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The impact of successive mass selection on population genetic structure in the Pacific oyster (*Crassostrea gigas*) revealed by microsatellite markers

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Abstract To evaluate the impact of mass selection on genetic structure in artificially closed populations of the Pacific oyster *Crassostrea gigas*, we performed mass selection over six generations on two stocks from Japan and Korea and analyzed their temporal genetic variation and structure using 18 microsatellite makers, which were compared with the base populations of the two selected lines and one wild population from China. The average numbers of alleles (*N*a), mean observed heterozygosities (*H*o), and expected heterozygosities (*H*e) varied over generations in the two selected lines (selected lines of Japan, *N*a = 10.7–14.9, *H*o = 0.757–0.846, *H*e = 0.778–0.871; selected lines of Korea, *N*a = 9.4–17.3, *H*o = 0.736–0.865, *H*e = 0.744–0.854). There was no significant reduction in heterozygosity in the two selected lines. However, the average number of alleles per locus was significantly lower in the fifth and sixth generations of the two selected lines compared with that in the base population and wild population ($P < 0.05$), suggesting that the successive mass selection in closed populations may increase the sensibility of rare alleles to genetic drift. Equalizing the sex ratio of parents and reducing the selection intensity properly with the increase of selective generations is recommended to minimize the deleterious effect of genetic drift and bottleneck caused by successive mass selection. The information obtained in this study is useful for the design of appropriate management strategies for selective breeding of *C. gigas*.

Keywords Mass selection · Microsatellite · Genetic variation · Pacific oyster · Population genetic structure

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Introduction

The Pacific oyster, *Crassostrea gigas*, is endemic to the Pacific coast of Asia but has become the primary oyster species supporting the worldwide shellfish industry (Ruesink et al. 2005). According to the FAO (2016), the Pacific oyster is grown in 27 countries and is the most highly produced mollusk species in the world. China, Korea, and Japan are the top three oyster-producing countries worldwide and the production of the Pacific oyster in the three countries reached 1550 thousand metric tons in 2015 (FAO 2016).

Due to the large spread of their cultivation worldwide, the Pacific oyster is cultured using a broad variety of rearing techniques, from intensive to semi-intensive. Intensive culture is restricted to early stages (i.e., larvae in hatcheries and seed in nurseries) because large-scale production of their food (i.e., phytoplankton) is not cost-effective at later stages, which means that it is in an excellent position to benefit from the development of genetically improved strains. During the past decades, several selective breeding programs for the Pacific oyster have been launched in couple of countries (Ward et al. 2000; Langdon et al. 2003; Degremont et al. 2010). Quantitative genetic selection has been used in these programs to improve any traits associated with additive genetic variance and obtained encouraging results, particularly in enhanced commercial yields and resistance to disease. While mass selection can improve given traits quickly, the selection of individuals on the basis of best performance of certain traits without regarding to pedigree information can result in inbreeding and loss of potentially valuable alleles and net additive genetic variation (Bentsen and Olesen 2002). However, in most cases, little attention has been paid to the variation of genetic diversity among the mass selection lines, especially for the successive selection within a closed population.

Because of the high fecundity and high variance in reproductive success of oyster species, relatively few animals can form the contributing parents of the next generation (Boudry et al. 2002). During the successive selection process, a challenging problem is how to avoid the loss of genetic variability, particularly for the breeding population without introducing any genetic material. Increasing the selection intensity within breeding programs could increase the rate of inbreeding to as much as 6–8% per generation; genetic drift and a further loss of genetic variation are likely to occur because of inbreeding depression (Bentsen and Olesen 2002). So, there are concerns that genetic diversity might have been lost in the closed populations by selective pressure and inbreeding. The consequence of the declining genetic diversity accompanied by inbreeding depression is lowering fitness-related characters in the species of oysters (Bierne et al. 1998; Evans et al. 2004). In which case, the understanding of genetic diversity and differentiation among generations in successive selection strains is essential for the sustainable achievement of desired gains, long-term sustainability of the breeding program, and to avoid genetic erosion in the oyster.

To improve the productivity traits of *C. gigas*, a breeding program concentrated on the establishment of selected lines by mass selection for faster growth has also been initiated in 2007 in China (Li et al. 2011). The first generation of mass selection for shell height was carried out in three cultured stocks from China, Japan (stock J), and Korea (stock K) in 2007, and the improvement for the shell height at harvest on day 360 was 12.2, 12.2, and 7.9%, respectively.

In the present study, to evaluate the impact of mass selection on genetic structure in artificially closed populations of the Pacific oyster, we performed mass selection over six generations on stock J and stock K from 2007 to 2012, analyzed their temporal genetic variation and structure using 18 microsatellite loci, and compared them with the base populations of the two selected lines and one wild population from China.

Materials and methods

Sample collections

In our selective breeding program, six generations of two *C. gigas* populations were produced from 2007 to 2012 in a private hatchery located in Laizhou, Shandong, China. In 2007, 2-year-old Pacific oysters collected from two cultured stocks in Onagawa Bay in Miyagi Prefecture, Japan (stock J; 38.3° N, 141.3° E), and Pusan, South Korea (stock K; 35.1° N, 129.1° E), were used to establish the first generation selection line (JG1 and KG1) separately for fast growth. The oysters were derived from natural seed collected on the coast and cultured on ropes suspended from rafts along the coastal regions.

Size-frequency distribution of shell height was determined for both stocks before selection; then, individuals in stocks J and K were selected from the top end of the size distribution of each stock with the selection intensity of the top 10% to serve as parents for the selected lines JG1 and KG1 (Li et al. 2011). Similarly, the second to sixth generations of the selected lines of the stocks J and K (JG2–6 and KG2–6) were constructed in 2008–2012, respectively. The wild oysters were sampled from Rushan, Shandong Province, China (WR; 36.4° N, 121.3° E). Hatchery, nursery, and grow-out followed the method described by Li et al. (2011). Sample information and the number of the parents used to produce each generation of selected lines are shown in Table 1.

DNA extraction and microsatellite analysis

Genomic DNA was extracted from adductor muscles which were kept at − 80 °C according to the phenol-chloroform method with a modification (Li et al. 2002). About 100 mg of the tissue was digested overnight at 37 °C in 0.5 ml of lysis buffer (6 M urea, 10 mM Tris–HCl, 125 mM NaCl, 1% SDS, 10 mM EDTA, pH 7.5) and 35 μl of proteinase K (20 mg/ml). The reaction mixture was extracted with phenol/chloroform (1:1), precipitated with isopropanol, and dissolved in 1× TE buffer.

Table 1 Sample information for the selected lines of *C. gigas*

Population	Number of parents	*Nf*	*Nm*	Sample size	Date of sampling
WR	–	–	–	47	September 2013
JG0	–	–	–	47	January 2007
JG1	80	38	42	48	October 2008
JG2	70	34	36	48	October 2009
JG3	88	37	51	48	October 2010
JG4	74	32	42	47	October 2011
JG5	83	35	48	48	September 2012
JG6	91	50	41	48	September 2013
KG0	–	–	–	48	January 2007
KG1	74	38	36	47	October 2008
KG2	65	30	35	48	October 2009
KG3	84	49	35	48	October 2010
KG4	87	46	41	48	October 2011
KG5	83	38	45	48	September 2012
KG6	107	59	48	48	September 2013

Nf number of female broodstock, *Nm* number of female broodstock, *WR* wild Rushan population, *J* selected lines of Japan, *K* selected lines of Korea, *G0* the base generation, *G1–G6* first to sixth generations

The extracted genomic DNA was stored at − 30 °C. Six multiplex PCRs containing 18 microsatellites were used for genetic analysis, and the reaction system of each multiplex PCRs were performed as the description of Liu et al. (2017). For genotyping, the PCR product was combined with formamide and a LIZ500 size standard (Applied Biosystems) and then electrophoresed using an ABI PRISM 3130 Automated DNA Sequencer (Applied Biosystems). Fragment lengths were assessed with the GeneMapper v4.0 software.

Data analysis

Possible null alleles and genotyping errors caused by stuttering or large-allele dropout were tested with MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004). Number of alleles per locus (*N*a), allele frequencies, the observed heterozygosity (*Ho*), and expected heterozygosity (*He*) among populations were assessed using MICROSATELLITE ANALYSER 4.0 (Dieringer and Schlötterer 2003). Fisher's exact test of deviations from Hardy–Weinberg equilibrium (HWE) for each locus were tested using GENEPOP 4.0 (Raymond and Rousset 1995). The inbreeding coefficient for each population and locus were calculated by FSTAT 2.9.3 (Goudet 2001). In addition, differences in genetic diversity parameters were tested using a nonparametric analysis (Wilcoxon signed-rank test; Wilcoxon 1945).

The program Bottleneck 1.2.02 was used to investigate the presence of recent bottlenecks in 15 Pacific oyster populations according to Cornuet and Luikart (1996). For each sample and locus, the distribution of heterozygosity expected from the observed number of alleles was computed under the infinite allele model (IAM), stepwise-mutation model (SMM), and two-phase model of mutation (TPM) with 1000 iterations to verify the existence of bottlenecks inferred by heterozygosity excess or deficiency. Significance was tested using the Wilcoxon signed-rank test (Wilcoxon 1945).

The extent of population subdivision among the different populations was assessed by *F*-statistics (*F*st) (Weir and Cockerham 1984). In consideration of the possible influence of microsatellite null alleles, the ENA (excluding null alleles) correction method was used to correct the positive bias on *F*st estimation. The adjusted value of *F*st using ENA was calculated by the software FreeNA (Chapuis and Estoup 2007). Values of *F*st were tested for significant departure from zero using random permutation procedures (1000 permutations). Hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used to partition the genetic variance among subsamples within and among groups (the base population versus the six mass-selected populations) using ARLEQUIN 3.5 (Excoffier and Lischer 2010). In all cases with multiple tests, corrections of the significance level were performed following the sequential Bonferroni procedure (Rice 1989).

The effective population size of each generation was calculated for unequal sex ratio after Falconer and Mackay (1996) as follows: $Ne = 4N_mN_f/(Nm + N_f)$, where N_m and N_f are the number of males and females used to produce that generation. Then, the effective population size was estimated using the linkage equilibrium method with NeEstimator 2.01 (*N*em) (Do et al. 2014). This method is premised on the principle that the effective population size decreases as genetic drift with few parents generates nonrandom associations among alleles at unlinked loci. The 95% confidence intervals (CIs) of *N*em were also calculated (Waples and Do 2008; Luikart et al. 2010).

Results

Genetic variability

The MICRO-CHECKER analysis revealed that null alleles might exist in 13 of 18 loci screened, and our data demonstrated that no locus was affected by null alleles in all 15 populations, and no evidence of "stutter peaks" which may have resulted in scoring errors or large allele dropout at any locus was observed. All 18 microsatellite loci were highly polymorphic in the 15 populations of *C. gigas* sampled and the level of polymorphism varied among the loci.

Average numbers of alleles (*N*a) for the selected lines of Japan declined from 14.9 to 10.7, while for the selected lines of Korea declined from 17.3 to 9.4 (Table 2). A significant reduction in allele number was observed in JG5 (20.1%), JG6 (28.2%), KG5 (32.7%), and KG6 (38.6%), compared with those observed in the base populations of the two selected lines (Wilcoxon matched-pairs signed-rank test, $P < 0.01$). The highest mean *N*a values were observed in the WR population (*N*a = 18.6). Mean observed heterozygosities (*H*o) per generation of the two closed selected lines were high and comparable, ranging from 0.757 to 0.846 in the JG0–JG6 populations and from 0.736 to 0.865 in the KG0–KG6 populations. The average expected heterozygosities (*H*e) ranged from 0.778 to 0.871 in the JG0–JG6 populations and from 0.744 to 0.854 in the KG0–KG6 populations (Table 2). No significant difference in the average value of *H*o was observed between the base population and selected strains (Wilcoxon matched-pairs signed-rank test, $P > 0.01$). The inbreeding coefficient value (*F*is) was close to zero for all 15 populations, varying from − 0.039 to 0.091 in the selected lines of Japan, and from − 0.067 to 0.018 in the selected lines of Korea.

Most of the alleles (44.0–68.6%) were in low frequencies (< 0.05) and 16.9–31.1% of the alleles in moderate frequencies (0.05–0.10) (Fig. 1). The number of alleles in low frequency (< 0.05) tend to decline with the increase of selective generations in the two selected lines, and a significant difference was observed in JG6 and KG6, compared with those of the base populations (Wilcoxon matched-pairs signed-rank test, $P < 0.01$, Fig. 1). The observed

Table 2 Analysis of genetic diversity in different populations of *C. gigas*

Population	*N*a	*H*o	*H*e	*F*is
WR	18.6 ± 6.3	0.814 ± 0.155	0.851 ± 0.111	0.045 ± 0.141
JG0	14.9 ± 3.6	0.845 ± 0.097	0.871 ± 0.049	0.027 ± 0.135
JG1	14.7 ± 4.8	0.757 ± 0.167	0.828 ± 0.096	0.091 ± 0.157
JG2	13.8 ± 5.5	0.809 ± 0.117	0.819 ± 0.103	0.012 ± 0.083
JG3	13.1 ± 5.8	0.789 ± 0.212	0.809 ± 0.102	0.029 ± 0.229
JG4	13.1 ± 3.9	0.846 ± 0.142	0.817 ± 0.099	− 0.039 ± 0.160
JG5	11.9 ± 4.3	0.787 ± 0.135	0.778 ± 0.139	− 0.025 ± 0.146
JG6	10.7 ± 3.0	0.822 ± 0.131	0.807 ± 0.096	− 0.027 ± 0.170
KG0	15.3 ± 7.0	0.843 ± 0.099	0.854 ± 0.074	0.007 ± 0.136
KG1	17.3 ± 5.2	0.836 ± 0.123	0.853 ± 0.110	0.018 ± 0.107
KG2	13.4 ± 5.5	0.865 ± 0.128	0.821 ± 0.082	− 0.058 ± 0.151
KG3	13.1 ± 3.7	0.860 ± 0.117	0.811 ± 0.088	− 0.067 ± 0.149
KG4	11.8 ± 4.1	0.811 ± 0.153	0.790 ± 0.120	− 0.025 ± 0.129
KG5	10.3 ± 2.9	0.794 ± 0.128	0.793 ± 0.081	− 0.005 ± 0.158
KG6	9.4 ± 3.0	0.736 ± 0.130	0.744 ± 0.102	0.006 ± 0.151

Na observed number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, *Fis* inbreeding coefficient

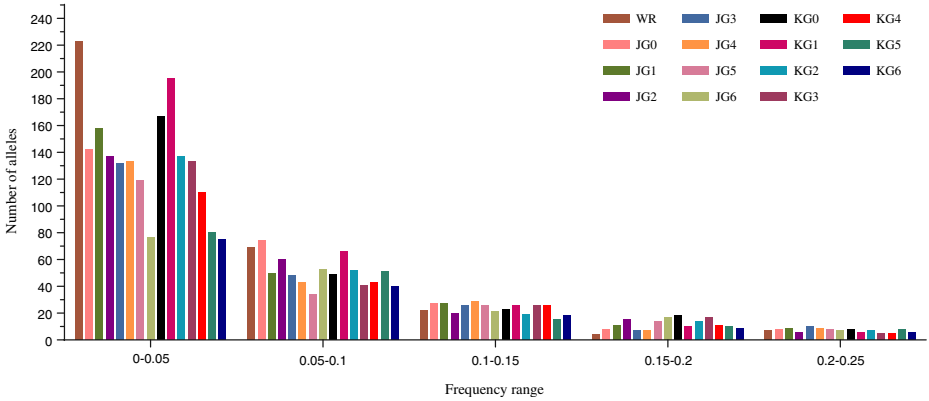

Fig. 1 Allele frequency distribution at 18 microsatellite loci in each population

genotype frequencies were tested for agreement with HWE. After adjusting the *P* values across the 18 loci using the sequential Bonferroni method for multiple observations (Rice 1989), the tests of HWE revealed that 46 of 270 population-locus cases (15 populations × 18 loci) exhibited significant deviation from HWE ($P < 0.05$). This deviation was not systematic, occurring at different loci for different populations, and only JG2 were at HWE in all 18 loci.

Bottleneck signature tests showed that most populations have experience bottlenecks under the IAM and SMM ($P < 0.05$). However, all the two selected lines were found to be at mutation-drift equilibrium under TPM ($P > 0.05$, Table 3).

Population differentiation

The genetic differentiation among the successive selected lines of Japan and Korea was demonstrated by the pairwise *F*st analysis separately (Table 4). A higher genetic differentiation

Table 3 Test for mutation drift equilibrium in the selected lines and wild population of *C. gigas*

Population	*P* values of Wilcoxon's sign-rank test (two tails for *H* excess or deficiency)		
	IAM	TPM	SMM
WR	0.86504	0.05374	*0*
JG0	*0.00002*	0.26453	*0.00401*
JG1	0.06654	0.30379	*0*
JG2	0.18147	0.96612	*0.00233*
JG3	*0.02081*	0.41711	*0.00064*
JG4	*0.00336*	0.79870	*0.00007*
JG5	*0.04828*	0.16735	*0.00016*
JG6	*0.00193*	0.21214	*0.03036*
KG0	*0.00401*	0.83173	*0.04317*
KG1	0.05386	0.05386	*0.00005*
KG2	*0.01203*	0.14152	0.14152
KG3	*0.04317*	0.08977	*0.00004*
KG4	*0.00769*	0.83173	0.83173
KG5	*0.00016*	0.46829	*0.00336*
KG6	*0.04317*	0.39274	*0.00021*

Italics represent significant difference ($P < 0.05$)

Table 4 *Fst* estimation of genetic differentiation using ENA during the closed breeding of *C. gigas*

	JG0	JG1	JG2	JG3	JG4	JG5		KG0	KG1	KG2	KG3	KG4	KG5
JG1	0.0513						KG1	0.0621					
JG2	0.0378	0.0369					KG2	0.0665	0.0307				
JG3	0.0303	0.0515	0.0264				KG3	0.0742	0.0284	0.0246			
JG4	0.0294	0.0550	0.0270	0.0219			KG4	0.0753	0.0216	0.0349	0.0239		
JG5	0.0711	0.0662	0.0685	0.0616	0.0721		KG5	0.0472	0.0682	0.0598	0.0796	0.0764	
JG6	0.0714	0.0834	0.0798	0.0835	0.0979	0.0947	KG6	0.0680	0.1004	0.0874	0.1064	0.1123	0.0490

was observed in KG0–KG6 populations, compared with the average pairwise *F*st values in JG0–JG6 populations. Furthermore, multilocus *F*st values were all highly significant from zero ($P < 0.01$ after Bonferroni correction). The pairwise *F*st values among the two base populations and the wild population ranged from 0.0189 to 0.0609, which showed a low level of genetic differentiation.

AMOVAs revealed that significant molecular genetic variance was associated with subdivisions between the base populations and the six successive selected lines of Japan ($P = 0.729$) and Korea ($P = 0.290$). Moreover, most of the variance was distributed within individuals (93.60 and 95.12% in selected lines of Japan and Korea) (Table 5).

Effective population sizes

For each generation, the effective population size estimates based on the sex ratio method (*N*e) ranged from 69.9 to 90.1 in selected lines of Japan, and from 64.6 to 105.9 in selected lines of Korea (Table 6). However, the estimates using the linkage disequilibrium method (*N*em) showed a larger range of variation than *N*e in the two selected lines (selected lines of Japan, *N*em = 67.9–205.2; selected lines of Korea, *N*em = 59.8–147.9) (Table 6).

Discussion

In the successive selective breeding program, a major concern for selected strains is the loss of genetic variability and inbreeding, especially for the selection of fecund animals (Gaffney et al. 1992; Yu and Guo 2004; Wang et al. 2016). Heterozygosity is often used as a primary genetic

Table 5 Analysis of molecular variances (AMOVA) of microsatellites for two closed selective breeding lines of *C. gigas*

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation (%)	*P* value
Selected lines of Japan					
Among groups	1	38.807	− 0.09677	− 1.25	0.729
Among populations within groups	5	275.763	0.49855	6.42	0
Among individuals within populations	327	2438.931	0.09527	1.23	0.019
Within individuals	334	2427.500	7.26796	93.60	0
Total	667	5181.001	7.76502	–	–
Selected lines of Korea					
Among groups	1	61.344	0.06140	0.80	0.290
Among populations within groups	5	255.559	0.46080	5.99	0
Among individuals within populations	328	2305.548	− 0.14664	− 1.90	0.999
Within individuals	335	2453.000	7.32239	95.12	0
Total	669	5075.451	7.69795	–	–

Each mass selection lines was divided into two groups, the base population and the six mass-selected populations

Table 6 The effective population size in the selected lines and wild population of *C. gigas*

Population	Sex ratio method	Linkage disequilibrium method	
	Ne	*Nem*	95% CI
WR	–	127.6	92.9–196.2
JG0	–	104.9	82.1–142.0
JG1	79.8	67.9	55.3–86.4
JG2	69.9	205.2	131.2–434.7
JG3	85.8	117.7	85.8–180.2
JG4	72.6	130.6	91.9–214.5
JG5	81	83.2	63.9–115.2
JG6	90.1	130.1	105.2–168.3
KG0	–	105.8	79.9–151.6
KG1	73.9	119.6	89.3–175.4
KG2	64.6	106.8	88.5–133.1
KG3	81.7	74.4	59.1–97.9
KG4	86.7	147.9	101.3–258.1
KG5	82.4	66.9	54.4–85.3
KG6	105.9	59.8	48.4–76.5

Ne effective population size estimates based on the numbers of males and females used to produce that generation Falconer and Mackay (1996), *Nem* effective population size estimates using the linkage disequilibrium method of Do et al. (2014), *95% CI* 95% confidence intervals of *N*em

parameter to reflect the overall genetic diversity of populations (Beaumont et al. 2010). In this study, the observed or expected heterozygosity varied over generations; however, no significant loss of heterozygosity was observed between the base population and selected lines. Similar results have been detected in selected strains of eastern oyster *Crassostrea virginica* (Yu and Guo 2004). Also, in the hard clam *Mercenaria mercenaria*, no association was observed between selection for fast growth and loss of heterozygosity (Dillon and Manzi 1987). This result was seen maybe because an estimate of heterozygosity could be inflated if a strain was founded using heterozygous parents, and because loss of low-frequency alleles seems to have not much effect on estimates of heterozygosity (Allendorf 1986).

The two selected lines over six generations in this study, however, showed a significant reduction in allele number in JG5–JG6 and KG5–KG6, compared with the respective base populations. There are many factors that contribute to the loss of alleles, such as genetic drift, bottlenecks, and selection. Consistent with many studies of allele loss in selected strains and cultured populations of aquatic species (Dillon and Manzi 1987; Hedgecock and Sly 1990; Appleyard and Ward 2006), we found that the loss of alleles was generally more easily observed than decrease of heterozygosity. Furthermore, a significant loss of rare alleles was detected in the sixth generations of selected lines (JG6 and KG6), suggesting that successive mass selection in closed populations may increase the sensibility of rare alleles to genetic drift. As an alternative, the loss of alleles is likely to be a better indicator of genetic variability in successive mass selected lines (Yu and Guo 2004).

In this study, the decline in *N*a and rare alleles over generations also suggested that additive genetic variation was subject to selection, and the selected strains carried a high risk of random genetic drift over six generations. Depletion of genetic variance and genetic drift may limit selection response for *C. gigas*. Actually, rapid declines in selection response for economic traits after generations of mass selection was reported in European oyster, *Ostrea edulis* (Newkirk and Haley 1983), and Sydney rock oyster, *Saccostrea glomerata* (Nell et al. 1999), and other aquatic animal species such as Silver barb *Puntius gonionotus* (Pongthana et al. 2006). James (1969) found the reduction in response to selection caused by a small number of founders is great for large populations under intense selection, especially when the

desirable alleles are rare in the base population. Although Li et al. (2011) and Wang et al. (2012) reported that 7.2–13.2 and 8.4–10.4% gains in first and second generations for general yield across different rearing stage were obtained in the three selected lines of *C. gigas*, a further study on selection response of subsequent generations are ultimately required to confirm the potential of the selected lines for increased gains in selective breeding program.

In total, 46 of 270 population-locus cases deviated from the Hardy–Weinberg expectations in our study. Li et al. (2006) surveyed five cultured populations of Pacific oyster in China and found that 32 of 35 population-locus cases deviated from HWE. A deviation from HWE could result from the existence of null alleles, inbreeding, nonrandom mating, and artificial and natural selection during seed production and cultivation (McGoldrick 2000; Li et al. 2003; Hedgecock et al. 2004; Li et al. 2006). In this study, a relatively high selection intensity (top 10%) was applied in the successive mass selection, which increased the risk of nonrandom mating, and could result in the deviation from HWE in the two selected lines.

The overall genetic differentiation (*F*st) among the base population and the two selected lines of K and J were significantly different from zero ($p < 0.05$). Within the selected lines of K and J, slight genetic differentiations ($Fst < 0.06$) were detected among the first four generations. However, moderate genetic differentiation ($0.06 < Fst < 0.15$) were detected between the fifth or sixth generation with the other selected lines, suggesting potential barriers to gene exchange may appear with the progress of successive mass selection. The existence of potential barriers may be mainly due to the long-term process of successive artificial selection and the high mortality of *C. gigas* at larval stage. Similar phenomenon has also been documented with respect to microsatellite loci in selected strains of the Pacific oyster (Appleyard and Ward 2006) and common carp (Napora-Rutkowski et al. 2017). The results revealed by AMOVA in this study also indicated that significant molecular genetic variance was associated with subdivisions between the base populations and the six successive selected lines.

Many researchers have indicated that the estimates of effective population size were probably more sufficient to achieve accurate genetic information for small populations (especially for artificially selective populations), compared with the general analysis of those genetic parameters (such as *H*o and *H*e) (Waples 1989; Consuegra et al. 2005). In this study, the values of *N*e and *N*em in the two selected lines revealed by the temporal analysis were still at a relatively high level ($Ne > 60$, $Nem > 50$) over generations (Bentsen and Olesen 2002), indicating that the effective population size was not affected greatly by successive mass selection. However, the decline of the effective population size was observed in many other selected strains and cultivated stocks of oysters (Hedgecock and Sly 1990; Gaffney et al. 1992). The decrease of effective population size may result from artificially selecting the best offspring produced by few parents or due to asymmetric reproduction and survival rates of broodstock (Appleyard and Ward 2006; Lallias et al. 2010). The effective population size can also be reduced by drift resulting from the high variances of reproductive success known to exist in hatchery-propagated oysters and shrimp (Boudry et al. 2002; Goyard et al. 2003). The successive mass selection was not sufficient to affect the effective population size of the two selected lines, which may result from artificial fertilization applied in our mass selective breeding program, and all the males and females can be guaranteed to contribute gametes to the next generation.

Our results based on linkage disequilibrium showed that there was a fluctuation in the effective population size of the two selected lines as the selection processes progressed. The

bottleneck analysis based on IAM and SMM indicated that the two selected lines could have experienced a recent bottleneck. Therefore, the fluctuation of *N*em might have arisen from a recent bottleneck, which might be due to successive artificial selection or summer and larval mortality of *C. gigas*. In addition, it is possible that the selected lines which have gone through a bottleneck may be more sensitive to a reduced population size during subsequent selection than a line which has not, especially when desirable alleles are at high frequencies in selected lines (James 1969). Hence, reducing the selection intensity properly with the increase of selective generations is recommended to minimize the deleterious effect of genetic drift and bottleneck caused by successive mass selection, even though the effective population size of the two selected lines were still at a high level over six selective generations.

In conclusion, there was no significant reduction in heterozygosity in the two selected lines. However, the average number of alleles per locus was considerably lower in the fifth and sixth generations of the two selected lines, compared with those of the base populations and wild population, suggesting that the selected strains carried a high risk of random genetic drift over six generations and successive mass selection in closed populations may increase the sensibility of rare alleles to genetic drift or selection. The selection intensity of the top 10% or higher can accelerate the loss of alleles even in selected lines with large effective population size. Crossing multiple lines selected from different geographical population is possible to become a hedge against loss of diversity. To maintain the effective population size and avoid inbreeding, equalizing the sex ratio of parents and reducing the selection intensity are a couple of measures generally recommended in the process of long-term mass selection of the closed populations. The results obtained in this study provide important information for the design of appropriate management strategies for selective breeding of *C. gigas*.

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