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Full Length Research Paper

Comparison of growth performance and genetic diversity of pearl oyster (*Pinctada martensii)* families in a breeding program

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In May of 2009, 28 full-sib families were established by selecting mature breeders in the third generation selected line. Growth performances of the families were compared on the basis of shell height and shell length at juvenile and adult stages. Four families were randomly sampled from the 28 families and their genetic variation was evaluated by using seven microsatellite markers. Significant differences in mean shell length and height among the families were observed at days 110 and 320. A total of 23 alleles were detected at seven microsatellite loci in the four families and the number of the alleles at each locus ranged from 2 to 4, with an average of 3.3. The average observed (Ho) and expected (He) heterozygosity of the four families varied from 0.476 to 0.573 and from 0.584 to 0.679, respectively. The results of the analysis of molecular variance (AMOVA) indicated that 88.4% of the total variations was attributed to the genetic variations within family, whereas only 11.6% variations among families. The results that there exists highly genetic diversity and genetic differentiation among the four families indicates desirable conditions for developing superior lines of the species by family mating.

Key words: *Pinctada martensii*, families, growth performance, genetic variation.

INTRODUCTION

Pearl oyster (*Pinctada martensii)* is one of the most important components of molluscan mariculture in Guangdong, Guangxi and Hainan Provinces of China. The species is primarily cultured for pearl production, and pearls produced by the species are referred to as “South China Sea Pearl”. However, pearl oyster culture in these provinces has recently suffered a lot from slow growth and mass mortalities. Some breeding studies have been recently initiated to improve growth and the resistance to diseases as well as increasingly deteriorating environments. These studies include crossing breeding (Wang et al., 2003), selective breeding (Wang et al., 2004; He et al., 2006; Deng et al., 2009ab; Wang et al., 2011), and marker-assisted breeding (Yu et al., 2007; Shi et al., 2009; Zhao et al., 2010).

Crossing distinct and isolated populations or stocks is a classical approach to improving economically important traits of shellfish species (Zhang and Liu, 2006). According to Falconer (1989), high heterosis can be expected in a hybrid if the parental populations have a high frequency of genes with partial or complete dominance and/or maximum differences in gene frequencies of overdominant loci. Consequently, knowledge of genetic diversity and differentiation among parental populations or stocks is a perquisite for an optimum exploitation of heterosis. For pearl oyster (*P. martensii)*, several researchers have investigated wild populations or stocks by using various marker systems (Li et al., 2002; Yu and Chu, 2006; Tong et al., 2007; Wang et al., 2010; Zhao et al., 2010). Compared with allozyme, RAPD and AFLP, the SSR marker is suitable for studies on genetic diversity of populations, with the advantages of reliability, reproducibility, discrimination, standardization, and cost effectiveness.

In the present study, we carried out evaluation of growth performance of 28 full-sib families with parental stocks

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Table 1. Primer sequence and amplification information of seven microsatellite loci.

Loci	Primer sequences (5'-3')	Size	Annealing temperature	Mg^{2+} (mmol/µl)
M3	F: CCAAGAAAGTCGATCTACCA R: ACAATCCTGACAAGCATAAA	144-162	50	1.6
M7	F: GGACCAGACGTGTTGGTCATT R: TGATTCCTTCTCCCTTTCTC	154-168	53	1.6
M12	F: TAATAAGTACTGTGGATAGGC R: CTCCATTGTTATGTCTTTATC	135-160	50	1.6
M113	F: TGCAGTCATTTGTTCGTG R: TTGCTTTGTCTCCTATGCTATT	202-210	52	1.6
M114	F: GATGCTCAAAATTCTGCTTTA R: TCTTGGGTTGTTCCTCTT	195-263	61	1.6
M287	F: TTAGACCCAATGAAAATCTG R: TTGAAGTTGAACATAGCCAC	230-244	51	1.6
HNUPM45	F: CATGGTTTTGGCCCATTATC R: GGAGGGATGGTGCTAAGATG	110-130	62	1.5

The SSR primers were developed by Qu et al. (2009) and Wang et al. (2010).

sampled from the third generation selected line at juvenile and adult stages, and then analyze genetic variation among four families randomly sampled from the families by SSR marker system, with an attempt to obtain some information for selecting breeders to produce future generation progeny or establishing superior lines by family mating.

MATERIALS AND METHODS

Establishment of families

The breeders were sampled from the third-generation selected line of pearl oyster. The establishment of the selected line was detailed by Wang et al. (2011). Briefly, a based population was established by sampled breeders from Liushagang and Beibuwan stocks in April of 2004. During the period of 2005 to 2008, a three successive generation selection for superior shell length growth in the base population was carried out to produce the third generation selected line. In May of 2009, mature individuals were sampled from the third generation selected line and used as breeders to produce 28 full-sib families by single pair mating. The selected spawners were each killed. Gametes from each sex were obtained and kept separately in 10-L polyethylene tanks. Fertilization was initiated by pouring an adequate amount of sperm of a male into eggs of a female in the tanks.

Larval rearing was the same as previously described by Deng et al. (2009a). The density per tank was kept at 1 individual per ml. Daily feeding consisted of *Isochrysis galbana* from Day 2 to 7, and a mixture of *I. galbana* and *Isochrysis zhanjiangensis* from Day 7 to 50. Feeding ration was increased with ages. Every other day 300 L filtrated sea water were replaced in each tank. Water temperature was at 26.2 ± 1.7°C and salinity was 30‰. At approximately 50 days, juveniles (1 to 2 mm at shell length) were removed from the plastic film, put into 45 × 45 cm^2 nets and reared in Chengwu, Xuwen of Zhanjiang. Five nets (replicates) were designed for each family in the experiment. Shells were cleaned and placed in new nets at appropriate internals. Densities adjusted at sampling time were 400 per net at days 45, 100 per net at days 110, and 30 per net at days 180 and 320. All individuals within a line were mixed at each sampling time and randomly distributed again in the nets.

Growth trait measurement

At days 110 and 320 post-fertilization, 50 individuals were randomly sampled from each net. Shell length, Shell height and shell length of each individual were measured using vernier calipers (0.02 mm accuracy).

Genetic evaluation of four families

At days 320, four families (F_2, F_4, F_{18} and F_{24}) were randomly sampled from the 28 full-sib families and subjected the experiment where genetic variation of the families was evaluated by SSR marker system. Total 30 individuals were sampled from each of the four families. Genomic DNA was extracted from muscle tissue according to the method of Zhao et al. (2010). A total of 7 pairs of polymorphic SSR primers developed by Qu et al. (2009) and Wang et al. (2010) were used in the study. Table 1 listed the sequences of the primers. PCR was composed of 10 ng DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.1 µM dNTPs, 0.2 µM of each primer and 1.0 U of Taq DNA polymerase, in a total volume of 10 µl. The following thermal

profile was used: initial denaturation at 94°C for 3 min, 10 cycles of 40 s at 94°C, 40 s at 60 to 50°C (1°C decrease per cycle), 1 min at 72°C and 25 cycles of 40 s at 94°C, 40 s at 50°C, 1 min at 72°C and final extension at 72°C for 10 min (Zhao et al., 2010).

Statistical analysis of data

A one-way analysis of variance (ANOVA) was used to analyze differences in mean shell height and length among the 28 full-sib families at juvenile and adult stages, and then followed by Tukey's multiple range tests to determine significant differences among means. All analyses were performed by SPSS (Statistical Program for Social Sciences) 11.0 software for Windows. Significance level was declared at $P < 0.05$. SSR is a codominant marker system. Family genetic analysis was performed using the model for codominant markers with diploid individuals in two levels: within families and among families.

The following parameters were estimated: effective number of alleles per locus (*Ne*), number of detected alleles (*Na*), observed (*Ho*) and expected (*He*) heterozygosities. These parameters were calculated using GENEPOP version 3.1c (Raymond and Rousset, 1995). Hardy–Weinberg expectations were tested by the exact P values and calculated by a Markov chain randomization method (Guo and Thompson, 1992) using GENEPOP version 3.1c. Analysis of molecular variance (AMOVA) was used to partition total genetic variation among individuals within families and performed in GENALEX (Peakall and Smouse, 2006).

RESULTS

Growth comparison

There existed significant differences in mean shell length and height among the families at two sampling ages. The F_{24} had the consistently larger shell length growth than the other families at days 110 and 320. The F_2 had the slowest shell length growth at days 110, while the F_{26} had the slowest shell length growth at days 320. At days 320, 13 of 28 families had larger mean shell length and height than the average, with the mean shell length and height of the all families measured being 42.68 ± 4.15 mm and 40.59 ± 4.19 mm, respectively (Table 2).

Genetic variation among the four families

Seven SSR markers were used in PCR of 120 individuals from the four families. A total of 23 alleles were detected and amplified fragments ranged from 110 to 260 bp. For each SSR locus, the number of alleles ranged from 2 to 4, with an average of 3.3. Four alleles were detected at M3, M114 and M287 loci, three alleles at M7, M12 and HNUPM45 loci and two alleles at M113. Genetic variation among the families were observed by genetic diversity parameter analysis and listed in Table 3. The F_{24} had the highest levels of observed and expected heterozygosity. Deviation from HWE tested for all family-locus combinations revealed significant heterozygote deficit. The F_2 and F_{24} had the highest number of loci (five) deviating from Hardy-Weinberg equilibrium (Tables 3 and 4).

AMOVA was carried out to investigate the significance of genetic separate of the four families. The results showed that the variations between individuals within family accounted for 88.4% of the total variations, whereas only 11.6% were resulted among families (Table 5). *N*ei's genetic distance was measured using the tables allele frequencies. As listed in Table 6, the genetic distance in each family combination ranged varied from 0.1649 between F_2 and F_{24} to 0.0306 between F_2 to F_4, respectively, with an average of 0.098.

DISCUSSION

Growth comparison of the families

In the present experiment, the results of ANOVA showed that there existed significant differences in growth traits examined among the families at two sampling ages (Table 1), which were similar with the reports of He et al. (2006) and Gu et al. (2010). For example, He et al. (2006) found that significant differences in growth and survival of seven full-sib families of pearl oyster *P. martensii*. High variation among the families indicated a desirable condition for future family selection or family crossing in the breeding programs. Due to similar rearing conditions, the effect of environmental factors on adult growth performance of the four families could be minimized. Consequently, there might be genetic factors contributing to growth performance differences among the families.

Genetic variation of the families

Several researchers have used SSR marker system to detect genetic diversity of cultivated stocks of pearl oyster *P. martensii* (Tong et al., 2007; Qu et al., 2009; Wang et al., 2010; Zhao et al., 2010). For example, Qu et al. (2009) reported that effective number of alleles (*Ne*), observed (*Ho*) and expected (*He*) heterozygosities were 3.334, 0.425 and 0.600 for Dayawan stocks, 2.770, 0.393 and 0.590 for Beihai stocks, 2.578, 0.415 and 0.615 for Sanya stock, respectively. *Ne*, *Ho* and *He* of the Liushagang stock were 2.1315, 0.2727 and 0.4645, respectively (Tong et al., 2007). An evaluation on deviation from Hardy-Weinberg equilibrium at each locus for each family found a significant deficiency of heterozygotes.

The breeders for the families were sampled from the third-generation selected lines. The significant deficiency of heterozygotes can be explained in part by the existence of inbreeding resulted from a limited number of breeders were used to produce each generation (Deng et al., 2009a). Several laboratory errors can also contribute to an overestimation of homozygosity:

(i) Alleles remained undetected due to competition for polymerase and other reagents during amplification in the PCR reaction;

Table 2. Growth comparison of 24 families of pearl oyster (*P. martensii)* at different ages.

Families	Age (days)			
	110		320	
	SH (mm)	SL (mm)	SH (mm)	SL (mm)
F_1	18.74 ± 2.37	16.92 ± 2.27	41.48 ± 3.56	42.22 ± 3.55
F_2	11.76 ± 1.33	9.82 ± 1.17	36.78 ± 5.53	33.79 ± 3.31
F_3	29.27 ± 3.38	26.07 ± 3.22	43.62 ± 2.83	40.58 ± 2.90
F_4	19.08 ± 1.80	15.79 ± 1.33	42.76 ± 4.05	41.02 ± 4.93
F_5	25.14 ± 2.73	21.69 ± 2.59	40.30 ± 4.86	38.40 ± 4.51
F_6	26.82 ± 3.45	26.14 ± 3.48	44.45 ± 4.41	42.95 ± 4.54
F_7	22.17 ± 2.32	18.33 ± 2.23	48.76 ± 5.43	42.72 ± 4.12
F_8	23.72 ± 2.48	21.18 ± 2.17	38.14 ± 4.44	38.28 ± 3.43
F_9	26.30 ± 3.41	23.64 ± 2.63	41.77 ± 3.47	40.98 ± 4.29
F_{10}	23.52 ± 2.68	19.65 ± 2.62	41.16 ± 2.90	40.02 ± 4.18
F_{11}	31.10 ± 3.70	27.23 ± 2.78	45.29 ± 4.23	46.10 ± 4.69
F_{12}	23.72 ± 2.17	21.18 ± 1.89	41.72 ± 4.79	42.78 ± 4.48
F_{13}	26.3 0± 3.31	23.64 ± 2.12	41.76 ± 3.61	39.14 ± 4.24
F_{14}	20.47 ± 2.18	15.91 ± 1.74	39.10 ± 3.65	38.43 ± 5.01
F_{15}	27.38 ± 3.52	24.12 ± 2.20	40.10 ± 4.42	39.76 ± 3.11
F_{16}	30.97 ± 3.14	27.59 ± 2.86	46.12 ± 4.53	43.18 ± 3.75
F_{17}	21.24 ± 2.13	19.92 ± 2.38	43.88 ± 3.94	42.66 ± 4.53
F_{18}	24.80 ± 2.43	20.55 ± 2.46	45.48 ± 4.74	40.72 ± 4.14
F_{19}	21.17 ± 2.17	18.19 ± 2.14	48.38 ± 4.99	48.13 ± 6.61
F_{20}	26.69 ± 2.78	22.29 ± 2.56	49.40 ± 3.81	47.94 ± 4.69
F_{21}	23.79 ± 2.65	20.68 ± 2.08	43.14 ± 4.80	42.78 ± 3.98
F_{22}	25.32 ± 2.32	22.21 ± 2.36	49.27 ± 5.68	46.80 ± 6.07
F_{23}	24.52 ± 2.65	21.66 ± 2.27	40.13 ± 4.71	38.37 ± 5.72
F_{24}	31.30 ± 2.89	29.08 ± 3.42	51.62 ± 5.26	50.68 ± 5.76
F_{25}	21.70 ± 2.10	19.91 ± 2.24	39.28 ± 4.20	36.26 ± 4.03
F_{26}	15.75 ± 1.50	12.49 ± 1.27	35.29 ± 3.07	32.85 ± 3.44
F_{27}	27.36 ± 2.83	22.26 ±2.54	40.35 ± 4.02	38.12 ± 3.72
F_{28}	12.75 ± 1.50	12.49 ± 1.27	35.68 ± 5.48	34.95 ± 3.89
Average	23.67 ± 4.95	20.74 ± 4.66	42.68 ± 4.15	40.59 ± 4.19
P-value	< 0.05	< 0.05	< 0.05	< 0.05

S L: Shell length; SH, shell height.

Table 3. Genetic diversity of four families at seven microsatellite loci.

Families	Parameter	M3	M7	M12	M113	M114	M287	HNUPM45
F_2	*N*a	2	3	3	2	3	4	2
	*N*e	1.557	2.539	1.514	1.763	2.662	2.998	1.684
	*H*e	0.358	0.833	0.339	0.633	0.744	0.864	0.567
	*H*o	0.333	0.606	0.333	0.433	0.624	0.666	0.406
	P_{HW}	ns	*	ns	*	***	**	*
F_4	*N*a	3	3	2	2	3	3	3
	*N*e	1.729	2.597	1.338	1.724	2.990	2.790	2.632
	*H*e	0.500	0.767	0.296	0.600	0.960	0.785	0.620
	*H*o	0.422	0.615	0.252	0.420	0.666	0.663	0.578
	P_{HW}	ns	ns	ns	*	*	**	***
F_{18}	*N*a	3	3	2	2	2	2	3

Table 3. Contd.

	*N*e	2.436	2.469	1.342	1.867	1.942	1.835	2.651
	*H*e	0.833	0.800	0.300	0.733	0.345	0.455	0.623
	*H*o	0.589	0.595	0.255	0.464	0.485	0.367	0.574
	P_{HW}	**	ns	ns	**	ns	ns	***
F_{24}	*N*a	4	3	3	2	4	2	2
	*N*e	3.375	2.486	2.666	1.998	3.930	1.963	1.600
	*H*e	0.756	0.667	0.725	0.433	0.812	0.862	0.500
	*H*o	0.734	0.598	0.625	0.449	0.746	0.490	0.375
	P_{HW}	**	**	**	ns	***	**	ns

Na: Number of detected alleles; Ne: effective number of alleles per locus; Ho: observed and He: expected heterozygosity; P_{HW} = Hardy-Weinberg probability test: ns not significant, *P < 0.05, **P < 0.01, ***P < 0.001.

Table 4. Mean allelic pattern analyzed families of pearl oyster (*P. martensii)*.

Families	*Ho*	*He*	*A*	*Ar*	*Fis*
F_2	0.485	0.619	2.42	8.59	0.185
F_4	0.516	0.646	2.28	9.35	0.265
F_{18}	0.476	0.584	2.00	5.73	0.113
F_{24}	0.573	0.679	2.57	6.92	0.174

Ho: Observed and He: expected heterozygosity; A: average number of alleles per family; Ar: allelic richness; Fis: fixation indice.

(ii) The setting of the threshold of band intensity to detect alleles was too strict, and
(iii) A heterozygous locus carrying a null allele was scored as homozygous.

Implication for breeding programs

The breeders used to produce the families in the present studies were sampled from the third-generation selected lines. The selected line exhibited 20.9% increase in adult shell length growth after a three successive generation selection for superior growth in the base population compared to the control (Wang et al., 2011). A major concern on selective breeding programs, however, is the existence of inbreeding resulted from a small number of breeders and consequently causes inbreeding depression on fitness-related traits. How to control the inbreeding level in the stocks and maximize selection response? In our breeding programs, some strategies were applied as follows:

(i) A combination of family and mass selection. A total of 64 families (28 families in May of 2009 and 36 families in September of 2009) were established by sampled parental animals from the third generation selected lines in 2009. Family performance was evaluated based on growth measurement at adult stages, and then superior families over the average were selected into a breeding pool and used as breeders to produce future generation.
(ii) To make use of heterosis. A large number of lines were designed to establish by family mating and superior combinations were identified.

The identification of superior combinations in hybrid programs is generally a rather costly and time-consuming task due to the great number of lines normally generated. However, several studies have been carried out to evaluate the potential to predict the hybrid performance from genetic distance estimates generated by molecular data, due to the fact that the magnitude of heterosis often depends on the level of genetic diversity or heterozygosity between the populations and/or strains used for the crossing program (Bentsen et al., 1998; Shikano and Taniguchi, 2002; Wang and Xia, 2002; Hulak et al., 2010). By using SSR and RAPD markers to estimate the amount of heterosis in the guppy *Poecilia reticulate*, Shikano and Taniguchi (2002) found that the amount of heterosis in salinity tolerance was correlated with the *N*ei's genetic distances and dissimilarity values.

In common carp, several researchers detected that the performance of hybrid lines was corresponded with genetic distances. For example, Wang and Xia (2002) crossed a German and a Russian mirror stocks with a local Xingguo red carp. They found that the heterosis from the hybrid progeny from Russian mirror carp and Xingguo red carp was significantly higher than that from German mirror carp and Xingguo red carp. The amount of heterosis was consistent with genetic distances. These studies aforestated have demonstrated that crossing population/or lines with higher genetic distances could result in greater heterosis in the progeny.

Conclusion

In the present study, we have observed significant

Table 5. Analysis of molecular variance (AMOVA) of four families of pearl oyster (*P. martensii).*

Source of variation	Degree of freedom	Source of squares	Variance component	Percentage of variation
Among families	3	38.121	0.338 Va	11.6%
Within families	117	299.500	2.559 Vb	88.4%
Total	120	337.621	2.897	
*F*st = 0.116				

Table 6. *N*ei's genetic distance among four families of pearl oyster (*P. martensii)* measured by microsatellite markers.

Families	F_2	F_4	F_{18}
F_4	0.0306		
F_{18}	0.0331	0.0415	
F_{24}	0.1649	0.1627	0.1574

differences in growth performance among the 28 full-sib families. Several superior families (over the average) have been selected for candidate based on their growth performance to produce future generation progeny.

Molecular data showed that highly genetic differentiation and genetic distances among the four families randomly sampled from the 28 full-sib families, indicating a desirable condition for developing lines by family mating. Our results also showed that SSR marker is highly appropriate for investigating genetic diversity of pearl oyster families and assisting in selecting breeders in the breeding programs.

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