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Studies on Genetic Diversity of Selected Population of Hybrid Scallop *Chlamys farreri* (♀) × *Patinopecten yessoensis* (♂) by Microsatellites Markers

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Abstract

The growth superiority of hybrid scallop *Chlamys farreri* (♀) × *Patinopecten yessoensis* (♂), as the following successive generation selection have been reported. However, the data about the genetic diversity in those population remains unexplored. In this study, the genetic structure analysis of F_1, F_2 and F_3 were conducted by PCR with 10 Simple Sequence Repeats (SSR) primers. It showed that a total of 68 alleles were detected, and the number of alleles per locus ranged from 3 to 11, Polymorphism Information Content (*PIC*) per locus ranged from 0.4729 to 0.8429. And, the average observed heterozygosity (H_o) of the three populations were 0.6100, 0.6975 and 0.7750, while the average expected heterozygosity (H_e) were 0.7607, 0.7751 and 0.7379 respectively. F_{st} values among the three populations were also low (F_{st}<0.05) which suggested low genetic differentiation between each two populations. In all, those data indicated the genetic structure challenge caused by hybridization and selection, supplying a new angle to understand artificial selective breeding.

Keywords: scallop, hybrid breeding, genetic diversity, microsatellite DNA markers

1. Introduction

The scallop *Chlamys farreri*, a native bivalve in China, is one of the most important marine farming species in Northern China. However, massive mortality has caused catastrophic losses to its aquaculture since 1996, which resulted in a sharp production decline. The germplasm quality degeneration have been considered as one major reason for the massive scallop mortality. Therefore, to breed new scallop species with high resistance is an effective method to change the current status quo.

The research on distant hybridization breeding of scallop *C. farreri*(♀) × *Patinopecten yessoensis*(♂) have been carried out (Zhou, Yang, & Liu, 2003; Lv, Yang, Wang, Liu, & Zhou, 2006), and surprisingly, the first hybrid generation owned prominent heterosis performance which has been cultivated in large-scale area. The second and third generation individuals with stronger resistance, faster growth than scallop *C. farreir* have also been selected for farming (Yang, Wang, Liu, & Zhou, 2003). However, during the process of selective breeding, many uncertain factors, such as increasing risk of inbreeding, decreased number of effective groups, may lead to lower genetic diversity, even the genetic effects. Therefore, it is necessary to detect the genetic variation, to understand the changes of genetic structure for developing appropriate scientific measures so that we can smoothly control the progress of selective breeding.

Microsatellite marker (Simple Sequence Repeats, SSR), due to its simple, fast, good stability, higher polymorphism, informative genetic variation and followed Mendelian codominant genetic, has been widely used in various fields as a molecular marker (Li, Park, Endo, & Kijima, 2004; Sakamoto, Danzmann, Okamoto, Ferguson, & Ihssen, 1999; Liu et al., 2004). In terms of hybrid scallop, isoenzyme (He, Yang, Wang, Liu, & Zhou, 2006), RAPD and other labeling techniques were particularly used, however, SSR analysis of different hybrid scallop populations generated from *C. farreri* (♀) × *P. yessoensis* (♂) have not been reported. In this study, SSR was employed to analyze the genetic variation of three selective breeding population, to explore the impact of

selection process on its genetic structure, which could provide a theoretical basis for molecular marker-assisted breeding.

2. Materials and Methods

2.1 Sample Collection

Mature female *C.farreri* and male *P. yessoensis* were collected as parents from Changdao, Shandong Province. The F_1 hybrid were reproduced from mother *C. farreri* and father *P. yessoensis* while F_2 generation from F_1 by self-fertilized, F_3 from F_2, respectively. 30, 40, 40 individuals were selected randomly from F_1, F_2, F_3 population, and stored at -80 °C for DNA extracted, respectively.

2.2 Preparation of Template DNA

The genome DNA was extracted from adductor muscles of hybrid individual using Phenol-chloroform method. In detail, about 100 mg tissue was sampled into a 1.5 ml centrifuge tube, and then 500 µl homogenization buffer (10 mM Tris-Cl, pH 8.0; 100 mM EDTA, pH 8.0), 50 µl 10% SDS and proteinase K with final concentration of 50 µg/ml were also added. After being mixed adequately, the sample were digested at 55 °C for 3 h, and then the proteins were extracted using phenol, phenol: chloroform (1:1), chloroform, isoamyl:alcohol (24:1), successively. And following, the nucleic acid was precipitated with ethanol, and then dissolved in ddH_2O. The concentration and quality of extracted DNA were detected by RNA/DNA quantitative analysis using Nanodrop 2000 and agarose gel electrophoresis, respectively. The concentration of genome DNA was diluted to 50 ng/µl and then stored at -20 °C.

2.3 PCR Amplification

10 pairs of primers (Table 1) were selected from reported SSR primers of *C.farreri* and *P. yessoensis*, to ensure its availability for amplification in hybrid offspring in this study. PCR reactions were carried out in a 10 µl reaction volume on a PCR amplification instrument, including 1 µl 10 × buffer, 0.6 µl Mg^{2+} (25 mM), 1 µl dNTP (each 2 mM), 1 µl forward/reverse primer (10 µM) each, 50 ng template DNA, 0.5 U Taq DNA polymerization enzyme (Promega), and PCR-grade water was replenished to 10 µl. PCR reaction program consisted of 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 72 °C for 30 s, and finally 72 °C for5 min. The PCR products were detected by 6 % denaturing polyacrylamide gel electrophoresis, silver staining for detecting polymorphism. The amplified brands were counted by manual mothed for analyzing by software.

Table 1. Microsatellite primers used in this experiment

Loci	Primer sequence	Annealing temperature	Repeat	GenBank Accession
CFMSM009	F:GTAGTCACATGATGACATAGAG R:CACAACTCCGTCAATCATTCTC	56	$(AG)_4G(AG)_5$… $(AG)_5$	DQ104704
CFMSM014	F:CATCTGATATGGCAGCTGATAC R: GAACTAACGAGGAGACAACTG	60	$(AG)_{10}$ …$(AC)_4$	DQ104705
CFMSM020	F: CAAAGGCATTTGTAGGAAGGC R: ACGGCACTTCGTTGATTAAC	62	$(CAC)_{11}$	DQ104709
CFMSP011	F: CAAAACCAACTCCTTCACAAC R: GGCGATATTCCACCTGACC	62	$(ACAAA)_5$	AY682110
CFAD213	F: ATTAGTTGTGAAGCAGTCCT R:CTTCTCTCAATCATTTCACTATC	56	$(GA)_{14}$	EF148875
CFFD143	F: CGCCAACCTTGCAGTATCTG R: TTCTTTCCCTCTTCTGTCCC	58	(GA)3CA$(GA)_3$ GGAA$(GA)_{11}$	EF148943
CFBD075	F: TTACTATCCCTACCCCAGAG R:CACTAACCCATTACAAACACAAG	60	$(TGTC)_9(TC)_5TG(TC)_{23}$ … $(TC)_6$ … $(CT)_7$	EF148893
CFAD053	F:CATTGACACAGTTACAGTTCAC R: GCAACAGGATTAGGCACAAG	56	$(CT)_{20}$	EF148859
S7259	F: CGTCCTTAAATGACCTTA R: GAAATTCCAGTGTTCGTA	60	$(AACC)_6$	AY164679
S9090	F: GAGGAAGAAACATAGTAA R: CTACATCAGCTACATCTC	58	$(TTAA)_5$	AY164680

2.4 Data Analysis

According to the genotype, the value of polymorphic information content (*PIC*), heterozygosity (*Ho*), heterozygosity (*He*), allele number (a), effective number of alleles (a_e), similar coefficient and genetic distances among, and F-statistics of the three groups were calculated using Popgen 32 (Version 1.31). F_{st} value range from 0 to 0.05 was considered as low population genetic differentiation, while 0.05-0.15 as middle level, 0.15-0.25 as high level and above 0.25 as significantly high level.

3. Results

3.1 Genetic Diversity of Loci

10 pairs of primers with high polymorphism were used to perform PCR amplification with 110 individuals from F_1, F_2 and F_3 populations. In all, 68 alleles were obtained, and the numbers of allele for each locus ranged from 3 to 11. *PIC* value was from 0.4729 to 0.8429. The average observed heterozygosity was F_2>F_1>F_3, with the values of 0.7025, 0.6893, 0.6900, respectively, while the average expected heterozygosity were 0.8315, 0.7751, 0.7379, respectively. According to *P* values of genotype, the value of the Hardy-Weinberg equilibrium deviated significantly in three generations (as shown in Table 2), such as CFMSM009 in the F_1 and F_2 populations, CFMSP011 in F_3 population and CFAD213 in F_2 and F_3. Some representative amplified brands were exampled in Figure 1.

Table 2. Genetic variability at 10 microsatellite loci in three populations

loci	populations	a	a_e	*PIC*	*Ho*	*He*	*P*-values
CFMSM009	F1	3	2.3407	0.5089	0.3333	0.5825	0.0068**
	F2	3	2.0901	0.4613	0.2750	0.5282	0.0146*
	F3	3	2.0566	0.4380	0.1500	0.5203	0.0000**
CFMSM014	F1	7	4.6512	0.7613	0.6667	0.7983	0.2517
	F2	7	5.9590	0.8110	0.7000	0.8427	0.0942*
	F3	6	4.1995	0.7244	0.7000	0.7715	0.3040
CFMSM020	F1	11	7.2581	0.8468	0.7333	0.8768	0.2247
	F2	10	6.8085	0.8365	0.9000	0.8963	0.4101
	F3	10	6.4000	0.8258	0.9750	0.8544	0.0823*
CFMSP011	F1	7	3.7657	0.6956	1.0000	0.7469	0.0110*
	F2	7	4.3096	0.7327	0.9750	0.7775	0.0162*
	F3	6	3.2196	0.6387	0.9737	0.6986	0.0001**
CFAD213	F1	6	3.1304	0.6363	0.4667	0.6921	0.0315*
	F2	6	3.7915	0.6964	0.3750	0.7456	0.0001**
	F3	6	2.6801	0.5663	0.3750	0.6348	0.0005**
CFFD143	F1	8	4.6036	0.7509	0.8667	0.7960	0.2007
	F2	7	4.1290	0.7227	0.8500	0.7674	0.4974
	F3	6	3.8508	0.7009	0.8000	0.7497	0.7390
CFBD075	F1	6	4.2857	0.7328	0.6333	0.7797	0.0106*
	F2	6	3.6036	0.6885	0.6750	0.7316	0.2184
	F3	6	3.1809	0.6466	0.5500	0.6943	0.0290*
CFAD053	F1	8	6.7924	0.8213	0.7667	0.7469	1.0000
	F2	7	6.7368	0.8331	0.7000	0.8623	0.0158*
	F3	8	5.9530	0.8118	0.7692	0.8423	0.4489
S7259	F1	6	4.9451	0.7670	0.4333	0.8113	0.3268
	F2	6	4.9231	0.7664	0.5750	0.8070	0.0392*
	F3	6	4.3716	0.7390	0.6000	0.7810	0.0837
S9090	F1	6	4.2254	0.7279	1.0000	0.7763	0.0280*
	F2	6	5.3872	0.7873	1.0000	0.8247	0.1639
	F3	6	5.5817	0.7957	1.0000	0.8315	0.1643
Mean	F1	6.8000	4.5998	0.7241	0.6900	0.8315	
	F2	6.5000	4.7736	0.7336	0.7025	0.7751	
	F3	6.2000	4.1494	0.6930	0.6893	0.7379	

Note: * indicates significant deviation (*P* <0.05); ** indicates highly significant deviation (*P* <0.01).

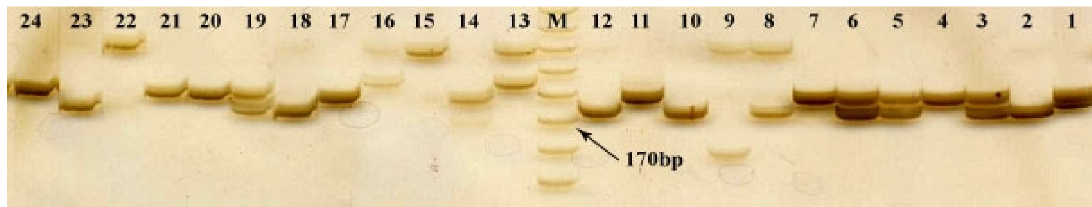

Figure 1. Demonstration of microsatellite locus amplified by CFMSM020 primer pairs in three populations
M: Marker; 1-8: F_1 population; 9-16: F_2 population; 17-24: F_3 population

3.2 Genetic Similarity Index, Genetic Distance and Cluster Analysis

The genetic distance and genetic similarity in three population was shown in Table 3. It showed that the genetic distance between F_1 and F_3 generation was the largest, while the value between F_2 and F_3 populations was the smallest. According to the genetic distance among groups, UPGMA were used to analyze relationship among three generations populations (Figure 2). It indicated that the F_2 and F_3 clustered firstly and then cluster with F_1.

Table 3. Genetic identity and genetic distance in three populations

Population	F_1	F_2	F_3
F_1	—	0.9465	0.9176
F_2	0.055	—	0.9716
F_3	0.086	0.0288	—

Notes: data below diagonal are genetic distance; data above diagonal are genetic identity.

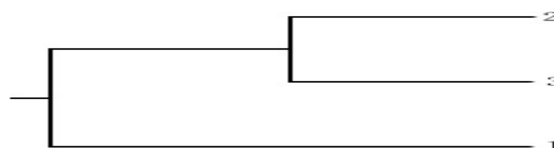

Figure 2. UPGMA dendrogram among three populations
1: F_1 population; 2: F_2 population; 3: F_3 population

3.3 Population Variation

The F_{st} values between F_1 and F_2, F_1 and F_3, F_2 and F_3 were 0.0079, 0.0196, 0.0028, respectively, which indicated that the genetic differentiation among the three generations was weak. The overall genetic differentiation coefficient value was 0.0169, which revealed that only 1.69 % genetic variation was from groups, while 98.31% variation was from individuals.

Table 4. *F*-statistica for three populations of hybrid scallop at ten microsatellite

loci	F_{is}			F_{st}
	F1	F2	F3	
CFMSM009	0.4180	0.4727	0.7080	0.0082
CFMSM014	0.1507	0.1588	0.0812	0.0148
CFMSM020	0.1495	-0.0549	-0.1556	0.0075
CFMSP011	-0.3616	-0.2698	-0.4124	0.0135
CFAD213	0.3143	0.4907	0.4018	0.0376
CFFD143	-0.1072	-0.1216	-0.0806	0.0235
CFBD075	-0.1739	0.0657	0.1978	0.0090
CFAD053	-0.0439	0.1780	0.0755	0.0355
S7259	0.4568	0.2784	0.2220	0.0042
S9090	-0.3100	-0.2279	-0.2180	0.0132
mean	0.0775	0.0822	0.0539	0.0169

Table 5. F_{st} values of pairwise comparison among different population at 10 microsatellite

Groups	F_1	F_2	F_3
F_1	—	0.0079	0.0196
F_2		—	0.0028
F_3			—

4. Discussion

In this study, the data revealed that the genetic diversity among three populations was not significantly different, although the proportion of polymorphic loci and genetic diversity decreased from F_1 to F_3. This situation was similar to some other reports (Hedgecock, Chow, & Waples, 1992; Mgaya, Gosling, Mercer, & Donlon, 1995; V. Sbordoni, De Matthaeis, M. C. Sbordoni, La Rosa, & Mattoccia, 1986). For example, the genetic diversity in several generations of Chinese shrimp were reported by Zhang et al. (2005) using SSR technology, which showed that the average observed heterozygosity dropped down from the first generation to the sixth. And it was same as the studied in American oysters (Yu and Guo, 2004) and Japanese flounder (Liu et al. 2005). Selective breeding is a complex process, the external environment and artificial selection pressure may cause fluctuations of population genetic variation. And on the other hand, during the artificial breeding process, due to the small effective population the inbreeding rate might increase which caused inbreeding depression and bottleneck effect. And also, high-intensity artificial directional selection might lead to the genetic deterioration and introgression, that these two factors could cause the loss of some particularly alleles, especially some rare gene allele in the breeding population. Therefore, genetic variation of breeding populations should be detected timely in the process of selective breeding.

Coefficient of genetic differentiation is an important parameter reflecting the degree of genetic differentiation among populations. Under controlled conditions, artificial selection, mutagenesis, hybridization could damage balance of genetic, which caused changes of genes and genotype, so the genetic characteristics within a population will also change. In this study, from the first generation to the third generation of breeding populations, the genetic structure within populations changed. Although the genetic structure had a certain differentiation, the degree of differentiation was not significant. The genetic variation analysis showed that genetic differentiation among generations of artificial breeding populations was smaller and differentiated was mainly from individuals. As studies in Chinese shrimp reported by Li et al. (2006), the genetic differentiation coefficient between adjacent groups showed a decreasing trend, and genetic similarity degrees of individuals within a population showed an upward trend with the increase in generation, which indicated the breeding populations tend stability after years of breeding.

Heterozygosity is an important parameter to measure the genetic diversity of populations. In this study, the mean observed heterozygosity were from 0.6893 to 0.7025 among the three generations. High significant deviation phenomena of Hardy-Weinberg equilibrium were observed, and the F_{is} values indicated that seven loci in three populations showed a certain degree loss of heterozygosity, which indicated dumb allele might exist, which was similar to the report of Chinese shrimp (Zhang et al., 2005)). This study enriches the data of hybrid breeding of scallop, opening a new angle to understand genetic diversity change of different generation during artificial selective breeding.

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