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Genetic Differentiation in Geographical Populations of the Pacific Oyster (*Crassostrea gigas*) around Japan

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Summary

Estimates of genetic distance were determined for local populations of Crassostrea gigas. The estimates are based on examination of 10 genetic loci using starch gel electrophoresis. The genetic distance between different geographical populations, Hokkaido, Miyagi, Shizuoka, Hiroshima and Kumamoto, varied from 0.0023 to 0.0099. The values indicate that genetic differentiation between local populations is low. Genetic differentiation within wild populations seems to be smaller than in cultured populations.

The amount of genetic variability found in *C. gigas* is higher than those values reported for other oyster species. This may be correlated with the fact that *C. gigas* has been spread widely and rapidily to other countries in the world by the transplantation of Japanese seeds.

Electrophoretic data are a valuable asset to measure the degree of genetic differentiation between populations. If two populations are isolated from each other for geographical or reproductive reasons, then the two populations tend to accumulate different genes. This differentiation of genes may occur through factors such as mutation, selection, random genetic drift and founder effect. Genetic differentiation, as measured by enzyme polymorphisms, is greater between cultured populations than natural populations in fish (1).

The Pacific oyster, *Crassostrea gigas*, is native to Japan, where it is widespread in coastal waters, and classified into four local races, Hokkaido, Miyagi, Hiroshima and Kumamoto strains on the basis of differences in morphological and physiological characteristics (2). Additional evidence from electrophoretic data in these strains tend to support Imai and Sakai's classification (3, 4). Miyagi and Hiroshima are the most important districts where the larvae are collected, and seed-oysters ongrown in raft culture. Miyagi seed in particular has been transported to other culture-districts in Japan and exported to several countries in the world. Transplantations of Miyagi seed were sent to the north-western coasts of

America and to British Columbia, Canada, at the begining of this century (5, 6). These transplants have been repeated several times, and to-day spawning populations of *C. gigas* are established in several areas along the west coasts of North America. Oysters were also exported from Miyagi to Europe, mainly France (7). Recently, oyster plantings have been tried in Australia and South-east Asia. In 1979, *C. gigas* spat were imported from British Columbia into a hatchery in North Wales, United Kingdom. *C. gigas* was accidentally introduced into New Zealand (8) where it now forms the base of an oyster farming industry. Therefore, the Pacific oyster has been spread to several countries in the world.

The purpose of this study was to examine 1) genetic differentiation within Japanese populations, and 2) to compare the degree of genetic differentiation and the amount of genetic variability in wild and cultured populations.

Materials and Methods

Five populations from the four main Japanese oyster populations, Hokkaido, Miyagi, Hiroshima and Kumamoto, of *Crassostrea gigas* were sampled (Fig.). Cultured oysters were collected from three different locations in Miyagi and one location in Hiroshima, where seed oysters are produced. In addition cultured oysters were collected from Hokkaido and Shizuoka. Most cultured oysters had been grown on raft culture strings on which about 200-500 spat were attached. Natural oysters had grown on rocks or mud banks. These latter samples were tentatively grouped as wild populations for comparative purposes with the cultured populations. Locations and date of collection are shown in Fig.

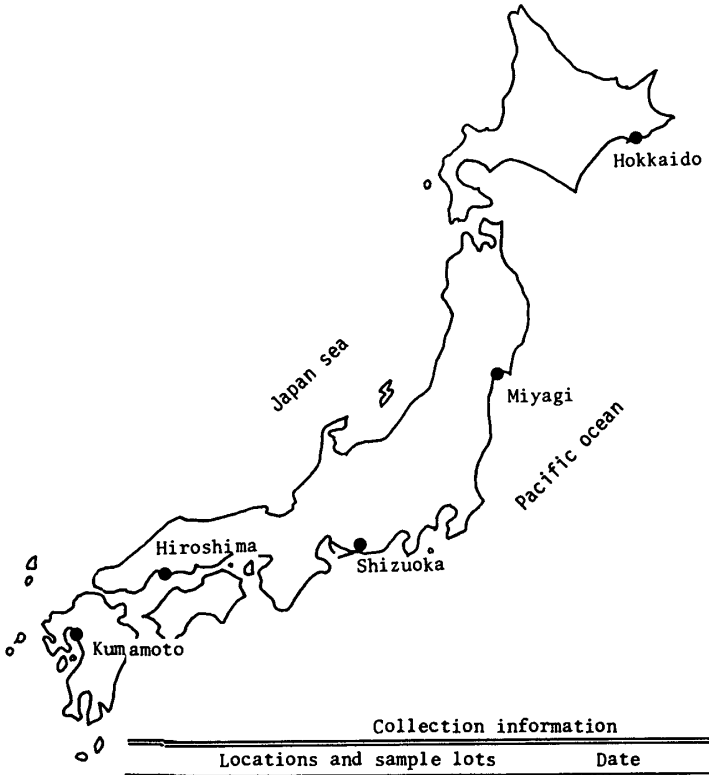
The samples were transported alive to the laboratory where the visceral mass and adductor muscle were removed from the shells and frozen at $-80°C$ until electrophoresis. Adductor muscle and digestive diverticular were homogenized with an approximately equal weight of distilled water, and the homogenates were centrifuged at 3,500 rpm for 30 minutes. The supernatants were subjected to horizontal starch gel electrophoresis. A tris-citrate buffer system was used as follows: electrode buffer 135 mM Tris-43 mM citrate (pH 7.0) and gel buffer a 1 : 10 dilution of electrode buffer in distilled water. Starch gels (10%) were run for 4 hrs. at 35.7 V/cm^2 in a cold box (4°C). After the run, the gel was sliced horizontally and the cut surface was stained following the recipes of Shaw and Prasad (9).

Eight enzymes and one muscle protein were examined in all of the samples. The eight enzymes were aspartate aminotransferase (AAT), glucosephosphate isomerase (GPI), isocitrate dehydrogenese (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), octanol dehydrogenase (ODH), phosphoglucomutase (PGM), and superoxide dismutase (SOD). Loci, which are shown in italics, were numbered after Fujio (3). The alleles were designated alphabetically in order of electrophoretic mobility from the most anodal to the most

cathodal.

Results

Ten genetic loci wer examined, five of which were found to be polymorphic (frequency of most common allele <0.95) and the other five were observed to be monomorphic. The proportion of polymorphic loci was 0.500 in all populations. Allele frequencies at the 10 genetic loci are tabulated in Table 1. An attempt was



Collection information

	Locations and sample lots	Date	Age
Cultured oysters	Akkeshi, Hokkaido	5/17/1984	2 years old?
	Ojika-1, Miyagi	4/ 6/1979	2 years old
	Ojika-2, Miyagi	4/ 6/1979	2 years old
	Ojika-3, Miyagi	3/19/1980	2 years old
	Mangokuura-1, Miyagi	4/25/1978	2 years old
	Mangokuura-2, Miyagi	4/25/1978	2 years old
	Mangokuura-3, Miyagi	6/13/1979	2 years old
	Mangokuura-4, Miyagi	6/13/1979	1 years old
	Mangokuura-5, Miyagi	5/ 7/1980	2 years old
	Mangokuura-6, Miyagi	5/ 7/1980	2 years old
	Mangokuura-7, Miyagi	7/ 1/1983	2 years old
	Mangokuura-8, Miyagi	7/ 1/1983	1 years old
	Matsushima-1, Miyagi	4/21/1978	2 years old
	Matsushima-2, Miyagi	3/26/1980	2 years old
	Matsushima-3, Miyagi	3/26/1980	2 years old
	Matsushima-4, Miyagi	3/11/1983	2 years old
	Hamanako(Miyagi seed), Shizuoka	12/12/1978	2 years old
	Hamanako(Native seed), Shizuoka	12/12/1978	2 years old
	Ondo, Hiroshima	6/ 1/1984	2 years old
Natural oysters	Akkeshi, Hokkaido	5/17/1984	
	Mangokuura, Miyagi	6/13/1979	
	Watari, Miyagi	5/11/1979	
	Ariake, Kumamoto	6/27/1983	

FIG. Location of five local populations (Hokkaido, Miyagi, Shizuoka, Hiroshima, and Kumamoto) of *Crassostrea gigas* in Japan

Tale 1. *Allele Frequencies at Several Localities in Crassostrea gigas*

		Cultured oyster					
		Akkeshi Hokkaido	Ojika-1 Miyagi	Ojika-2 Miyagi	Ojika-3 Miyagi	Mangokuura-1 Miyagi	Mangokuura-2 Miyagi
Aat-2	*N*	36	223	176	315	116	149
	A	0	0	0	0	0	0
	B	0.389	0.357	0 349	0.297	0.297	0.258
	C	0.555	0.574	0.555	0.625	0.690	0.642
	D	0.056	0.069	0.096	0.078	0.013	0.101
Bai-1b	*N*	24	223	176	315	128	149
	A	0	0.004	0.003	0 011	0.007	0 010
	B	0.042	0.054	0 017	0.040	0.019	0.044
	C	0.916	0 900	0.946	0.886	0.918	0.906
	D	0 042	0.038	0.034	0.057	0.052	0.040
	E	0	0.002	0	0.006	0 004	0
Idh-1	*N*	40	223	176	315	130	149
	A	0.050	0 038	0.026	0.044	0.076	0.044
	B	0.612	0.765	0 721	0.691	0.704	0.768
	C	0.338	0.197	0.253	0.265	0.220	0.188
Idh-2	*N*	50	59	58	60	58	57
	A	0	0	0	0	0	0
	B	1 000	1.000	1.000	1.000	1.000	1.000
Lap-1b	*N*	40	223	176	315	130	149
	A	0.013	0.018	0 011	0.025	0.027	0 013
	B	0.125	0.047	0 077	0.044	0.100	0.023
	C	0.750	0.807	0.765	0.817	0.761	0.816
	D	0.050	0.013	0.011	0.011	0.004	0.010
	E	0.025	0.099	0.116	0.089	0.085	0.111
	F	0 013	0.016	0.020	0 014	0.023	0.027
	G	0 024	0	0	0	0	0
Mdh	*N*	30	60	60	60	60	60
	A	1.000	1.000	1 000	1.000	1.000	1.000
Odh	*N*	45	60	60	60	60	60
	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
	C	0.011	0	0	0	0	0
	D	0.980	1.000	1.000	1.000	1.000	1.000
	E	0	0	0	0	0	0
Pgm	*N*	59	223	176	315	104	149
	A	0.154	0.152	0 202	0.187	0.231	0.158
	B	0	0.036	0.031	0.013	0	0.023
	C	0.641	0.606	0.565	0.607	0.583	0 591
	D	0.179	0.108	0.074	0 103	0.102	0.087
	E	0.013	0.092	0.091	0 079	0.080	0.074
	F	0.013	0.011	0.037	0 011	0.004	0.067
Sod	*N*	26	60	60	60	60	60
	A	1 000	1.000	1.000	1.000	1.000	1.000
Pt	*N*	42	60	60	60	60	60
	A	1.000	1 000	1 000	1.000	1.000	1.000

Table 1. (continued)

		Cultured oyster					
		Mangokuura -3 Miyagi	Mangokuura -4 Miyagi	Mangokuura -5 Miyagi	Mangokuura -6 Miyagi	Mangokuura -7 Miyagi	Mangokuura -8 Miyagi
Aat-2	*N*	108	127	80	113	53	57
	A	0	0	0	0	0	0
	B	0.245	0.346	0.350	0.314	0.264	0.272
	C	0.639	0.575	0.582	0.598	0.627	0.632
	D	0.116	0.079	0.068	0.088	0.075	0.096
Gpi-1b	*N*	108	127	80	113	53	45
	A	0.005	0.008	0	0.009	0	0
	B	0.046	0.071	0.050	0.058	0.019	0.122
	C	0.926	0.882	0.875	0.902	0.914	0.789
	D	0.018	0.039	0.075	0.031	0.067	0.078
	E	0.005	0	0	0	0	0.011
Idh-1	*N*	108	127	80	113	53	48
	A	0.046	0.051	0.050	0.018	0.057	0.063
	B	0.764	0.673	0.681	0.692	0.697	0.583
	C	0.190	0.276	0.269	0.290	0.246	0.354
Idh-2	*N*	59	60	60	55	57	45
	A	0	0	0	0	0	0
	B	1.000	1.000	1.000	1.000	1.000	1.000
Lap-1b	*N*	108	127	80	112	53	59
	A	0.023	0.020	0.006	0.004	0.008	0
	B	0.042	0.043	0.050	0.049	0.127	0048
	C	0.847	0.826	0.807	0.832	0.755	0.865
	D	0.005	0.008	0.031	0.044	0.059	0.029
	E	0.083	0.079	0.100	0.071	0.051	0.058
	F	0	0.024	0.006	0	0	0
	G	0	0	0	0	0	0
Mdh	*N*	60	60	60	60	59	30
	A	1.000	1.000	1.000	1.000	1.000	1.000
Odh	*N*	60	60	59	54	58	30
	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	1.000	1.000	1.000	1.000	1.000	1.000
	E	0	0	0	0	0	0
Pgm	*N*	108	127	80	112	53	67
	A	0.241	0.213	0.175	0.151	0.240	0.193
	B	0.005	0.024	0.019	0	0	0
	C	0.550	0.566	0.625	0.656	0.625	0.620
	D	0.093	0.150	0.138	0.090	0.087	0.120
	E	0.079	0.047	0.031	0.076	0.048	0.037
	F	0.032	0	0.100	0.027	0	0.030
Sod	*N*	60	58	56	57	53	53
	A	1.000	1.000	1.000	1.000	1.000	1.000
Pt	*N*	60	60	60	60	56	53
	A	1.000	1.000	1.000	1.000	1.000	1.000

Table 1 (continued)

		Cultured oyster				
		Matsushima -1 Miyagi	Matsushima -2 Miyagi	Matsushima -3 Miyagi	Matsushima -4 Miyagi	Hamanako (Miyagi seed) Shizuoka
Aat-2	*N*	117	154	132	232	347
	A	0	0	0	0	0
	B	0.291	0.302	0 227	0 248	0.252
	C	0.602	0.604	0.666	0.677	0 663
	D	0 107	0 094	0 057	0.075	0 085
Gpi-1b	*N*	119	154	132	232	347
	A	0.013	0	0 004	0 009	0.007
	B	0 046	0 055	0 049	0 047	0.060
	C	0.903	0.897	0 894	0 910	0.882
	D	0 025	0 045	0 049	0 034	0 047
	E	0.013	0 003	0.004	0	0
Idh-1	*N*	118	154	132	232	347
	A	0 076	0.052	0 015	0 045	0.052
	B	0.704	0.708	0.674	0.617	0.672
	C	0.220	0.240	0 311	0.338	0 276
Idh-2	*N*	60	60	60	60	60
	A	0	0	0	0	0
	B	1 000	1 000	1 000	1.000	1.000
Lap-1b	N	119	154	132	232	347
	A	0	0.006	0 011	0.013	0 017
	B	0 088	0 052	0 053	0.060	0 063
	C	0 782	0.777	0 827	0 786	0 793
	D	0 004	0.019	0.011	0.026	0 015
	E	0 109	0 120	0 087	0 091	0 101
	F	0 017	0 026	0 011	0.024	0 011
	G	0	0	0	0	0
Mdh	*N*	60	60	60	60	60
	A	1 000	1 000	1 000	1 000	1.000
Odh	*N*	59	60	56	67	60
	A	0	0	0	0	0
	B	0	0	0	0	0
	C	0	0	0	0	0
	D	1 000	1.000	1 000	1 000	1 000
	E	0	0	0	0	0
Pgm	*N*	104	154	132	221	347
	A	0 322	0 301	0 152	0 120	0 201
	B	0	0 013	0.011	0 086	0.010
	C	0 520	0.604	0 634	0 577	0.606
	D	0 106	0 114	0 110	0.077	0 119
	E	0 038	0 068	0 083	0 106	0.057
	F	0 014	0	0 011	0 031	0 007
Sod	*N*	60	60	53	60	60
	A	1.000	1 000	1 000	1 000	1.000
Pt	*N*	60	60	60	60	60
	A	1 000	1 000	1 000	1 000	1 000

Table 1. (continued)

		Cultured oyster		Natural oyster			
		Hamanako (Native seed) Shizuoka	Ondo Hiroshima	Akkeshi Hokkaido	Mangokuura Miyagi	Watari Miyagi	Ariake Kumamoto
Aat-2	*N*	90	51	55	58	228	57
	A	0	0	0	0	0	0
	B	0.341	0.118	0.291	0.207	0.300	0.219
	C	0.591	0.764	0.591	0.724	0.627	0.702
	D	0.140	0.118	0.118	0.069	0.079	0.079
Gpi-1b	*N*	90	50	29	58	228	56
	A	0.006	0.020	0	0.026	0.009	0
	B	0.033	0.080	0.052	0.052	0.050	0.036
	C	0.938	0.890	0.896	0.870	0.889	0.910
	D	0.023	0.010	0.052	0.052	0.048	0.054
	E	0.006	0	0	0	0.004	0
Idh-1	*N*	90	56	55	59	228	57
	A	0.030	0.027	0.065	0.008	0.024	0.036
	B	0.730	0.624	0.704	0.738	0.733	0.606
	C	0.240	0.349	0.231	0.254	0.243	0.358
Idh-2	*N*	60	30	30	30	60	30
	A	0	0.027	0	0	0	0
	B	1.000	0.972	1.000	1.000	1.000	1.000
Lap-1b	*N*	90	56	41	57	228	57
	A	0.022	0	0.009	0.044	0.015	0.026
	B	0.044	0.089	0.055	0.026	0.050	0.044
	C	0.812	0.776	0.864	0.825	0.830	0.851
	D	0.008	0.036	0.018	0.009	0.024	0.070
	E	0.110	0.063	0.018	0.070	0.004	0.009
	F	0.004	0.036	0.027	0.026	0.004	0
	G	0	0	0.009	0	0	0
Mdh	*N*	60	45	55	30	60	49
	A	1.000	1.000	1.000	1.000	1.000	1.000
Odh	*N*	60	30	42	30	60	37
	A	0	0	0.012	0	0	0
	B	0	0	0.012	0	0	0
	C	0	0	0	0	0	0
	D	1.000	1.000	0.964	1.000	1.000	1.000
	E	0	0	0.012	0	0	0
Pgm	*N*	90	49	42	56	228	56
	A	0.143	0.071	0.215	0.225	0.246	0.197
	B	0.012	0	0	0.010	0.018	0
	C	0.578	0.572	0.559	0.618	0.587	0.606
	D	0.128	0.245	0.214	0.078	0.090	0.179
	E	0.106	0.102	0.012	0.059	0.059	0
	F	0.011	0.010	0	0.010	0	0.018
Sod	*N*	60	29	57	29	60	44
	A	1.000	1.000	1.000	1.000	1.000	1.000
Pt	*N*	60	29	30	30	60	38
	A	1.000	1.000	1.000	1.000	1.000	1.000

Table 2 *Estimates of Genetic Distance within and between Locations in Miyagi Cultured Populations of Crassostrea gigas*

Location (Number of Samples)	Ojika (3)	Mangokuura (8)	Matsushima (4)
Ojika (3)	0.0012±0.0001		
Mangokuura (8)	0.0022±0.0003	0.0028±0.0003	
Matsushima (4)	0.0025±0.0003	0.0027±0.0002	0.0032±0.0007

Table 3 *The Genetic Distance between the Miyagi Cultured Population and Other Cultured and Wild Populations of Crassostrea gigas*

	Local population	Genetic distance
Cultured population	Akkeshi, Hokkaido	0.0047±0.0004
	Hamanako (Miyagi seeds), Shizuoka	0.0016±0.0002
	Hamanako (Native seeds), Shizuoka	0.0023±0.0003
	Ondo, Hiroshima	0.0084±0.0005
Wild population	Akkeshi, Hokkaido	0.0033±0.0003
	Mangokuura, Miyagi	0.0028±0.0003
	Watari, Miyagi	0.0020±0.0002
	Ariake, Kumamoto	0.0046±0.0004

made to calculate the genetic distance (10) between every pair of samples on the basis of the allele frequencies. The values within the Miyagi cultured population are given in Table 2. They vary from 0.0011 to 0.0040 with a mean of 0.0012 for Ojika, from 0.0008 to 0.0062 with a mean of 0.0028 for Mangokuura, and 0.0007 to 0.0057 with a mean of 0.0032 for Matsushima. The average genetic distance is 0.0022 between Ojika and Mangokuura, 0.0025 between Ojika and Matsushima, and 0.0027 between Mangokuura and Matsushima. These values are similar to those obtained within locations, indicating no genetic differentiation among localities in the Miyagi cultured population. Therefore the Miyagi cultured oysters are treated as one population.

The genetic distances between the Miyagi cultured population and the other local cultured and wild populations were calculated for comparative purposes. The results are shown in Table 3. Mean values based on the 15 samples were used for the Miyagi cultured population. Genetic distances between Miyagi cultured and Miyagi wild populations (Mangokuura and Watari) are similar to those obtained within the Miyagi cultured population (Table 2) indicating no significant difference between cultured and wild populations in Miyagi. Genetic distances between Miyagi cultured oysters and two cultured samples from Shizuoka are smaller than the within Miyagi values (Table 2). However the Hamanako

Table 4. *Estimates of Genetic Distance between Local Populations of Crassostrea gigas*

		Cultured population			Wild population		
		Hokkaido	Miyagi	Hiroshima	Hokkaido	Miyagi	Kumamoto
Cultured population	Hokkaido				0.0077±0.0015		
	Miyagi	0.0047			0.0051±0.0006		
	Hiroshima	0.0099	0.0084				
Wild population	Hokkaido	0.0043	0.0033	0.0082			
	Miyagi	0.0063	0.0025	0.0078	0.0029	0.0033±0.0002	
	Kumamoto	0.0045	0.0046	0.0042	0.0034	0.0036	

native seed has a greater distance from the Miyagi oysters than does the Hamanako Miyagi-seed (Table 3). The Hokkaido and Hiroshima cultured populations show greater genetic distances from the Miyagi population; with the Hiroshima population showing a greater difference than the Hokkaido population (Table 3). The wild populations of Hokkaido and Kumamoto also show greater distances than the within Miyagi distances (Tables 2+3).

A matrix of genetic distances between averaged local populations is summarized in Table 4. There is a genetic distance of 0.0043 between wild and cultured oysters in Hokkaido and a genetic distance of 0.0099 between Hokkaido and Hiroshima cultured oysters. The degree of genetic differentiation was measured as an average of gennetic distances. The distance is 0.0077 ± 0.0015 within cultured populations, 0.0033 ± 0.0002 within wild populations, and 0.0051 ± 0.0006 between wild and cultured populations. These values suggest that genetic differentiation within wild populations is smaller than that in cultured populations.

The mean heterozygosity is given for each sample in Table 5. Genetic variation measured as the observed heterozygosity (Ho) varies from 0.151 to 0.224 with an average of 0.194 for the cultured population and from 0.176 to 0.211 with an average of 0.187 for the wild populations. The expected heterozygosity (He) varies from 0.178 to 0.243 with an average of 0.209 for the cultured populations and from 0.191 to 0.211 with an average of 0.199 for the wild populations. There is no significant difference in genetic variation between cultured and wild populations. The overall values are 0.193 for the observed heterozygosity and 0.207 for the expected heterozygosity in *Crassostrea gigas*. In most samples, the observed heterozygosity is lower than the expected heterozygosity, that is, an excess of homozygotes is observed. The excess of homozygotes from that expected under Hardy-Weinberg equilibrium was measured as $D=(Ho-He)/He$. A negative value indicates an excess of homozygotes and a positive value an excess of heterozygotes. There is an overall excess of homozygotes in all but 2 of the 23 samples (column 4, Table 5).

Table 5 *The Levels of Genetic Variation found within Samples of Crassostrea gigas*

	Sample	Heterozygosity		D
		Ho	He	
Cultured population	Akkeshi Hokkaido	0.197	0 224	−0.121
	Ojika-1, Miyagi	0 224	0 202	0.107
	Ojika-2, Miyagi	0 182	0.210	−0 133
	Ojika-3, Miyagi	0.202	0.208	−0 029
	Mangokuura-1, Miyagi	0 151	0 178	−0.152
	Mangokuura-2, Miyagi	0 166	0 199	−0 166
	Mangokuura-3 Miyagi	0 169	0.192	−0 120
	Mangokuura-4, Miyagi	0 207	0.215	−0.037
	Mangokuura-5, Miyagi	0.218	0.210	0.038
	Mangokuura-6, Miyagi	0 198	0 206	−0.039
	Mangokuura-7, Miyagi	0 189	0 198	−0.045
	Mangokuura-8, Miyagi	0.206	0 243	−0 152
	Matsushima-1, Miyagi	0.197	0.207	−0.048
	Matsushima-2, Miyagi	0 198	0.212	−0.066
	Matsushima-3, Miyagi	0 195	0 203	−0 039
	Matsushima-4, Miyagi	0 195	0 227	−0 141
	Hamanako (Wiyagi seeds) Shizuoka	0 210	0.215	−0.023
	Hananako (Native seeds), Shizuoka	0 199	0.208	−0.043
	Ondo, Hhiroshima	0 185	0 215	−0 140
	Mean	0 194	0 209	−0 071
Wild population	Akkeshi, Hokkaido	0.211	0.211	0
	Mangokuura Miyagi	0 179	0.195	−0 082
	Watari, Miyagi	0.176	0 191	−0.079
	Ariake, Kumamoto	0.181	0 200	−0.095
	Mean	0 187	0 199	−0 064
Overall mean		0 193	0.207	−0 070

Discussion

The lack of genetic differentiation between three localities in Miyagi cultured population can be explained in two ways. One is an artificial gene flow arising from the fact that seed oysters are circulated between localities by oyster farmers, and the other is natural gene flow arising from the fact that the oyster larvae can disperse up to 50 km (5) or possibly up to 1,300 km (11) riding water currents and tidal cycles The small genetic distance between the Miyagi population and the other local populations, Shizuoka, Hokkaido, and Kumamoto supports the

hypothesis that Miyagi oysters have been spread widely and rapidly to other locations by transplantation of Miyagi seeds. Such circumstances are considered from the following facts. The majority of oyster seed is obtained from the Miyagi and Hiroshima populations in Japan. The Hiroshima seed is used only for local production, while the Miyagi seed is used not only throughout Japan but also is exported to America and Europe in considerable quantities. During the years 1965-1974, the annual output of oyster seed from Miyagi was 3,250,000 rens of which 970,000 rens were transported to other districts in Japan. Frequently oyster seed has been exchanged between Miyagi and Hiroshima. The exports to America and Europe have reached ca. 414,000 rens and ca. 1,140,000 rens, respectively (7). The "ren" is a string of oysters in which 70-100 collector shells are punched with a hole and strung on wires 15 m in length. The genetic distance between Miyagi and Shizuoka populations suggests that the native population of Shizuoka has been replaced by the Miyagi population through annual transplantation of Miyagi seed. Therefore, several local populations have been genetically modified to become similar to the Miyagi population by the effective transplantation of Miyagi seed. However, the genetic distance between the Hiroshima and Miyagi populations seems to remain at present at the local level.

Buroker *et al.* (4) reported that the genetic distances between the Kumamoto population (supposed to be *C. sikame*) and the Hiroshima and Miyagi population of *C. gigas* were 0.1888 and 0.4521, respectively. Our data are significantly different from these values. One possibility for this difference is that we have sampled different populations, since it is said that there are two types of *C. gigas* in Kumamoto district (12). Numachi (12) described the two types as having a patchy distribution in Ariake, extending along Kumamoto, Fukuoka, Saga, and Nagasaki, by cross experiments in which one (A type) failed a fertilization test with other local oysters while the other (B type) succeeded. The A type may be corresponded to *C. sikame*.

It is difficult to say what is the real reason of the widespread success of *C. gigas*. One possibility for the success may be that the ecological capacity of *C. gigas* is superior to that of other oyster species. Such capacity may correlate with the higher genetic variability in *C. gigas* than in other species. As expected, the genetic variation of *C. gigas* is higher in magnitude than those obtained from other species (Table 6). Buroker *et al.* (4) reported that a high genetic similarity was observed between *C. gigas* and *C. angulata* and that *C. angulata* should be classified as *C. gigas*. Neverthless, considering all these facts, the native Japanese *C. gigas*, especially the Miyagi type is an excellent oyster for culture and is widely used in several countries of the world.

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TABLE 6 *Summary of the levels of genetic variation found within the species in Crassostrea, Saccostrea, and Ostrea species*

Species	No of sample lots or populations	No of loci studied	Proportion of polymorphic loci	Heterozygosity		Reference
				Ho (observed)	He (expected)	
C gigas	25	10	0 500	0 193(0.151-0 224)	0 207(0 178-0 243)	the present study
	1	30	0 500	0 186	0.205	Fujio *et al* (1983)(13)
	3	26	0 602(0 583-0 630)	0.205(0 195-0 222)	0.218(0.201-0.238)	Buroker *et al* (1979)(4)
	1	12	0.533	0 210	0.245	Buroker *et al* (1975)(15)
	1	19	0 526	0.176	0 203	Gosling (1982)(6)
C angulata	1	25	0 600	0.234	0 238	Buroker *et al* (1979)(4)
C virginica	2	33	0 470(0 469-0 471)	0.179(0 166-0.191)	0.185(0 175-0 195)	Buroker *et al* (1979)(14)
C rivularis	1	32	0 375	0.098	0 111	Buroker *et al* (1979)(14)
	1	12	0 238	0 089	0 127	Fujio *et al* (1983)(13)
	1	17	0 300	0 091	0 115	Ozaki (unpublished)
C. rhizophorae	1	30	0 300	0 100	0 111	Buroker *et al* (1979)(14)
C iredalei	1	28	0 393	0 100	0 105	Buroker *et al.*(1979)(14)
C belcheri	1	25	0 200	0 062	0 068	Buroker *et al* (1979)(14)
C nippona	1	40	0 425	0 098	0.109	Fujio *et al* (1983)(13)
S glomerata	1	27	0 519	0 182	0.193	Buroker *et al* (1979)(4)
	1	17	0 467	0 182	0.205	Ozaki (unpublished)
S commercialis	3	28	0 464	0 180(0 172-0.195)	0.190(0 184-0.196)	Buroker *et al* (1979)(4)
S malabonensis	1	30	0 533	0 192	0 213	Buroker *et al* (1979)(14)
S mandai	1	30	0 467	0 187	0.194	Buroker *et al.*(1979)(14)
S cucullata	1	27	0 481	0 185	0 180	Buroker *et al* (1979)(14)
O edulis	1	31	0 226	0 084	0 081	Fujio *et al* (1983)(13)
O lurida	1	21	0 190	0 063	0.060	Fujio *et al* (1983)(13)
O denselamellosa	1	24	0 333	0 078	0.085	Fujio *et al* (1983)(13)
O circumpicta	1	21	0 143	0 059	0 063	Fujio *et al* (1983)(13)

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