Aquaculture 508 (2019) 159–166

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Genetic variability of an orange-shell line of the Pacific oyster *Crassostrea gigas* during artificial selection inferred from microsatellites and mitochondrial COI sequences

Ziqiang Han[a], Qi Li[a,b,*], Shikai Liu[a], Hong Yu[a], Lingfeng Kong[a]

[a] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

ARTICLE INFO

Keywords:
Rare breeds
Crassostrea gigas
Mass selection
Genetic variability
Microsatellite
mtCOI

ABSTRACT

Rare breeds represent a valuable resource for current or future market demands, but their low census compromises the genetic variability and future utilization of these breeds. Whether genetic variability of rare breeds with low initial genetic variation can be maintained during an intense mass selection becomes the key to applying mass selection to the genetic improvement of these breeds. The genetic variability among three generations of successive mass selection of the orange-shell line of *Crassostrea gigas* (MS1–MS3) were evaluated by both 20 nuclear microsatellite loci and mitochondrial cytochrome oxidase I sequences (mtCOI) compared to four wild populations. In this study, the orange-shell line exhibited very low genetic variability. Only one mtCOI haplotype was detected in all individuals of MS1–MS3. Significant reductions in average number of alleles (N_a: 69.55–76.92%), allelic richness (A_r: 68.17–74.91%) and expected heterozygosity (H_e: 34.21–39.24%) as well as increased mean pairwise genetic relatedness (R: 6.87–25.79 times) were observed in MS1–MS3 when compared to wild populations. However, the genetic variability of orange-shell line with very low initial variation successfully maintained during three generations of mass selection. No significant difference in N_a (3.60–4.40), A_r (3.51–4.08) and H_e (0.48–0.50) occurred among MS1–MS3. Pedigree reconstructions (no full-sib group from MS1–MS3 was larger than 16% of the whole group) revealed artificial spawning used in this study has a better control over contribution of gametes than traditional mass spawning. In addition, effective population size of MS1–MS3 calculated by linkage disequilibrium methods increased from 29.3 to 67.0 indicating the linkage disequilibrium decays over time. This study provides important insights in the genetic consequences of a rare variant line of *C. gigas* with very low genetic variation over generations of mass selection. This will provide a reference for carrying out genetic improvement programs on rare breeds where small populations are inevitable.

1. Introduction

Rare breeds, as valuable germplasm resources, always exhibit some specific agricultural traits that meet current or future market demands. However, their low census compromises the genetic variability and future of these breeds (Cervantes et al., 2016). This makes it difficult to use the rare resources to achieve sustainable profitability, especially when some rare breeds need further improvement in production performance. Genetic variability is the fundamental resource on which stock improvements rely. It therefore should be a major priority for breeding operations to not only capture, but also maintain, as much of genetic variation as possible within domesticated populations (Lind et al., 2009).

Achieving this, however, can be problematical in some breeding practices, especially in a small population, such as a breeding program with rare breeds as founder population. Firstly, at the beginning of the breeding program, the founding stock size is limited and difficulty are faced in sourcing wild germplasm lead to non-random mating between individuals that are closely related (inbreeding) and consequently to a reduction of genetic variation. Secondly, most marine invertebrates and fish are highly fecund, allowing stringent selection and faster gains (Gjedrem, 2012). However, the great fecundity is always accompanied by high variance in reproduction success of such organisms (Boudry et al., 2002; Hedgecock et al., 2007). These characteristics of aquatic species could result in inbreeding and loss of potentially beneficial alleles and net additive genetic variation during successive selection, especially without individual tagging and pedigree records, and when there are high selection intensities (In et al., 2016). The effects of

* Corresponding author at: Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China.
E-mail address: qili66@ouc.edu.cn (Q. Li).

https://doi.org/10.1016/j.aquaculture.2019.04.074
Received 3 March 2019; Received in revised form 14 April 2019; Accepted 27 April 2019
Available online 09 May 2019
0044-8486/

traditional selection processes upon the genetics of aquatic populations is a topic that has received substantial research attention. Many studies have shown that genetic variation typically declines over successive generations of domestication, such as shrimp (Dixon et al., 2008), fish (Sawayama and Takagi, 2016) and shellfish (Fu et al., 2017; In et al., 2016; Rhode et al., 2014). Decreasing genetic variation will not only cause significant negative effect on stock performance and production traits, but also limit the potential for genetic gains and response to selection (Bentsen and Olesen, 2002; Zhang et al., 2010).

It is because of these factors mentioned above that breeders are not optimistic about the selection of rare breeds. Indeed, there are few reports or examples of reactions to effects of artificial selection on the genetic variability of rare breeds. Recently, in the case of an increasing understanding of the cause of loss of genetic diversity during selection, some measures to prevent losses have been proposed. Some of the easy-to-operate and low-cost measures have been applied to the oyster breeding process, such as artificial spawning, balanced sex ratio and large size of broodstock, and successfully maintained the genetic diversity during artificial selections in two strains of oyster selected for growth (Wang et al., 2016; Xu et al., 2019). Artificial spawning permits a better control of the number, sex ratio and contribution of gametes of real parents who provide gametes than the traditional mass spawning mentioned above. Therefore, mass selection is promising for the genetic improvement of rare breeds, if the genetic variability of small populations can be maintained by improved selection process. This information will be helpful in the sustainable use of rare breeds where small populations are inevitable.

The Pacific oyster (*Crassostrea gigas*) is one of the most widely farmed aquaculture species worldwide (FAO, 2016). A rare orange-shell variant of *C. gigas* (Fig. 1) was obtained through family selection in our breeding practice. Since orange-shell variant not only has a unique shell color, but also its soft tissue has twice the zinc content of commercial population of *C. gigas* with a common shell color (Zhu et al., 2018), it may become a valuable germplasm resources for further market demands. To further improve the growth performance of the orange-shell line, successive three-generation improved mass selection with a selection intensity of about 1.9 has been conducted since 2014 (Table 1). Rare variant, as typical small populations, provide an opportunity to assess whether genetic variability of rare breeds can be maintained over generations of intense selection with improved mass selection. This study aims at (1) assess the current level of genetic diversity of the orange-shell line compared to wild populations of *C. gigas*, (2) evaluating whether the genetic variability is maintained in orange-shell line over mass selection generations using nuclear microsatellite loci as well as mitochondrial cytochrome oxidase I sequences.

2. Materials and methods

2.1. Selection, sample collections and DNA extraction

Three generations of the orange-shell selection and four wild populations of *C. gigas* were surveyed in this study (Table 1). Four orange-shell individuals (about 0.2%) were found in the offspring of purple-black shell color individuals, which were produced by crossing females with black shell color and males with purple shell color selected from the cultured population of *C. gigas* in Rushan, Shandong province, China. These four individuals (two males and two females) with orange left and right shell color were collected and used to produce two full-sib families as the first generation. Next, two consecutive generations of family selection were established from 2012 to 2013 to fix the shell color. After three generations of family selection, we obtain genetically stable orange-shell line. To enhance the growth performance of the orange-shell line, truncation selection for shell height was initiated in 2014 to construct the first generation of mass selections (MS1) using individuals of families from the third generation with greatest shell height as broodstock. Similarly, truncation selections were implemented for the next two successive generations of mass selection (MS2 and MS3) in 2015 and 2016, respectively. The number of parents, truncation point and selection intensity for each generation are shown in Table 1. The eggs and sperm were collected from the matured males and females by dissection. For the purpose of providing equal mating chances for each parent, equal amounts of eggs and sperm from each female and male were mixed well after estimating concentrations using a microscope. Samples of the MS1 (14 months), MS2 (12 months) and MS3 (12 months) were collected randomly at harvest. Four wild populations were collected from Dongying (DY), Qingdao (QD), Penglai (PL) and Rushan (RS), Shandong province, China between 2013 and 2017 (Fig. 2). The sample size and sample time of each studied populations are shown in Table 1. Samples for mtDNA analysis were collected from the same individuals used for nuclear DNA analysis. The adductor muscle was collected from fresh specimens and immediately stored at −30 °C until DNA preparation. Genomic DNA was extracted

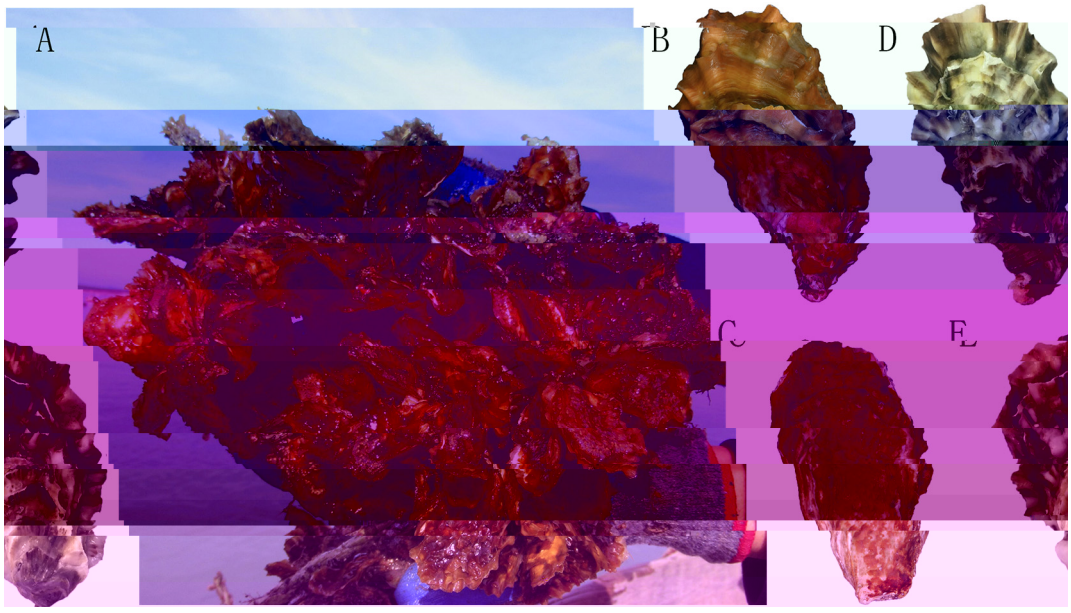

Fig. 1. Phenotypes of the orange-shell variant (A: living bodies, B: left shell, C: right shell) and wild-type (D: left shell, E: right shell) of *C. gigas*.

Table 1
List of sample information.

Population	Number of parents		Truncation point (mm)	Selection intensity	Sample time	Sample size	
	Female	Male				Microsatellite	mtCOI
RS	–	–			2016.09	48	20
PL	–	–			2013.02	50	20
DY	–	–			2013.07	51	20
QD	–	–			2017.11	50	20
MS1	60	60	51.45	1.87	2015.10	50	20
MS2	50	50	62.23	1.95	2016.06	48	20
MS3	50	47	62.50	1.98	2017.06	50	20

RS, Rushan wild population; PL, Penglai wild population; DY, Dongying wild population; QD, Qingdao wild population; MS1, 1st mass selected generation; MS2, 2st mass selected generation; MS3, 3st mass selected generation.

from approximately 100 mg of muscle tissue according to the phenol-chloroform method as described in Li et al. (2006) and preserved in 1 × TE buffer. DNA samples were quantified by Nanodrop 2000 and diluted to 100 ng/μl for PCR.

2.2. Microsatellites analysis

Six multiplex PCRs containing 17 microsatellite loci (ucdCg-117, ucdCg-120, ucdCg-146, ucdCg-152, ucdCg-170, ucdCg-198, ucdCg-199, ucdCg-200, uscCgi-210, Crgi3, Crgi4, Crgi39, Crgi45, otgfa0_0007_B07, otgfa0_0129_E11, otgfa0_408293, and otgfa0_0139_G12) (Liu et al., 2017) and three additional loci (ucdCg-140, ucdCg-153 and ucdCg-177) (Li et al., 2003) were used to genotype 347 individuals in total.

Alleles size was performed utilizing GeneMapper software v.4.0 (Applied Biosystems). Micro-Checker v.2.2.3 (Van Oosterhout et al., 2004) was used to assess genotypic errors caused by stuttering or large-allele dropout. The presence of null alleles was tested using FREENA software (Chapuis and Estoup, 2007), in which loci with estimated frequencies of null alleles above 0.2 were potentially problematic for calculations (Napora-Rutkowski et al., 2017). Fisher's exact test of deviations from Hardy-Weinberg equilibrium for each locus was tested using Genepop v.4.0 (Raymond and Rousset, 1995). Number of alleles per locus (N_a), observed heterozygosity (H_o), excepted heterozygosity (H_e) and fixation indices (F_{is}) were calculated using GenAlEX v.6.5 (Peakall and Smouse, 2012). Allelic richness (A_r: the number of alleles adjusted for the smallest sample size) was calculated using FSTAT v.2.9.3.2 (Goudet, 1995).

To understand the genetic similarities among individuals in a population, mean pairwise relatedness estimate (R) of each population in this study was calculated using the methods of Queller and Goodnight (1989) and Lynch and Ritland (1999). To standardize the range of Lynch and Ritland (1999) estimators (range of −0.5 to 0.5) with Queller and Goodnight estimators, Lynch and Ritland (1999) estimators were multiplied by 2 to give a maximum value of 1 and minimum of −1. Pedigree reconstruction (Number of iterations: 1000000; Full-sib Constraint: 0 and 1; Temperature: 10; Weight: 1; Seed: −1) on three generations of the orange-shell selection populations was performed with PEDIGREE 2.2 (online, http://herbinger.biology.dal.ca:5080/Pedigree), which aimed at reconstructing full pedigree in a group of individuals base on their genotype data in the absence of parental information. The three selected populations and four wild populations were analysed with full-sib (Full-sib Constraint = 1) and kin group (Full-sib Constraint = 0) partition algorithm, and the setting of weight and temperature is based on the highest score.

Effective population size (N_e) of MS1–MS3 and four wild populations was calculated using linkage disequilibrium methods implemented in NeEstimator v.2.0 (Do et al., 2014). This method was shown to perform well in estimating N_e in non-ideal populations with a skewed sex ratio or non-random variance in reproductive success (Waples, 2006). N_e estimates were generated excluding alleles with frequencies < 0.05 to reduce bias related to rare alleles and 95% confidence intervals (95% CI) are reported. Beside the estimates based on genetic data, the unequal sex ratio correction method was used to calculate theoretical effective population size of each generation as: $N_e = 4N_mN_f/(N_m + N_f)$, with N_f and N_m the number of females and males broodstock respectively (Falconer and MacKay, 1996).

Pairwise F_{st} estimation (significance testing: 1000 permutations at 5% nominal level) and hierarchical analysis of molecular variance (AMOVA significance testing: 1000 permutations at 5% nominal level) were utilized to assess population differentiation and partition the genetic variance within/among populations. Also, a pairwise matrix assessing allele frequency heterogeneity among samples was constructed

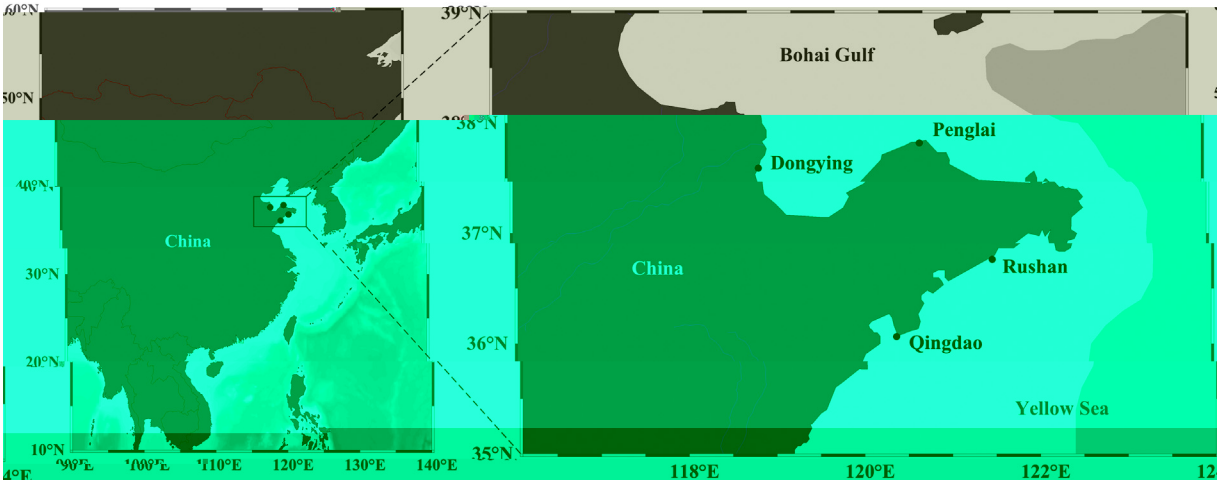

Fig. 2. Map of sampling sites of four wild populations of *C. gigas*.

using *Nei's* unbiased genetic distance (*Nei's D*) (Hedrick, 2000). These analyses were also conducted in GenAlEX v.6.5 (Peakall and Smouse, 2012). Then *Nei's D* was used to contrast neighbor-joining tree by Mega v.5.0 (Tamura et al., 2011).

2.3. Mitochondrial DNA sequencing and analysis

The mitochondrial cytochrome C oxidase subunit I (COI) was amplified using LCO1490 and HCO2198 universal prime (Folmer et al., 1994). The sequences were edited and aligned using Mega v.5.0 (Tamura et al., 2011). DNASP v.5.10.01 (Librado and Rozas, 2009) was used to calculate the total number of haplotypes, haplotype diversity and nucleotide diversity.

3. Results

3.1. Genetic variability

No evidence of stuttering error or large allele dropout was identified by the Micro-checker. Only the locus ucdcg-199 in MS3 (0.2227) and loci Crgi4 (0.35547) and ucdcg-152 (0.29782) in QD showed estimated frequencies of null alleles above 0.2. However, inclusion or exclusion of these loci did not qualitatively change the outcome, hence analysis was performed based on all loci.

There were no significant differences in average number of allele (N_a), allelic richness (A_r), observed (H_o) and expected heterozygosity (H_e) and fixed index (F_{is}) among the four wild populations (Table 2). However, significant reductions in N_a (69.55–76.92%), A_r (68.17–74.91%) and H_e (34.21–39.24%) were observed in MS1–MS3 comparing with the wild populations ($P < .05$). Within MS1–MS3, no significant difference in N_a (3.60–4.40), A_r (3.51–4.08), H_o (0.60–0.62), H_e (0.48–0.50) and F_{is} was observed, except that the A_r reduced slightly from 4.08 (MS1) to 3.51 (MS3) with successive mass selection.

Observed heterozygosity did not differ markedly across populations overall, with mean H_o of all seven populations ranged between 0.60 (MS1 and MS3) and 0.87 (RS). F_{is} for all seven populations ranged between −0.23 (MS1) and 0.15 (QD). Only DY and QD populations have positive F_{is}, while other populations have negative F_{is} values. Similarly, each of DY and QD had two loci deviating from Hardy-Weinberg equilibrium (HWE), while the number of loci deviating from HWE of other five populations ranged between 5 (PL) and 10 (MS2 and MS3).

A total of 599-bp fragments of mtCOI gene generated from 140 specimens were used for the analysis. There were 20 mtCOI haplotypes considering all seven populations in this study (Table 3). The conspicuous unique high-frequency haplotype (B) was observed in all seven populations and had a prevalence of 85.7% (120/140) in all individuals. All sampled individuals in MS1–MS3 only have haplotype B. Thus, no variation was detected within the orange-shell line. Besides haplotype B, each wild population had another four or five private haplotypes which were not shared with each other. Average haplotype diversity (H_d) of the four wild populations ranged from 0.442 to 0.447, while the average nucleotide diversity (P_i) of the four wild populations ranged from 0.083% to 0.127%.

3.2. Relatedness and pedigree reconstruction

Mean pairwise relatedness coefficients (R), a measure of genetic similarity relative to the population mean, across all seven populations ranged from 0.019 (RS) to 0.490 (MS1) (Fig. 3A). R of MS1–MS3 calculated by the method of Queller and Goodnight (1989) (R_{QG}: 0.446–0.490) turned larger mean estimates than that from Lynch and Ritland (1999) (R_{LR}: 0.268–0.272), while R of the wild populations calculated by both methods turned similar estimators (R_{QG}: 0.019–0.047; R_{LR}: 0.032–0.039). For both methods, R of MS1–MS3 was about 6.87–25.79 times that of the wild populations.

For the MS1–MS3 populations, the best full-sib partition (score: 6841.67–7367.49) identifiod (sd)-338 0 /Tty(

D range 0.434–0.641), while differentiation within MS1–MS3 (F_{st} range 0.009–0.047; *Nei's D* range 0.009–0.058) and within wild populations (F_{st} range 0.009–0.046; *Nei's D* range 0.029–0.192) was relatively low (Table 5). Within MS1–MS3, pairwise F_{st} and *Nei's D* values between adjacent generations were gradually increasing. All the pairwise F_{st} were significantly different from zero ($P < .05$).

Neighbor-joining tree generated from the *Nei's D* is shown in Fig. 4. Seven populations fell into two clusters: one cluster includes four wild populations, and the other includes MS1–MS3. The limited variation detected in the mtDNA did not allow further analysis of genetic difference.

4. Discussion

Genetic variation is the fundamental resource on which stock improvements rely, thus capturing and maintaining as much of genetic variation as possible within domesticated populations should be the major priority for breeding operations (Lind et al., 2009). However, founding stock size is limited and difficulty are faced in sourcing wild germplasm when using rare breeds as based population. Therefore, whether genetic variability can be maintained during the mass selection with low initial diversity and high selection intensity becomes the key to applying mass selection to the genetic improvement of rare breeds. This study is the first to report genetic change of rare variant of oysters over several generations of mass selection.

Low census in rare breeds compromises the genetic variability (Cervantes et al., 2016). The current level of genetic variability of orange-shell line was assessed in reference to four outbred wild populations in China. As a result of mtDNA analysis, only one haplotype was detected in all individuals of MS1–MS3, while five or six haplotypes were detected in wild populations. Also, significant reductions in both of average allelic richness (A_r: 68.17–74.91%) and average expected heterozygosity (H_e: 34.21–39.24%) were observed in the whole orange-shell line. Reduced genetic variation in domesticated stocks is common when compared with outbred wild populations. However, the N_a of the orange-shell lines ranging from 3.60 to 4.40 was lower than that of most other strains of mollusk (about 6–26) in previous studies (Chen et al., 2017; Fu et al., 2017; Rhode et al., 2014; Wang et al., 2016; Xu et al., 2019; Zhang et al., 2018). There was no study so far on the analysis of the effects of mass selection on the genetic variability of mollusk population with such a low level of genetic variation.

Despite the large drop in N_a, A_r, and H_e between wild populations and orang-shell lines, no significant differences in observed heterozygous and fixation index were observed among all seven populations in this study. Both heterozygote deficiency (positive F_{is}) and heterozygote excess (negative F_{is}) observed in the wild populations indicate non-randomly mating in these populations (Dixon et al., 2008), probably because large-scale hatchery populations in those locations have diluted or deteriorated the natural gene pool of *C. gigas* or sampling effects (Zhang et al., 2010). Meanwhile, heterozygosity excesses were prevalent in the orange-shell lines. This pattern that allele reduction is not accompanied by a decrease in heterozygosity has also been reported in other mass selected lines of *C. gigas* (Zhang et al., 2018) and other cultured populations of aquatic species (Hillen et al., 2017; Lind et al., 2009), supporting the view that heterozygosity is not as susceptible to decline as alleles in the immediate term (Lind et al., 2009). Furthermore, heterozygotes excesses will break the direct correlation between heterozygosity and inbreeding, and F_{is} will be temporally deflated (Rhode et al., 2014f

large size of broodstock. These measures have been applied in some strains of *C. gigas* and achieved good results (Wang et al., 2016; Xu et al., 2019), but this study is the first to apply them to a small population with such low levels of variability. No significant differences occurred in *N*

Table 5
Pairwise F_{st} (lower diagonal) and *Nei's D* (upper diagonal) values between mass selected orange-shell lines and wild populations based on 20 microsatellite loci.

	DY	QD	PL	RS	MS1	MS2	MS3
DY		0.041	0.118	0.133	0.569	0.547	0.534
QD	0.013		0.179	0.192	0.641	0.614	0.581
PL	0.030	0.046		0.029	0.460	0.452	0.434
RS	0.031	0.045	0.009		0.508	0.483	0.457
MS1	0.192	0.197	0.177	0.184		0.009	0.058
MS2	0.178	0.184	0.166	0.170	0.009		0.034
MS3	0.180	0.185	0.166	0.169	0.047	0.028	

All F_{st} values were significantly different from zero ($P < .05$).

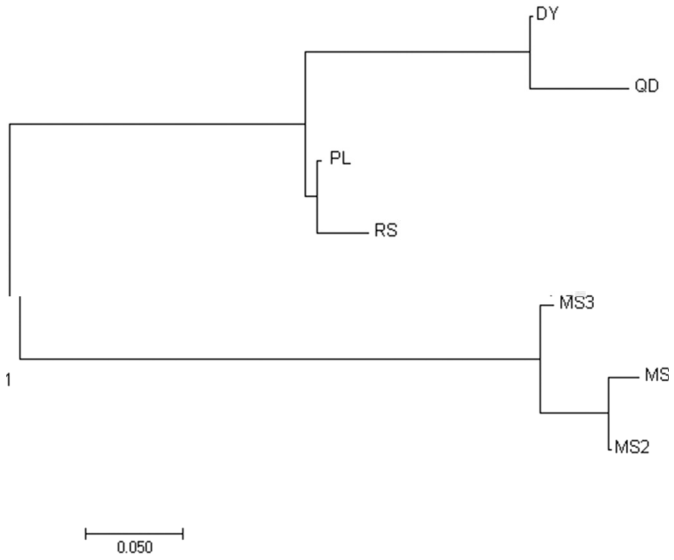

Fig. 4. Neighbor-joining tree of all seven populations based on *Nei's* unbiased genetic distance.

Acknowledgements

This work was supported by the grants from National Natural Science Foundation of China (31772843 and 31741122), the Earmarked Fund for Agriculture Seed Improvement Project of Shandong Province (2017LZGC009), Fundamental Research Funds for the Central Universities (201762014), and Taishan Scholars Seed Project of Shandong.

References

Bentsen, H.B., Olesen, I., 2002. Designing aquaculture mass selection programs to avoid high inbreeding rates. Aquaculture 204, 349–359. https://doi.org/10.1016/S0044-8486(01)00846-8.

Boudry, P., Collet, B., Cornette, F., Hervouet, V., Bonhomme, F., 2002. High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture 204, 283–296. https://doi.org/10.1016/S0044-8486(01)00841-9.

Cervantes, I., Gutierrez, J.P., Meuwissen, T.H.E., 2016. Response to selection while maximizing genetic variance in small populations. Genet. Sel. Evol. 48 (69). https://doi.org/10.1186/s12711-016-0248-3.

Chapuis, M.-P., Estoup, A., 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24, 621–631. https://doi.org/10.1093/molbev/msl191.

Chen, N., Luo, X., Lu, C., Ke, C., You, W., 2017. Effects of artificial selection practices on loss of genetic diversity in the Pacific abalone, *Haliotis discus hannai*. Aquac. Res. 48, 4923–4933. https://doi.org/10.1111/are.13311.

Dixon, T.J., Coman, G.J., Arnold, S.J., Sellars, M.J., Lyons, R.E., Dierens, L., Preston, N.P., Li, Y., 2008. Shifts in genetic diversity during domestication of Black Tiger shrimp, *Penaeus monodon*, monitored using two multiplexed microsatellite systems. Aquaculture 283, 1–6. https://doi.org/10.1016/j.aquaculture.2008.07.009.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Mol. Ecol. Resour. 14, 209–214. https://doi.org/10.1111/1755-0998.12157.

Falconer, D.S., MacKay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th ed. Longman House, Essex.

FAO, 2016. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Fu, J., Lue, W., Li, W., Shen, M., Luo, X., Ke, C., You, W., 2017. Comparative assessment of the genetic variation in selectively bred generations from two geographic populations of ivory shell (*Babylonia areolata*). Aquac. Res. 48, 4205–4218. https://doi.org/10.1111/are.13241.

Gjedrem, T., 2012. Genetic improvement for the development of efficient global aquaculture: A personal opinion review. Aquaculture 344–349, 12–22. https://doi.org/10.1016/j.aquaculture.2012.03.003.

Goudet, J., 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. J. Hered. 86, 485–486. https://doi.org/10.1093/oxfordjournals.jhered.a111627.

Han, Z., Li, Q., 2018. Different responses between orange variant and cultured population of the Pacific oyster *Crassostrea gigas* at early life stage to temperature-salinity combinations. Aquac. Res. 49, 2233–2239. https://doi.org/10.1111/are.13680.

Hedgecock, D., Launey, S., Pudovkin, A.I., Naciri, Y., Lapègue, S., Bonhomme, F., 2007. Small effective number of parents (N_b) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. Mar. Biol. 150, 1173–1182. https://doi.org/10.1007/s00227-006-0441-y.

Hedrick, P., 2000. Genetics of Populations, Second ed. Jones and Bartlett, Sudbury.

Hillen, J., Coscia, I., Vandeputte, M., Herten, K., Hellemans, B., Maroso, F., Vergnet, A., Allal, F., Maes, G., Volckaert, F., 2017. Estimates of genetic variability and inbreeding in experimentally selected populations of European sea bass. Aquaculture 479, 742–749. https://doi.org/10.1016/j.aquaculture.2017.07.012.

In, V.-V., O'Connor, W., Dove, M., Knibb, W., 2016. Can genetic diversity be maintained across multiple mass selection lines of Sydney rock oyster, *Saccostrea glomerata* despite loss within each? Aquaculture 454, 210–216. https://doi.org/10.1016/j.aquaculture.2015.12.030.

Lallias, D., Boudry, P., Lapegue, S., King, J.W., Beaumont, A.R., 2010. Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes. Conserv. Genet. 11, 1899–1910. https://doi.org/10.1007/s10592-010-0081-0.

Li, G., Hubert, S., Bucklin, K., Ribes, V., Hedgecock, D., 2003. Characterization of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. Mol. Ecol. Notes 3, 228–232. https://doi.org/10.1046/j.1471-8286.2003.00406.x.

Li, Q., Yu, H., Yu, R., 2006. Genetic variability assessed by microsatellites in cultured populations of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture 259, 95–102. https://doi.org/10.1016/j.aquaculture.2006.05.030.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187.

Lind, C.E., Evans, B.S., Knauer, J., Taylor, J.J., Jerry, D.R., 2009. Decreased genetic diversity and a reduced effective population size in cultured silver-lipped pearl oysters (*Pinctada maxima*). Aquaculture 286, 12–19. https://doi.org/10.1016/j.aquaculture.2008.09.009.

Liu, T., Li, Q., Song, J., Yu, H., 2017. Development of genomic microsatellite multiplex PCR using dye-labeled universal primer and its validation in pedigree analysis of Pacific oyster (*Crassostrea gigas*). J. Ocean Univ. China 16, 151–160. https://doi.org/10.1007/s11802-017-3121-2.

Lynch, M., Ritland, K., 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152, 1753–1766.

Napora-Rutkowski, L., Rakus, K., Nowak, Z., Szczygiel, J., Pilarczyk, A., Ostaszewska, T., Irnazarow, I., 2017. Genetic diversity of common carp (*Cyprinus carpio* L.) strains breed in Poland based on microsatellite, AFLP, and mtDNA genotype data. Aquaculture 473, 433–442. https://doi.org/10.1016/j.aquaculture.2017.03.005.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. Bioinformatics 28, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460.

Queller, D.C., Goodnight, K.F., 1989. Estimating relatedness using genetic markers. Evolution 43, 258–275. https://doi.org/10.1111/j.1558-5646.1989.tb04226.x.

Raymond, M., Rousset, F., 1995. GENEPOP (Version1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573.

Rhode, C., Maduna, S.N., Roodt-Wilding, R., Bester-van der Merwe, A.E., 2014. Comparison of population genetic estimates amongst wild, F1 and F2 cultured abalone (*Haliotis midae*). Anim. Genet. 45, 456–459. https://doi.org/10.1111/age.12142.

Sawayama, E., Takagi, M., 2016. Genetic diversity and structure of domesticated strains of red sea bream, *Pagrus major*, inferred from microsatellite DNA markers. Aquac. Res. 47, 379–389. https://doi.org/10.1111/are.12498.

Straus, K.M., Vadopalas, B., Davis, J.P., Friedman, C.S., 2015. Reduced genetic variation and decreased effective number of breeders in five year-classes of cultured geoducks (*Panopea generosa*). J. Shellfish Res. 34, 163–169. https://doi.org/10.2983/035.034.0120.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739. https://doi.org/10.1093/molbev/msr121.

Taris, N., Batista, F.M., Boudry, P., 2007. Evidence of response to unintentional selection for faster development and inbreeding depression in *Crassostrea gigas* larvae. Aquaculture 272, S69–S79. https://doi.org/10.1016/j.aquaculture.2007.08.010.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Wang, X., Li, Q., Yu, H., Kong, L., 2016. Genetic variation assessed with microsatellites in mass selection lines of the Pacific oyster (*Crassostrea gigas*) in China. J. Ocean Univ. China 15, 1039–1045. https://doi.org/10.1007/s11802-016-3056-z.

Waples, R.S., 2006. A bias correction for estimates of effctive population size based on linkage disequilibrium at unlinked gene loci. Conserv. Genet. 7, 167–184. https://doi.org/10.1007/s10592-005-9100-y.

Xu, L., Li, Q., Xu, C., Yu, H., Kong, L., 2019. Genetic diversity and effective population size in successive mass selected generations of black shell strain Pacific oyster (*Crassostrea gigas*) based on microsatellites and mtDNA data. Aquaculture 500, 338–346. https://doi.org/10.1016/j.aquaculture.2018.10.007.

Zhang, T., Kong, J., Wang, W., Wang, Q., 2010. Genetic variability assessed by microsatellites in the breeding populations of the shrimp *Penaeus* (*Fenneropenaeus*) *chinensis* in China. Aquaculture 310, 229–233. https://doi.org/10.1016/j.aquaculture.2010.07.025.

Zhang, J., Li, Q., Wang, Q., Cong, R., Ge, J., Kong, L., 2018. The impact of successive mass selection on population genetic structure in the Pacific oyster (*Crassostrea gigas*) revealed by microsatellite markers. Aquac. Int. 26, 113–125. https://doi.org/10.1007/s1049.

Zhu, Y., Li, Q., Yu, H., Kong, L., 2018. Biochemical composition and nutritional value of different shell color strains of Pacific oyster *Crassostrea gigas*. J. Ocean Univ. China 17, 897–904. https://doi.org/10.1007/s11802-018-3550-6.