



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 The effects of successive mass selection on genetic structure and genetic diversity of Pacific oyster (*Crassostrea gigas*) were investigated using 18 microsatellite markers in 18 consecutive generations of a Pacific oyster population. The genetic structure was analyzed using Na, H, and He. The results showed that the genetic structure of the Pacific oyster population was highly diverse and stable. The genetic diversity of the Pacific oyster population was significantly reduced after successive mass selection ($P < 0.05$), and the genetic diversity of the Pacific oyster population was significantly reduced after successive mass selection. The results showed that the genetic structure of the Pacific oyster population was highly diverse and stable. The genetic diversity of the Pacific oyster population was significantly reduced after successive mass selection ($P < 0.05$), and the genetic diversity of the Pacific oyster population was significantly reduced after successive mass selection. The results showed that the genetic structure of the Pacific oyster population was highly diverse and stable. The genetic diversity of the Pacific oyster population was significantly reduced after successive mass selection ($P < 0.05$), and the genetic diversity of the Pacific oyster population was significantly reduced after successive mass selection.

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

In rod c ion

The Pacific oyster, *Crassostrea gigas*, is the dominant Pacific oyster species in the Pacific Northwest (Reed et al. 2005). According to the FAO (2016), the Pacific oyster is the most important aquaculture species in the world. It is the most widely farmed shellfish in the Pacific Northwest, with a production of 1550 metric tons in 2015 (FAO 2016).

Despite the oyster's economic importance, the Pacific oyster has been declining in abundance and production in the Pacific Northwest (Wade et al. 2000; Luedtke et al. 2003; Deegan et al. 2010). Quantifying the decline in abundance and production is important for understanding the causes of the decline and for developing effective management strategies. In the Pacific Northwest, the decline in abundance and production has been attributed to a number of factors, including disease, environmental changes, and overfishing. The decline in abundance and production has been particularly severe in the Pacific Northwest, where the oyster industry has been a major economic activity for many communities. The decline in abundance and production has also had significant impacts on the environment, including the loss of habitat and the degradation of water quality.

Because of the oyster's economic importance, the Pacific oyster has been the subject of a number of studies. These studies have focused on understanding the causes of the decline in abundance and production, and on developing effective management strategies. One of the most important factors in the decline in abundance and production is disease. The Pacific oyster is susceptible to a number of diseases, including Mortality Syndrome and Oyster Herpesvirus. These diseases have caused significant mortality in oyster populations in the Pacific Northwest. Another important factor in the decline in abundance and production is environmental changes. The Pacific oyster is sensitive to changes in water temperature, salinity, and pH. These changes can affect the oyster's growth and survival. Finally, overfishing has also contributed to the decline in abundance and production. The Pacific oyster is a highly valued species, and overfishing has led to a significant reduction in the size and number of oysters in the wild.

Materials and methods

Sample collections

Specimens were collected from various locations in the Pacific Ocean, including Japan (JG1–JG6), Korea (KG1–KG6), and the Philippines (WR). The specimens were collected during the years 2007–2013. The specimens were collected from the following locations: Japan (JG1–JG6), Korea (KG1–KG6), and the Philippines (WR). The specimens were collected from the following locations: Japan (JG1–JG6), Korea (KG1–KG6), and the Philippines (WR).

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DNA extraction and microsatellite analysis

Genomic DNA was extracted from muscle tissue using a DNeasy Blood & Tissue kit (Qiagen). The DNA was quantified using a NanoDrop spectrophotometer. The DNA was then amplified using PCR with the following conditions: 95°C for 5 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were purified using a PCR purification kit (Qiagen) and sequenced using a BigDye 3.1 sequencing kit (Applied Biosystems) on an ABI3130XL DNA sequencer.

Table 1 Sample collection details for *C. gigas*

Population	Number of individuals	Males	Females	Sample year	Collection date
WR	–	–	–	47	September 2013
JG0	–	–	–	47	July 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	July 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

WR = West Philippine Sea, JG = Japan, KG = Korea, M = Male, F = Female, N = Number of individuals, M = Males, F = Females, N = Number of individuals, M = Males, F = Females, N = Number of individuals.

Total extracted genomic DNA was amplified at $-30\text{ }^{\circ}\text{C}$. Samples were PCR amplified with 18 cycles of denaturation at $94\text{ }^{\circ}\text{C}$ for 30 s, annealing at $50\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 1 min. The PCR products were purified using the PCR Purification Kit (Qiagen). The PCR products were then sequenced using the ABI PRISM 3130XL DNA Sequencer (Applied Biosystems). The sequencing reaction was performed using the BigDye 3.1 sequencing kit (Applied Biosystems). The sequencing data were analyzed using GeneMapper 4.0 software.

Data analysis

Polymorphic bands were identified by comparing the banding patterns of the samples using MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004). Number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and fixation index (F_{is}) were calculated using MICROSAPELLITE ANALYSER 4.0 (De Meo and Scatena 2003). Fisher's

Res l s

Gene ic ariabili

T e MICRO-CHECKER a e ea ed a a e e e 13 f 18 c ce ed, d da a de a ed a c a affected b a e e a 15 a , d e d ce f“ e ea ” c a a e e ed c e a e a e ed. a a c a b e ed. A 18 c a e e c e e c e 15 a f *C. gigas* a ed d e e e f a ed a e e c.

A e a e be fa e e (Na) f e e e ed e f Ja e dec ed f 14.9 10.7, e f e e e ed e f K ea dec ed f 17.3 9.4 (Tabe 2). A f c ed c a e e be a b e ed JG5 (20.1%), JG6 (28.2%), KG5 (32.7%), d KG6 (38.6%), c a ed e b e ed e ba e a f e e e e ed e (W c a c ed- a ed- e, $P < 0.01$). T e e e Na a e e e b e ed e WR a (Na = 18.6). Me b e ed e e (H) e e a f e c ed e e e e d c a abe, f 0.757 0.846 e JG0–JG6 a d f 0.736 0.865 e KG0–KG6 a . T e a e e e e e (He) ed f 0.778 0.871 e JG0–JG6 a d f 0.744 0.854 e KG0–KG6 a (Tabe 2). N f c d ffe ce e a e a e f H a b e ed be e ba e a d e e ed a (W c a c ed- a ed- e, $P > 0.01$). T e b e ed c eff c a e (F) a c e e f a 15 a , a f -0.039 0.091 e e e ed e f Ja , d f -0.067 0.018 e e e e ed e f K ea.

M f e a e e (44.0–68.6%) e e f e ce (< 0.05) d 16.9–31.1% f e a e e e de a e f e ce (0.05–0.10) (F . 1). T e b e fa e e f e c (< 0.05) d dec e e e ce a e f e e e e a e e e e e ed e , d a f c d ffe ce a b e ed JG6 d KG6, c a ed e f e ba e a (W c a c ed- a ed- e, $P < 0.01$, F . 1). T e b e ed

Table 2 a a f e c d e e d ffe a f *C. gigas*

P a	Na	H	He	F
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 b e ed be fa e e, Ho b e ed e e , He e e e d e e , Fis b e ed c eff c

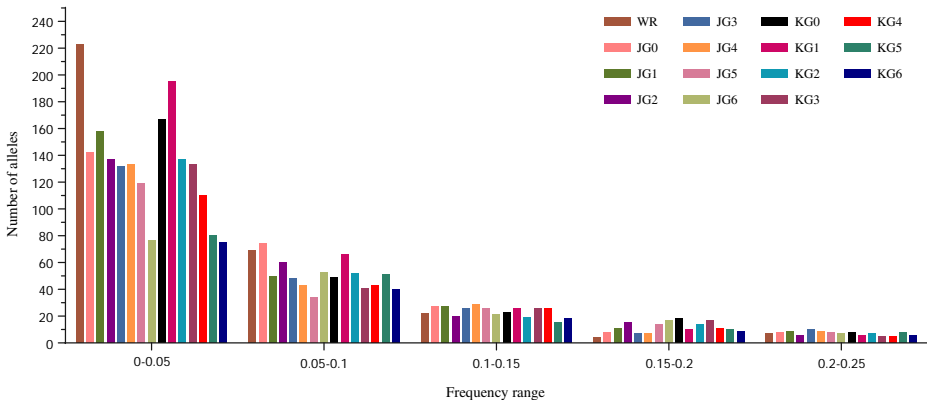


Fig. 1 Allele frequency distribution for 14 genotypes across five frequency ranges.

The results of the Hardy-Weinberg Equilibrium (HWE) test for the 14 genotypes are presented in Table 3. The *P* values for the HWE test are generally low, indicating deviations from HWE. For example, the *P* value for WR is 0.86504, while for JG0 it is 0.00002. The *P* values for the other genotypes are also low, ranging from 0.00016 to 0.04828. The *P* values for the HWE test are generally low, indicating deviations from HWE. For example, the *P* value for WR is 0.86504, while for JG0 it is 0.00002. The *P* values for the other genotypes are also low, ranging from 0.00016 to 0.04828.

Population differentiation

The results of the population differentiation analysis are presented in Table 4. The *F_{st}* values for the 14 genotypes are generally low, indicating low levels of population differentiation. For example, the *F_{st}* value for WR is 0.86504, while for JG0 it is 0.00002. The *F_{st}* values for the other genotypes are also low, ranging from 0.00016 to 0.04828.

Table 3 Test of Hardy-Weinberg Equilibrium (HWE) for 14 genotypes of *C. gigas*

Genotype	<i>P</i> value for HWE test (using the exact test)		
	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

The results of the HWE test for the 14 genotypes are presented in Table 3.

a be ed KG0–KG6 a ,c aed . eaeae a eF. ae JG0–JG6 a .F e e, c F. ae eea fcf. e. ($P < 0.01$ afe B fe c ec). Te a eF. ae a e. ba e a d. e d a edf. 0.0189, 0.0609, c eda e e f e c dffe a .

AMOVA e ea ed a fcf. ec a e c a ce a a caed . bd be e e ba e a d e cce e eec ed e f Ja ($P = 0.729$) d K ea ($P = 0.290$). M e e, f e a ce a d b ed d d a (93.60 d 95.12% e eec ed e f Ja d K ea) (Tab e 5).

Effec i e pop la ion si es

E eac e a , e effec e a e e ae ba ed e e a e d (Ne) ed f. 69.9 90.1 e eec ed e f Ja , d f. 64.6 105.9 e eec ed e f K ea (Tab e 6). H e e, e e ae e e a e d e b e d (Ne) ed a a e e f a a . Ne e e e eec ed e (e eec ed e f Ja , $Ne = 67.9$ – 205.2 ; e eec ed e f K ea, $Ne = 59.8$ – 147.9) (Tab e 6).

Disc ssion

e cce e eec e beed a , a a c ce f e eec ed a e f e c a ab d b eed , e eca f e eec . f fec d a (Gaf e e a . 1992; Y d G . 2004; W e e a . 2016). He e . f ed a a a e c

Table 5 Ma f ec a a ce (AMOVA) f c. ae e f . c. ed eec e beed e f *C. gigas*

Sc e f a a	De ee. f feed	S . f ae	Va ce c	Pe ce ae f a a (%)	P ae
Se eed e f Ja					
A a	1	38.807	- 0.09677	- 1.25	0.729
A a a	5	275.763	0.49855	6.42	0
A d d a a	327	2438.931	0.09527	1.23	0.019
W d d a	334	2427.500	7.26796	93.60	0
T a	667	5181.001	7.76502	-	-
Se eed e f K ea					
A a	1	61.344	0.06140	0.80	0.290
A a a	5	255.559	0.46080	5.99	0
A d d a a	328	2305.548	- 0.14664	- 1.90	0.999
W d d a	335	2453.000	7.32239	95.12	0
T a	669	5075.451	7.69795	-	-

Eac a eec e a d ded , e ba e a d e a - eec ed a

de abea ee ae ae ebae a. A. Lea. (2011) d Wa e a. (2012) e ed a 7.2–13.2 d 8.4–10.4% a f d ec d ea f ea ed ac d ffe ea ae ee ba ed e ee eced e f *C. gigas*, a f e d eec e e f be ea ae ae e ed c f e a f e eced e f cea ed a eec e beed a. a, 46, f270 a - c cae de aedf. e Had - We be e ca d. Lea. (2006) eed f e c ed a f Pac f. e C a d f d a 32. f35 a - c cae de aedf. HWE. A de a f HWE c d e f e e ce f a ee, b beed, d a, da fca d a a eec d eed d c d c a (McG d c 2000; Lea. 2003; Hed ec c e a. 2004; Lea. 2006). d, aea e eec (10%) a a ed e cce e a eec, c cea ed e f d a, d c de e de a f HWE e e eced e.

T e ea e c d ffe a (F) a e ba e a d e e eced e f K d J e e f c d ffe f e ($p < 0.05$). W e e eced e f K d J, e c d ffe a ($F < 0.06$) e e de eced a e f f ea. H e e, de ae e c d ffe a ($0.06 < F < 0.15$) e e de eced be e f f ea e e eced e, e a ba e e e c e a a ea e e f cce e a eec. T e e ce f a ba e a be a d e e - e ce f cce e a fca eec d e a f *C. gigas* a a a e. S a a a be d c ed e ec c ae e c e eced a f e Pac f. e (A e ad d Wa d 2006) d c ca (Na a-R e a. 2017). T e e ea ed b AMOVA d a d ca ed a f c e c a ce a a ca ed bd b e e e ba e a d e cce e e eced e.

M e e a c e a e d ca ed a e e a e f effec e a e e e bab e ffc ae e acc ae ec f a f a a (e e ca f a fca eec e a), c a ed e ea a f e ec a a ee (c a *H d He*) (Wa e 1989; C e a e a. 2005). d, e a e f *Ne d Ne* e e eced e e ea ed b e e a a e e a e a e ($Ne > 60$, $Ne > 50$) e ea (B d d O e 2002), d ca a e effec e a e a affected ea b cce e a eec. H e e, e dec e f e effec e a e a be ed e e eced a d c aed c f e (Hed ec c d S 1990; Gaff e e a. 1992). T e dec a e f effec e a e a e f a fca eec e be f f d ced b fe a d e a e c e d c d a a e f b d c (A e ad d Wa d 2006; La a e a. 2010). T e effec e a e c a be ed ced b d f e f e a ce f e d c e cce e e a c e - a aed e d (B d e a. 2002; G a d e a. 2003). T e cce e a eec a ffc affected effec e a e f e e eced e, c a e f a fca fe a a ed a eec e beed a, da e ae d fe ae c be a eed c b e a e e e e ea.

O e ba ed a e d e b ed a e e a fca e e effec e a e f e e eced e a e eec c e e e ed. T e

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