

: 1000-0615(2017)12-1838-09

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PCR

3

*

(266003)

10 3 4 PCR
6 7.2~12.6
6.8~11.0 0.672~0.769 0.486~0.542 3
60 — 39 —
 F_{is} 0.215~0.342 F_{st} 0.005~0.076

: PCR : A
: Q 786 S 968.3

(*Crassostrea gigas*)

[6]

[7]

“ 1 ”

457 t 2015 [1]

[8-10]

2010 4 [11-12]

3

[2-3]

Langdon [4]

Dégre-

mont [5]

4

(OsHV-1)

2016-11-12

2017-03-06

(2014GB2B020029)

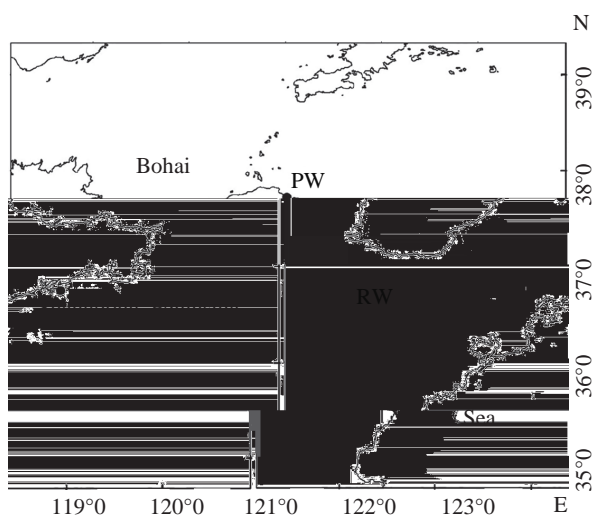
(2014GHY115002)

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1
1.1
 1 2
 (1)
 2010
 PCR (multiplex PCR) 4
 PCR 2014—2016 3
 [13-17] -80 °C /
 PCR DNA
 3 Li [18] NanoDrop 2000 DNA
 50 ng/μL DNA
 -20 °C
 1

Tab. 1 Details of sample locations, types, time and number of *C. gigas*

population	sampling site	sample time	population type	sample number (n)
G ₀	(36.5°N 121.3°E)	2010-06		50
G ₁	(36.5°N 121.3°E)	2015-08	1	50
G ₂	(36.5°N 121.3°E)	2016-03	2	50
G ₃	(36.5°N 121.3°E)	2016-11	3	50
RW	(36.8°N 121.7°E)	2016-03		50
PW	(37.7°N 120.0°E)	2016-02		50



1
 PW. wild Penglai population; RW. wild Rushan population

Fig. 1 Locations of wild populations and base stock samples collected

PW. wild Penglai population; RW. wild Rushan population

1.2 PCR
 Liu [19] 4 PCR 10
 PCR
 10 μL 50 ng DNA 10×PCR Buffer
 (Mg²⁺)1 μL 0.2 mmol/L dNTP 10 μmol/L
 0.15 μL 10 μmol/L 3
 0.06 μL 10 μmol/L 3 0.15 μL Taq
 (5 U/μL) 0.05 μL PCR 94 °C
 3 min 94 °C 30 s 60 s 72 °C
 75 s 35 94 °C 30 s 53 °C
 60 s 72 °C 75 s 8 72 °C 10 min
 12 °C PCR ABI3130
 GeneMarker

2.2.0

1.3

GenAIEx 6.502^[20]

2 **PCR**
Tab. 2 Multiplexes of microsatellite markers for *C. gigas*

group	locus	(5 ~ 3) primer sequence (5 - 3)	/°C annealing temperature	/bp size
panel 1	ucdCg-117	F-TGTAAAACGACGGCCAGTCCAAGCTTGCACTCACTCAA 6-FAM R-GAGTGTCTCTGGTGTGCCAAAT	58	290
	ucdCg-120	F-TGTAAAACGACGGCCAGTGGGTGAGATTTAGGGGAGAGA 6-FAM R-CTCCATCAAACCTGCCAAAC	58	152
	ucdCg-198	F-TGTAAAACGACGGCCAGTGAAAGACACGACCGGAGAGA 6-FAM R-CTGATGATGTCCACACCTG	58	230
panel 2	ucdCg-146	F-TGTAAAACGACGGCCAGTCGCTCTGGTCTTTGTTCCAT VIC R-ACCCCAACAGATCACAATCC	58	218
	Crgi3	F-TGTAAAACGACGGCCAGTTAGGATGAGGCTGGCACCTTGGA VIC R-GCCTGCCTTGCTTTGAGGAATA	58	161~173
	uscCgi-210	F-TGTAAAACGACGGCCAGTTTCACAATGAAGATGACAGTGC VIC R-CCTCCTCTGCCTCCATATCA	58	316
panel 3	ucdCg-170	F-TGTAAAACGACGGCCAGTTGGTGGTCAGTGAATGTGAGA NED R-CGGACAGTAGCCTTTTAAACACA	58	276
	ucdCg-199	F-TGTAAAACGACGGCCAGTGGGAAGAGTTGAATTCTGCAA NED R-AAACCGAGGCTCAGGAAAAT	58	270
panel 6	ucdCg-200	F-TGTAAAACGACGGCCAGTAAAGTTGCTTTGCTGTCGTC PET R-CGCTAACGTGCTTCATTCAA	54	254
	otgfa0_408293	F-TGTAAAACGACGGCCAGTACCCTGGTTTGATCTGAGAAATG PET R-TCTAAGGAGTGTGAGTGTAGTAG	54	118~122

(N_a)	(H_e)	(H_o)	7.2~12.6	6.8~11.0
—	(HWE)			0.486~0.542
(D_c)	FSTAT V2.9.3.2 ^[21]		0.672~0.769	Mann-Whitney U
(A_r)	(F_{is})		3	
(F_{st})		(Mann-Whitney U		$(P<0.05)$
test) ^[22]				
	MEGA 6 ^[23]	UPGMA		$(P>0.05)$
			0.215~0.342	6
2			50	10
			(H_e)	(H_o)
2.1			—	Bonferroni
			—	60
4	PCR10	2	—	39
3			—	$(P<0.05/10)$
		3 10	6	2.2
			6	F_{st}
	3~20	6	G_1 G_2	$(P<0.01)$
				F_{st}

0.005~0.045
 G_3 F_{st} D_c G_3 RW
 0.046~0.076
 Nei D_c 0.007~ G_1 G_2
 0.236 G_1 G_2 G_3 (2)

3

(n=50)

Tab. 3 Genetic diversity of successive mass-selection strains, and wild and base populations of *C. gigas*

locus	population						
		G_0	G_1	G_2	G_3	RW	PW
ucdCg-120	N_a	8.0	4.0	4.0	5.0	9.0	9.0
	H_o	0.673	0.600	0.694	0.844	0.660	0.571
	H_e	0.614	0.573	0.602	0.700	0.695	0.706
	A_r	6.0	4.0	4.0	4.6	7.4	7.9
	F_{is}	-0.087	-0.036	-0.142	-0.195	0.061	0.201
	P	1.000	0.154	0.078	0.373	0.843	0.000*
ucdCg-198	N_a	14.0	11.0	9.0	9.0	18.0	18.0
	H_o	0.521	0.429	0.548	0.571	0.520	0.375
	H_e	0.823	0.809	0.838	0.812	0.911	0.880
	A_r	11.2	9.0	8.5	8.3	15.7	15.7
	F_{is}	0.376	0.478	0.357	0.307	0.437	0.581
	P	0.000*	0.000*	0.001*	0.050	0.000*	0.000*
ucdCg-117	N_a	17.0	12.0	10.0	9.0	18.0	16.0
	H_o	0.296	0.512	0.639	0.677	0.500	0.459
	H_e	0.912	0.830	0.813	0.805	0.917	0.919
	A_r	17.0	10.4	9.6	8.9	16.0	15.5
	F_{is}	0.685	0.394	0.227	0.175	0.464	0.51
	P	0.000*	0.000*	0.000*	0.009*	0.000*	0.000*
Crgi3	N_a	7.0	5.0	3.0	3.0	7.0	6.0
	H_o	0.220	0.260	0.122	0.217	0.380	0.420
	H_e	0.389	0.346	0.360	0.232	0.479	0.404
	A_r	5.2	4.4	3.0	2.8	5.9	5.1
	F_{is}	0.442	0.258	0.665	0.072	0.217	-0.03
	P	0.002*	0.000*	0.000*	0.000*	0.112	0.872
ucdCg-146	N_a	17.0	13.0	11.0	11.0	20.0	20.0
	H_o	0.447	0.939	0.913	0.690	0.660	0.617
	H_e	0.903	0.862	0.871	0.877	0.922	0.918
	A_r	15.1	11.2	10.0	10.4	16.8	17.8
	F_{is}	0.513	-0.079	-0.038	0.224	0.294	0.337
	P	0.000*	0.261	0.015	0.000*	0.024	0.000*

locus		population					
		G ₀	G ₁	G ₂	G ₃	RW	PW
uscCgi-210	N_a	7.0	4.0	5.0	4.0	9.0	6.0
	H_o	0.604	0.313	0.295	0.361	0.521	0.587
	H_e	0.666	0.561	0.550	0.721	0.668	0.680
	A_r	6.0	4.6	4.5	4.0	7.4	5.8
	F_{is}	0.103	0.541	0.472	0.524	0.231	0.148
	P	0.000*	0.005	0.000*	0.000*	0.895	0.125
ucdCg-170	N_a	18.0	9.0	10.0	11.0	14.0	15.0
	H_o	0.583	0.440	0.408	0.429	0.440	0.408
	H_e	0.914	0.824	0.848	0.761	0.906	0.900
	A_r	15.4	8.0	9.5	9.5	13.5	13.1
	F_{is}	0.371	0.474	0.526	0.445	0.522	0.553
	P	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
ucdCg-199	N_a	7.0	8.0	8.0	7.0	8.0	7.0
	H_o	0.143	0.132	0.306	0.138	0.205	0.220
	H_e	0.390	0.521	0.590	0.337	0.323	0.592
	A_r	6.5	7.2	7.3	6.9	6.5	6.4
	F_{is}	0.641	0.753	0.493	0.601	0.377	0.636
	P	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
otgfa0_40829 3	N_a	9.0	6.0	6.0	7.0	10.0	11.0
	H_o	0.800	0.755	0.714	0.680	0.780	0.780
	H_e	0.820	0.614	0.653	0.654	0.785	0.828
	A_r	8.8	5.3	5.5	6.3	9.2	9.8
	F_{is}	0.035	-0.22	-0.083	-0.03	0.016	0.068
	P	0.797	0.900	0.418	0.000*	0.015	0.098
ucdCg-200	N_a	10.0	6.0	8.0	6.0	13.0	12.0
	H_o	0.574	0.596	0.653	0.809	0.592	0.646
	H_e	0.861	0.814	0.812	0.823	0.870	0.865
	A_r	9.2	7.1	8.1	6.0	11.8	10.5
	F_{is}	0.342	0.285	0.211	0.028	0.329	0.263
	P	0.000*	0.014	0.000*	0.466	0.000*	0.000*
mean	N_a	11.4±4.6 ^{ab}	7.8±3.3 ^a	7.4±2.8 ^a	7.2±2.8 ^a	12.6±4.7 ^b	12.0±5.1 ^b
	H_o	0.486±0.209	0.497±0.239	0.529±0.241	0.542±0.244	0.526±0.162	0.508±0.162
	H_e	0.729±0.206 ^a	0.676±0.176 ^a	0.694±0.169 ^a	0.672±0.216 ^a	0.748±0.208 ^a	0.769±0.171 ^a
	A_r	10.0±4.4 ^{ab}	7.1±2.5 ^{ab}	7.0±2.6 ^{ab}	6.8±2.5 ^a	11.0±4.3 ^b	10.8±4.6 ^b
	F_{is}	0.342±0.255	0.285±0.309	0.269±0.282	0.215±0.256	0.295±0.167	0.327±0.234

Notes: significance of Hardy-Weinberg departure: * $P < 0.05/10$; in the same row, values with the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$)

4

 F_{st} () Nei D_c ()Tab. 4 Pairwise F_{st} (under diagonal) and Nei unbiased genetic distances (above diagonal) of six *C. gigas* populations

	G ₀	G ₁	G ₂	G ₃	RW	PW
G ₀		0.092	0.109	0.186	0.026	0.033
G ₁	0.035		0.007	0.166	0.113	0.128
G ₂	0.036	0.005		0.165	0.140	0.145
G ₃	0.063	0.046	0.061		0.236	0.215
RW	0.005	0.040	0.045	0.076		0.039
PW	0.007	0.043	0.045	0.068	0.008	

: F_{st}

[26-28]

65%

 $(P < 0.05)$

Li [29] 51.9% (41/79)

[18]

6 F_{is} 0.215~0.342

83.3%

3

 F_{st}

[27]

3

 F_{st}

0.008~0.025

[30]

*(Pinctada**martensii)*

3

 F_{st} 0.0031~0.1478G₁ G₂G₃

3

31.6%~42.9% 29.0%~38.2%

G₃

3

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Analysis of genetic diversity in mass selection lines of white-shell Pacific oyster (*Crassostrea gigas*) using microsatellite fluorescent multiplex PCR technique

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Abstract: To investigate the genetic variances in our successive selection strains for fast growth and white shell color of the Pacific oyster (*Crassostrea gigas*) during mass selection process, genetic diversity of three successive mass selection strains from G₁ to G₃, one based stock and two wild populations was analysed using multiplex assays with ten microsatellite markers. The study showed that the average allele number (N_a) ranged from 7.2 to 12.6 and the mean allelic richness (A_r) ranged from 6.8 to 11.0, the expected heterozygosity (H_e) and observed heterozygosity (H_o) ranged from 0.672 to 0.769 and 0.486 to 0.542, respectively. Compared to wild populations, the three successive mass selection strains showed significantly lower in the mean number of alleles and the mean allelic richness, but there was no significant difference in the average expected heterozygosity among the populations. There were 39 cases deviating significantly from Hardy-Weinberg equilibrium among the 60 population-locus cases (6 populations \times 10 loci), and the inbreeding coefficient (F_{is}) ranged from 0.215 to 0.342. F_{st} values ranged from 0.005 to 0.076, showing low-moderate level genetic differentiation among the populations. The results suggest that the genetic variation has not been significantly affected by continuous mass selection and high genetic variability remains existing in the three successive selection strains, indicating that there is still potential for increased gains in future selective breeding programs.

Key words: *Crassostrea gigas*; white-shell; microsatellite; multiplex PCR; genetic diversity

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