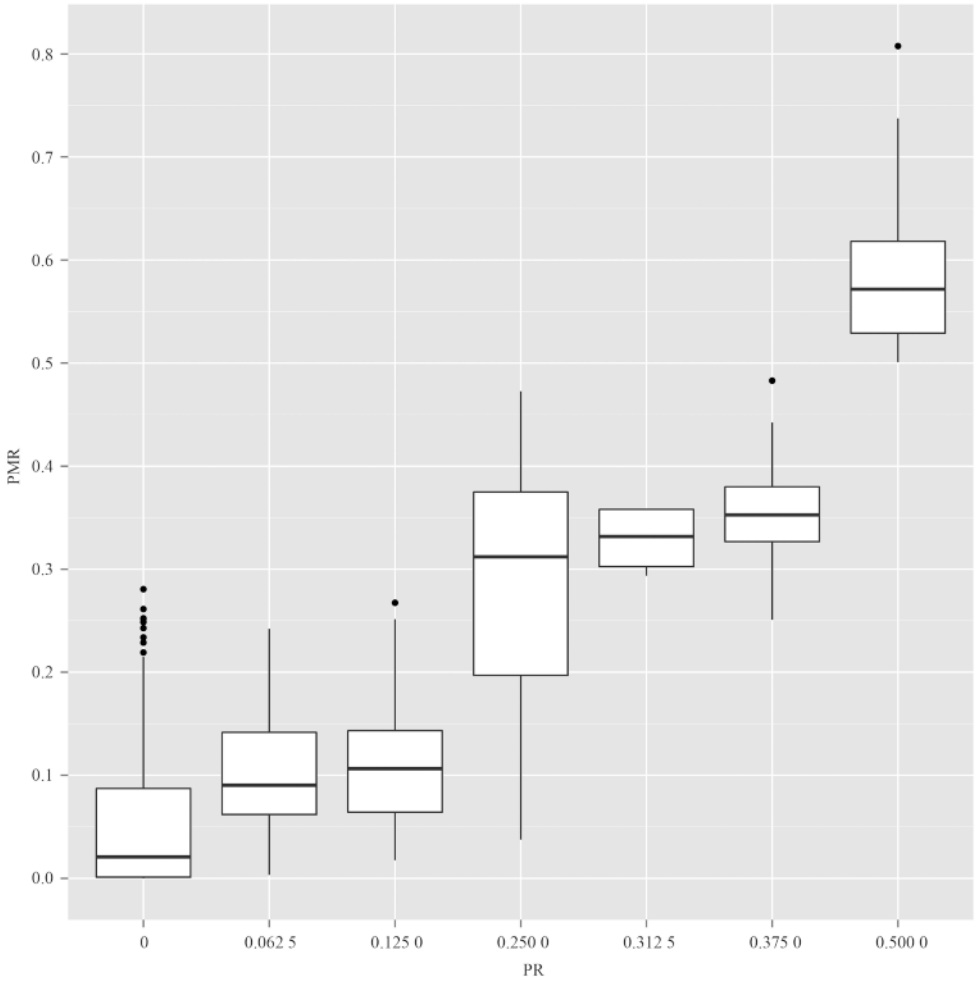

Fig. 1. The distribution of the PMR on each PR class. The x-axis denotes the values of the PR. The median for the PMR is indicated by the centerline, and the first and third quartiles are represented by the edges of the area, which is known as the interquartile range (IQR). The extreme values (within 1.5 times of the IQR from the upper or lower quartile) are represented by the ends of lines extending from the IQR. Points at a greater distance from the median than 1.5 times of the IQR are plotted individually as dark dots.

3.2 *Variance components and genetic parameters*

The estimates of variance components, heritability for the body mass using the PR and the PMR were given in Table 2. For PR, the additive genetic variance and maternal common environmental variance were 4.45 and 1.91. However, for PMR, the two variance components were lower (i.e., 3.15 and 1.91). Heritability estimates for body weight using PR and PMR were high and nearly identical (0.55 and 0.52). Moreover, the standard errors of latter one were lower. After the two heritability estimates were tested to be significantly different from zero (P<0.01), a two-tailed t-test (P>0.05) further showed that there was no significant difference between heritability estimates based on PR and PMR. In addition, Pearson correlation between estimated breeding values (EBVs) based on PR and PMR was 0.88 (P<0.01).

3.3 *Cross-validation*

Two hundred times of the ten-fold CV have been completed in order to compare the predictive ability of models using the PR and the PMR. The relationship between the observed phenotypes and the predicted phenotypes has been displayed in Fig. 2. The average Pearson correlations between the predictive values and observed values of a validation set were both 0.81 when the CV was implemented based on either PMR or PR (Table 2).

4 Discussion

4.1 *Relatedness estimators*

In this study, the two sorts of the relatedness were used for the same turbot population. The PR is estimated from a complete pedigree (three generations pedigree) and the PMR is calculated using the parental markers. The high Pearson correlation coefficient between the PMR and the PR shows that the PMR can recover the genetic relationship which the complete pedigree reflects. The accuracy of the molecular relatedness was affected by the quantity of the molecular markers. Oliehoek et al. (2006) proved that the correlation between the estimated molecular relatedness and the pedigree relatedness increased considerably (>80%) when the number of loci reached 100 loci with ten alleles in a simulated experiment. However, PMR method not only utilizes the information of the molecular markers but also the genetic information from the usable pedigree of two generations. Therefore, the relatively small number of SSR loci still can lead to a satisfying result.

4.2 *Genetic evaluation*

The heritability estimates using the PR and the PMR were close (see Table 2) and the difference between them was not significant (P>0.05) through the t-test. Moreover, the application of the PMR could generate smaller standard errors than those of the PR. Therefore, in terms of the estimation of the heritability, the performance of the PMR was comparable to that of PR. The study of Van Kleunen and Ritland (2005) also showed that, the heritability which is estimated with the pedigree would produce bigger standard errors than those based on the molecular markers.

In the predictive ability of the model, the model using the PMR displayed the equal predictive ability to one using the PR. In addition, from Fig. 2, a conclusion can be made that scatterplots

Table 2. Heritability (h^2), variance components (additive genetic variance, σ_a^2; maternal common environmental variance, σ_d^2; residual variance, σ_e^2), predictive ability (PA) of model estimated based on the PR and the PMR

Relatedness	h^2	σ_a^2	σ_d^2	σ_e^2	σ_p^2	PA
PR	0.55 (±0.22)	4.45	3.64	0.04	8.13	0.81
PMR	0.52 (±0.13)	3.15	1.91	0.97	6.04	0.81

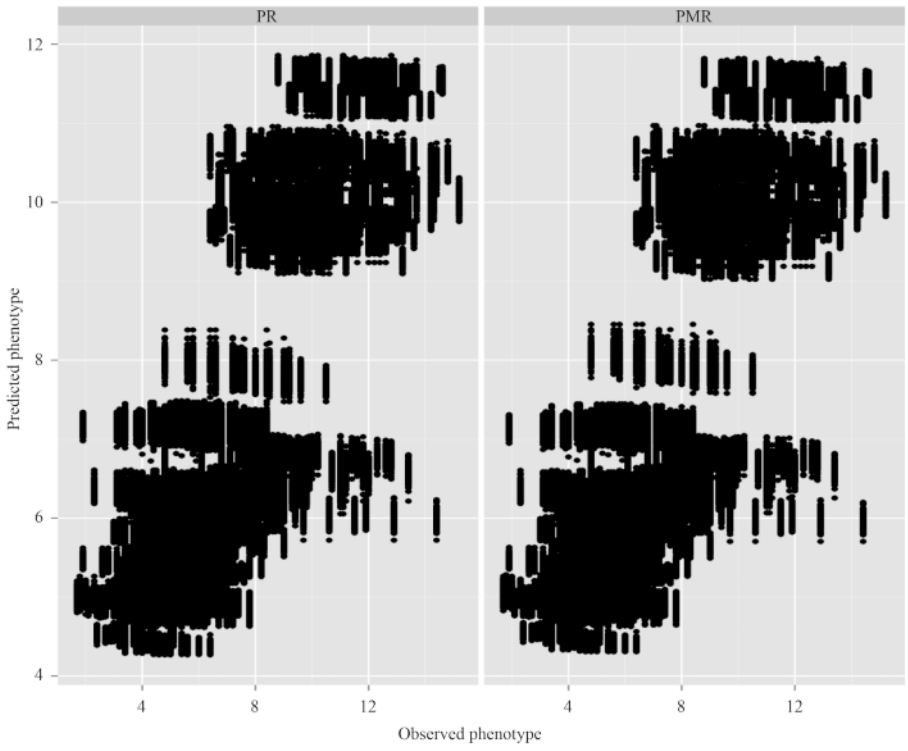

Fig. 2. Scatterplots between the observed phenotypes and the predicted phenotypes using the PR and the PMR. The x-axis and y-axis represent the observed phenotype and the predicted phenotype from 200 times of cross-validations.

between the predicted phenotypes and the observed phenotypes from the PMR and PR method were very similar. Moreover, the correlation between EBVs from the PMR and the PR was high. These all reflected that the PMR can be an alternative choice for PR when predicting phenotypes of individuals. Some research in a genomic selection has also found that models including marker information had higher predictive ability than pedigree-based models using thousands of markers (Crossa et al., 2010; Burgueño et al., 2012; Legarra et al., 2008). The correlations in the CV reflected the correlations between observed and the predicted genetic values assuming there is no environmental effect. Therefore the correlations can further measure the accuracy of the genetic values. The object of breeding is to improve the phenotypes of individuals. The observed genetic gains depended on the correlations in the CV, so the correlation was a direct measure of efficiency of a breeding scheme applying proposed model to this data set. The study of Meuwissen et al. (2001) has demonstrated that the selection on the genetic values predicted from markers could substantially increase the rate of genetic gains in animals and plants in a simulated experiment. In this study, due to few markers compared with the genomic selection, the predictive ability from the PMR did not own advantages over that from the PR. However, the results of this study were encouraging due to the comparable performance of the PMR. The further study including more markers and more families should proceed.

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