Tohoku Journal of Agricultural Research
Vol. 30, No. 1, August 1979
Printed in Japan

Enzyme polymorphism and Population structure of the Pacific oyster, *Crassostrea gigas*

Yoshihisa FUJIO

Department of Fishery Science, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received June 11, 1979)

Summary

Enzyme polymorphism of IDH, PGM, GPI, AAT, and LAP in the Pacific oyster, *Crassostrea gigas*, was exhibited by starch gel electrophoresis. The wild populations collected from 18 localities in Hokkaido, Miyagi, Fukushima, Hyogo, Hiroshima, and Kumamoto were analysed by using as markers the genes at the five polymorphic loci controlling the five enzymes. Any two of the 18 wild populations showed clear differences of gene frequencies, indicating that they are independent of each other.

The wild populations could be divided into 4 groups by dendrogram for relations among local populations. The grouping seemed to indicate a differentiation of local races which we named Hokkaido, Miyagi, Hiroshima, and Kumamoto, although some exceptions between Miyagi and Hiroshima were observed.

These results suggest that the population structure of the wild Pacific oyster as a whole has a tendency to split into a number of local subpopulations in which the effect of random genetic drift is prevailing.

There exist many species and local races of the oyster in suitable coastal areas through Japan. The native Pacific oysters, *Crassostrea gigas*, of different localities exhibit differences in their morphological characters, growth rate, spawning conditions, adaptability to environmental conditions and also in flavor. The extensive breeding studies by Imai and Sakai (1) disclosed the four local populations, called "Hokkaido-, Miyagi-, Hiroshima-, and Kumamoto-strains". They observed hereditary difference in their adaptability to environmental conditions.

Genetic variations detected by electrophoretic analysis have been reported in the Pacific oyster (2–4). Nagaya *et al.* (5) reported the geographical differences of the Pacific oyster, indicated by frequencies of the allele Lap^5 at the leucine aminopeptidase locus in the areas along the coasts of Hokkaido and the northern districts of Japan, and named "Hokkaido-northern Tohoku and southern Tohoku

* Supported in part by the Grant from the Ministry of Education, the Government of Japan.

groups". Previous paper (6) reported that genetic differentiation of wild Pacific oyster population was demonstrated by the allelic frequencies at the phosphoglucomutase locus. In the present work, enzyme polymorphism in the Pacific oyster was exhibited by starch gel electrophoresis and the population structure was discussed.

Materials and Methods

The wild Pacific oyster, Crassostrea gigas was collected from the 18 localities in the Pacific coasts of Hokkaido, Miyagi, Fukushima, Hyogo, Hiroshima, and Kumamoto as shown in Fig. 1. Extracts of tissues were analysed by starch gel electrophoresis for the five enzymes, isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), glucosephosphate isomerase (GPI), asparate aminotransferase (AAT), and leucine aminopeptidase (LAP). Tissue extraction and starch gel electrophoresis were carried out by the methods previously reported (7).

Gene frequencies were calculated from phenotypic frequencies for each population. For the calculation of genetic distance, the following formula was employed. The genetic distance between two populations (j and k), $\bar{D}_{jk}$, is

$$\bar{D}_{jk} = \frac{1}{l} \sum_{m=1}^{l} \sqrt{\sum_{i=1}^{n} \frac{(q_{imj} - q_{imk})^2}{2}}$$

where q_{imj} and q_{imk} present the frequencies of i allele at the m locus in population j and k, respectively. A dendrogram expressing relative similarities among populations was drawn with an average distance between branches calculated by the unweighted pair-group method.

1. Akkeshi, Hokkaido
2. Kesennuma, Miyagi
3. Onagawa-1, Miyagi
4. Onagawa-2, Miyagi
5. Ojika, Miyagi
6. Ishinomaki, Miyagi
7. Mangokuura-1, Miyagi
8. Mangokuura-2, Miyagi
9. Watanoha, Miyagi
10. Matsushima-1, Miyagi
11. Matsushima-2, Miyagi
12. Matsushima-3, Miyagi
13. Haragama, Fukushima
14. Akoh, Hyogo
15. Yasuura, Hiroshima
16. Ondo-1, Hiroshima
17. Ondo-2, Hiroshima
18. Amakusa, Kumamoto

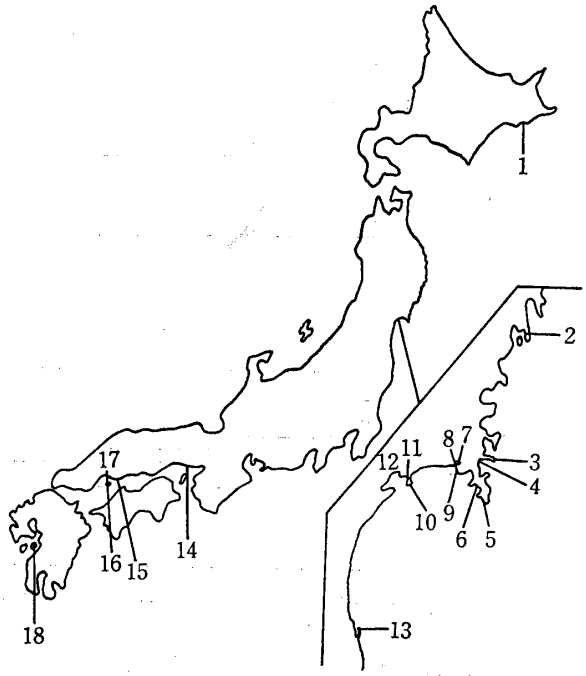

FIG. 1. Locations from which samples of the Pacific oyster were collected.

Results

1. *Genetic variations*

Five enzymes were examined and the polymorphic gene loci were detected. A locus was considered polymorphic if the frequencies of the most common allele were no greater than 0.95. The five enzyme systems have been interpreted in the following manner.

The IDH activity in the gill appeared in the two zones which were apparently coded by two gene loci (*Idh–1* and *Idh–2*). The *Idh–1* locus controlled the enzyme located in the more anodal migrating zone and exhibited polymorphism, and the *Idh–2* determined the enzyme located in the slower migrating zone and was monomorphic. The digestive diverticula showed activity coded by *Idh-1* locus and the adductor muscle activity coded by *Idh–2* locus. In the more migrating zone, heterozygous individuals showed three banded phenotype and homozygotes single banded phenotype, indicating a probable dimeric structure of the enzyme. The different phenotypes at the *Idh–1* locus indicated three alleles (Fig. 2a).

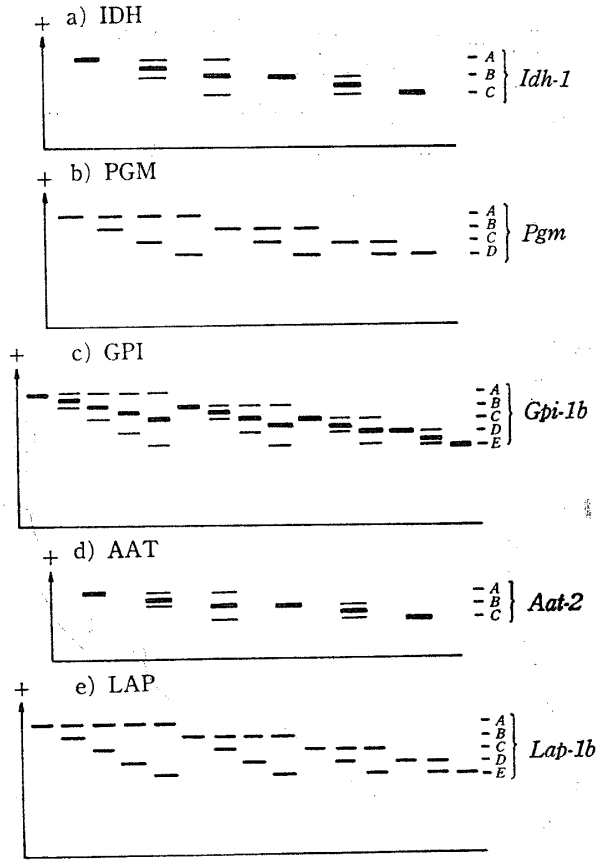

FIG. 2. The proposed phenotypic variation for five polymorphic enzymes of the Pacific oyster.
An italic letter was used to differentiate between alleles with the most anodal allele always designated at "*A*".

PGM was examined in the adductor muscle and showed the typical pattern of a monomer. The enzyme was coded by one gene locus (*Pgm*). Heterozygous individuals expressed two banded phenotype, while homozygotes showed single banded phenotype. The different phenotypes at this locus indicated four alleles (Fig. 2b).

The GPI activity in the adductor muscle was apparent in two zones which were coded by three gene loci (*Gpi-1a*, *Gpi-1b*, and *Gpi-2*). In the more anodal migrating zone, three bands were observed from which two loci have been designated as *Gpi-1a* (the more anodal) and *Gpi-1b*. The slower migrating band coded by *Gpi-1b* locus stained more intensely and developed first, and the more migrating band coded by *Gpi-1a* locus developed most slowly. The different phenotypes at the Gpi—*1*b locus indicated five alleles and showed the typical pattern of a dimer (Fig. 2c). If allowed to stain longer, the enzyme coded by the *Gpi-1b* locus became indistinguishable from those coded by the *Gpi-1a* locus because one of them was in the identical electrophoretical position with a band by the *Gpi-1a* locus. The slower migrating zone coded by the *Gpi-2* locus was also the slowest to develop and the different phenotypes suggested at least three alleles though it had not yet been clearly resolved.

The AAT activity in the adductor muscle appeared in the two zones which were coded by two gene loci (*Aat-1* and *Aat-2*). The *Aat-1* locus coded for the enzyme located in the more anodal migrating zone but had not yet been clearly resolved. The *Aat-2* locus coded for the enzyme located in the slowest migrating zone, and exhibited polymorphism. The different phenotypes indicated three alleles and showed the typical pattern of a dimer (Fig. 2d).

LAP was examined in the adductor muscle and exhibited activity in two zones which were controlled by three gene loci (*Lap-1a*, *Lap-1b*, and *Lap-2*). In the more anodal migrating zone, variant enzyme forms of LAP were observed and the polymorphic loci have been designated as *Lap-1a* (the more anodal) and *Lap-1*b. Because they were almost superimposed on each other, only the *Lap-1b* locus was analysed. This enzyme form stained more intesely and developed first. The enzymes located in the slowest migrating zone developed mostly slowly and had not yet been clearly resolved. The different phenotypes at the *Lap-1b* locus indicated five alleles and showed the typical pattern of a monomer (Fig. 2e).

2. *Population structure*

The electrophoretic data obtained from the five polymorphic loci has been tabulated for the 18 localities sampled in Tables 1–5. A geographical cline of gene frequencies was not clearly observed at the *Idh-1*, *Gpi-1b*, and *Lap-1b* loci. The *Pgm* and *Aat-2* loci, however, exhibited clinial tendencies along the Pacific doast. The frequencies of the *B* gene at the *Pgm* locus increased towards the lower latitudes along the Pacific coast and the *C* gene decreased inversely. Similar tendencies were

TABLE 1. *Gene Frequencies of IDH Variants in Wild Populations of the Pacific Oyster*

Populations	No. of shells tested	*Idh-1*		
		qA	*qB*	*qC*
Akkeshi, Hokkaido	16	0	0.500±0.088	0.500±0.088
Kesenuma, Miyagi	54	0.028±0.016	0.815±0.037	0.157±0.035
Onagawa-1, Miyagi	60	0.052±0.020	0.719±0.041	0.229±0.038
Onagawa-2, Miyagi	50	0.030±0.017	0.780±0.041	0.190±0.039
Ojika, Miyagi	50	0.010±0.010	0.730±0.044	0.260±0.044
Ishinomaki, Miyagi	50	0.061±0.024	0.755±0.043	0.184±0.039
Mangokuura-1, Miyagi	170	0.006±0.004	0.832±0.020	0.162±0.020
Mangokuura-2, Miyagi	125	0.036±0.012	0.756±0.027	0.208±0.026
Watanoha, Miyagi	120	0.058±0.015	0.725±0.029	0.217±0.027
Matsushima-1, Miyagi	50	0.043±0.012	0.787±0.042	0.170±0.039
Matsushima-2, Miyagi	65	0.032±0.016	0.762±0.038	0.206±0.036
Matsushima-3, Miyagi	50	0.020±0.014	0.780±0.041	0.200±0.040
Haragama, Fukushima	60	0.05. ±0.020	0.783±0.038	0.167±0.034
Akoho, Hyogo	60	0.050±0.021	0.683±0.042	0.267±0.040
Yasuura, Hiroshima	105	0.033±0.012	0.719±0.031	0.248±0.030
Ondo-1, Hiroshima	60	0.067±0.023	0.683±0.042	0.250±0.040
Ondo-2, Hiroshima	75	0.087±0.023	0.647±0.039	0.266±0.036
Amakusa, Kumamoto	30	0	0.583±0.063	0.417±0.063

TABLE 2. *Gene Frequencies of PGM Variants in Wild Populations of the Pacific Oyster*

Populations	No. of shells tested	*Pgm*			
		qA	*qB*	*qC*	*qD*
Akkeshi, Hokkaido	16	0.031±0.031	0.125±0.058	0.813±0.069	0.031±0.031
Kesenuma, Miyagi	54	0.009±0.009	0.111±0.030	0.806±0.038	0.074±0.025
Onagawa-1, Miyagi	60	0.033±0.016	0.183±0.035	0.667±0.043	0.117±0.029
Onagawa-2, Miyagi	50	0.010±0.010	0.230±0.042	0.560±0.050	0.200±0.040
Ojika, Miyagi	50	0.090±0.029	0.290±0.045	0.470±0.050	0.150±0.036
Ishinomaki, Miyagi	50	0.010±0.010	0.200±0.040	0.770±0.042	0.020±0.014
Mangokuura-1, Miyagi	170	0.056±0.012	0.177±0.021	0.626±0.026	0.141±0.019
Mangokuura-2, Miyagi	125	0.008±0.006	0.112±0.020	0.692±0.029	0.188±0.025
Watanoha, Miyagi	120	0.012±0.007	0.271±0.028	0.546±0.032	0.171±0.024
Matsushima-1, Miyagi	50	0.040±0.020	0.220±0.041	0.590±0..49	0.150±0.036
Matsushima-2, Miyagi	65	0.015±0.011	0.146±0.031	0.616±0.043	0.223±0.037
Matsushima-3, Miyagi	50	0.030±0.017	0.270±0.044	0.570±0.050	0.130±0.034
Haragama, Fukushima	60	0.066±0.023	0.167±0.034	0.592±0.045	0.175±0.035
Akoh, Hyogo	60	0.033±0.016	0.192±0.036	0.592±0.045	0.183±0.035
Yasuura, Hiroshima	105	0.076±0.018	0.176±0.026	0.548±0.034	0.200±0.028
Ondo-1, Hiroshima	60	0.133±0.030	0.292±0.041	0.442±0.045	0.133±0.030
Ondo-2, Hiroshima	75	0.007±0.007	0.127±0.027	0.680±0.038	0.186±0.032
Amakusa, Kumamoto	30	0.017±0.017	0.383±0.063	0.483±0.065	0.117±0.041

observed in the frequencies of the *C* and *B* genes at the *Aat-2* locus.

A matrix presented in Table 6 gives the results of calculations of the genetic distance under the diagonal and of the test of significant heterogeneities above the diagonal between every pair of the localities examined. In the matrix of

TABLE 3. *Gene Frequencies of GPI Variants in Wild Populations of the Pacific Oyster*

Populations	No. of shells tested	*Gpi-1b*				
		qA	*qB*	*qC*	*qD*	*qE*
Akkeshi, Hokkaido	16	0	0	0.937±0.042	0.063±0.042	0
Kesenuma, Miyagi	54	0	0.018±0.012	0.917±0.026	0.065±0.023	0
Onagawa–1, Miyagi	60	0.008±0.008	0.075±0.024	0.884±0.029	0.033±0.016	0
Onagawa–2, Miyagi	50	0.010±0.009	0.090±0.028	0.850±0.035	0.030±0.017	0.020±0.014
Ojika, Miyagi	50	0	0.160±0.036	0.760±0.042	0.080±0.027	0
Ishinomaki, Miyagi	50	0	0.100±0.030	0.810±0.039	0.090±0.028	0
Mangokuura–1, Miyagi	170	0.003±0.003	0.038±0.010	0.900±0.016	0.059±0.013	0
Mangokuura–2, Miyagi	125	0	0.070±0.016	0.889±0.020	0.041±0.013	0
Watanoha, Miyagi	120	0	0.058±0.015	0.871±0.021	0.071±0.016	0
Matsushima–1, Miyagi	50	0	0.200±0.040	0.750±0.043	0.050±0.021	0
Matsushima–2, Miyagi	65	0	0.054±0.019	0.900±0.026	0.038±0.016	0.008±0.007
Matsushima–3, Miyagi	50	0	0.110±0.031	0.790±0.040	0.100±0.030	0
Haragama, Fukushima	60	0.025±0.014	0.050±0.019	0.900±0.027	0.025±0.014	0
Akoh, Hyogo	60	0.008±0.008	0.042±0.018	0.917±0.025	0.033±0.016	0
Yasuura, Hiroshima	105	0.005±0.005	0.076±0.018	0.866±0.024	0.048±0.015	0.005±0.005
Ondo–1, Hiroshima	60	0	0.208±0.037	0.700±0.041	0.092±0.026	0
Ondo–2, Hiroshima	75	0	0.233±0.035	0.727±0.036	0.033±0.015	0.007±0.007
Amakusa, Kumamoto	30	0	0	0.967±0.023	0.033±0.023	0

TABLE 4. *Gene Frequencies of AAT Variants in Wild Populations of the Pacific Oyster*

Populations	No. of shells tested	*Aat-2*		
		qA	*qB*	*qC*
Akkeshi, Hokkaido	16	0.344±0.083	0.656±0.083	0
Kesenuma, Miyagi	54	0.333±0.045	0.621±0.046	0.046±0.020
Onagawa-1, Miyagi	60	0.250±0.039	0.683±0.042	0.067±0.022
Onagawa-2, Miyagi	50	0.170±0.037	0.780±0.041	0.050±0.021
Ojika, Miyagi	50	0.200±0.040	0.770±0.042	0.030±0.017
Ishinomaki, Miyagi	50	0.340±0.047	0.630±0.048	0.030±0.017
Mangokuura-1, Miyagi	170	0.285±0.024	0.697±0.025	0.018±0.007
Mangokuura-2, Miyagi	125	0.280±0.028	0.668±0.030	0.052±0.014
Watanoha, Miyagi	120	0.237±0.027	0.696±0.029	0.067±0.016
Matsushima-1, Miyagi	50	0.040±0.019	0.950±0.021	0.010±0.009
Matsushima-2, Miyagi	65	0.300±0.040	0.654±0.041	0.046±0.018
Matsushima-3, Miyagi	50	0.450±0.049	0.510±0.049	0.040±0.019
Haragama, Fukushima	60	0.367±0.043	0.592±0.044	0.041±0.018
Akoh, Hyogo	60	0.183±0.035	0.675±0.042	0.142±0.031
Yasuura, Hiroshima	105	0.157±0.025	0.757±0.030	0.086±0.019
Ondo-1, Hiroshima	60	0.175±0.034	0.717±0.041	0.108±0.028
Ondo-2, Hiroshima	75	0.087±0.023	0.853±0.029	0.060±0.019
Amakusa, Kumamoto	30	0.100±0.038	0.700±0.059	0.200±0.051

genetic distance, if the two localities are identical in regard of gene frequencies at the five loci, respectively, the distance between them will be zero. The larger the difference between them, the larger will be the distance. The maximum possible between localities would be 1.0. For example, the smallest distances were obtained between Mangokuura-2 and Matsushima-2, between Onagawa-1 and Mangokkuura-2, and between Onagawa-2 and Yasuura, that is, the values were 0.032, 0.035, and 0.038, respectively. On the other hand, the largest distances were obtained between Akkeshi and Matsushima-1, between Akkeshi and Ondo-1, and between Ishinomaki and Amakusa, that is, the values were 0.209, 0.205, and 0.200, respectively.

In the test of significant heterogeneities, significant differences were seen in the frequency distribution of alleles at the five loci between the localities sampled. This indicated that they were independent of each other. In other words, the population structure of the wild Pcific oyster has a tendency to split into a number of local subpopulations.

To summarize the relations among subpopulations, a dendrogram was drawn on the basis of similarity illustrated with an average of genetic distance as shown in Fig. 3. A vertical line was drawn across the dendrogram as an attempt to delimit a group having a distance of 0.1. Using this vertical line, the 18 subpopulations are divided into four groups. The grouping seemed to indicate the differentiation of local races such as Hokkaido, Miyagi, Hiroshima, and Kumamoto. But some exceptions between Miyagi and Hiroshima were observed, that is, Ojika and Matsushima-1 were grouped in Hiroshima and Yasuura and Akoh were grouped in

TABLE 5. *Gene Frequencies of LAP Variants in Wild Populations of the Pacific Oyster*

Populations	No. of shells tested	*Lap-1b*				
		qA	*qB*	*qC*	*qD*	*qE*
Akkeshi, Hokkaido	16	0	0	0.906±0.051	0.063±0.042	0.031±0.031
Kesenuma, Miyagi	54	0.028±0.015	0.028±0.015	0.861±0.033	0.083±0.026	0
Onagawa–1, Miyagi	60	0	0.008±0.008	0.808±0.035	0.167±0.034	0.017±0.011
Onagawa–2, Miyagi	50	0	0.020±0.014	0.830±0.037	0.140±0.034	0.010±0.009
Ojika, Miyagi	50	0	0	0.840±0.036	0.160±0.036	0
Ishinomaki, Miyagi	50	0.020±0.014	0.040±0.019	0.760±0.042	0.160±0.036	0.020±0.014
Mangokuura–1, Miyagi	170	0.041±0.011	0.050±0.012	0.768±0.023	0.129±0.018	0.012±0.006
Mangokuura–2, Miyagi	125	0	0.012±0.007	0.844±0.023	0.136±0.022	0.008±0.006
Watanoha, Miyagi	120	0	0.050±0.014	0.838±0.023	0.112±0.020	0
Matsushima–1, Miyagi	50	0	0	0.870±0.033	0.120±0.032	0.010±0.009
Matsushima–2, Miyagi	65	0.031±0.015	0.023±0.013	0.869±0.029	0.069±0.022	0.008±0.007
Matsushima–3, Miyagi	50	0.010±0.009	0.020±0.014	0.840±0.036	0.110±0.031	0.020±0.014
Haragama, Fukushima	60	0.017±0.011	0.083±0.025	0.725±0.040	0.175±0.032	0
Akoh, Hyogo	60	0.008±0.008	0.059±0.021	0.850±0.032	0.075±0.024	0.008±0.008
Yasuura, Hiroshima	105	0	0.014±0.008	0.833±0.026	0.129±0.023	0.024±0.011
Ondo–1, Hiroshima	60	0	0.083±0.025	0.775±0.038	0.142±0.031	0
Ondo–2, Hiroshima	75	0	0.053±0.018	0.807±0.032	0.140±0.028	0
Amakusa, Kumamoto	30	0	0	0.983±0.017	0.017±0.017	0

TABLE 6. *Genetic Distances under the Diagonal, and the Test of Significant Heterogeneities in Gene Frequencies above the Diagonal, between Every Pair of Local Populations of the Pacific Oyster*

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Akkeshi, Hokkaido		+1	+1	+2	+1	+3	+3	+2	+2	+4	+2	+3	+3	+2	+3	+4	+4	+2
2. Kesenuma, Miyagi	.095		+2	+3	+3	+1	+2	+2	+2	+4	+2	+3	+3	+3	+4	+4	+4	+4
3. Onagawa–1, Miyagi	.126	.081		—	+2	+1	+3	−	+2	+2	+2	+2	+2	+2	+2	+3	+3	+5
4. Onagawa–2, Miyagi	.170	.107	.060		+1	+2	+2	+2	−	+2	+1	+2	+3	+1	+1	+3	+3	+5
5. Ojika, Miyagi	.181	.146	.083	.060		+2	+3	−3	+2	+2	+3	+2	+4	+3	+3	+1	+2	+5
6. Ishinomaki, Miyagi	.125	.066	.067	.099	.115		+2	+1	+1	+4	+2	+1	+1	+5	+2	+3	+3	+5
7. Mangokuura–1, Miyagi	.140	.066	.054	.066	.104	.075		+4	+4	+3	+2	+2	+1	+3	+3	+4	+4	+5
8. Mangokuura–2, Miyagi	.122	.062	.035	.061	.097	.072	.055		+1	+3	+1	+3	+2	+2	+2	+4	+4	+5
9. Watanoha, Miyagi	.137	.095	.063	.047	.069	.088	.064	.055		+2	+1	+1	+4	+1	+3	+2	+3	+4
10. Matsushima–1, Miyagi	.209	.146	.112	.072	.085	.135	.115	.113	.103		+2	+1	+3	+3	+2	+2	+2	+4
11. Matsushima–2, Miyagi	.122	.061	.055	.065	.103	.085	.055	.032	.055	.117		+3	+2	+1	+2	+4	+4	+4
12. Matsushima–3, Miyagi	.165	.101	.093	.087	.096	.087	.092	.088	.077	.120	.083		+2	+3	+1	+4	+3	+4
13. Haragama, Fukushima	.155	.080	.096	.084	.123	.072	.052	.068	.080	.134	.062	.082		+3	+2	+3	+4	+4
14. Akoh, Hyogo	.132	.098	.059	.068	.097	.115	.077	.067	.051	.118	.056	.108	.085		+1	+2	+2	+2
15. Yasuura, Hiroshima	.154	.114	.052	.038	.061	.108	.076	.061	.048	.091	.065	.101	.090	.050		+3	+4	+5
16. Ondo–1, Hiroshima	.205	.170	.103	.097	.058	.126	.124	.126	.085	.114	.130	.125	.128	.095	.083		+2	+4
17. Ondo–2, Hiroshima	.181	.154	.098	.094	.098	.128	.128	.100	.111	.079	.122	.151	.144	.103	.090	.091		+5
18. Amakusa, Kumamoto	.142	.180	.149	.145	.144	.200	.170	.162	.125	.183	.148	.178	.185	.105	.129	.153	.181	

The sign +1 represents the significant difference at one locus.

Miyagi. These exceptions might be interpreted by the fact that the transplantation of the cultured oysters had been carried out between Miyagi and Hiroshima in the past.

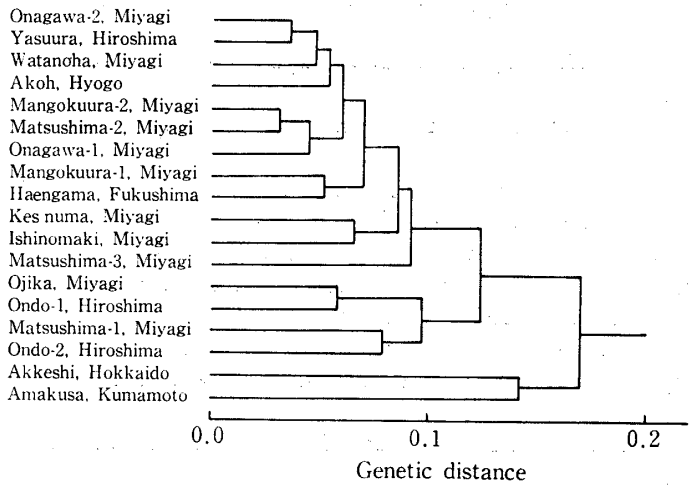

FIG. 3. The dendrogram of relations among local subpopulations of the Pacific oyster.

Discussion

From the result of the dendrogram, four genetically distinct groups might be identified with the four local races called Hokkaido, Miyagi, Hiroshima, and Kumamoto. Some exceptions between Miyagi and Hiroshima might have resulted from the transplantation of the cultured oysters between them. This problem must be clarified by a series of extensive genetic stidies on the cultured populations.

The analyses suggest that the population structure of the wild Pacific oyster as a whole has a remarkable tendency to split into a number of local subpopulations, although a question of whether each of the subpopulations sampled was truely wild or a mixture due to gene flow from the cultured population remains. In any event, it is certain that the wild population has a structure capable of being influenced by random genetic drift. Such circumstances are considered responsible for the marked genetic differentiation between localities and the preliminary observation of homozygote excess. Sveral studies have revealed that frequencies of heterozygotes were often lower than those expected under Hardy-Weinberg equilibrium in marine molluscs, such as *Mytilus edulis* (8–11), *Mytilus californianus* (12), *Modiolus demissus* (9, 13), and *Crassostrea virginica* (14). Singh and Zouros (14) reported that a homozygote excess was observed at the four loci (*Lap–1*, *Pgi*, *Pgm*, and *Est–3*) in the American oyster (*Crassostrea virginica*).

References

1) Imai, T., and Sakai, S., *Tohoku J. Agr. Res.*, **12**, 125–171 (1961)
2) Buroker, N.E., Hershberger, W.K., and Chew, K.K., *J. Fish. Res. Board Can.*, **32**, 2471–2477 (1975)
3) Fujino, K., and Nagaya, N., *Bull. Japan. Soc. Sci. Fish.*, **43**, 983–988 (1977)
4) Nagaya, N., and Fujino, K., *Bull. Japan. Soc. Sci. Fish.*, **43**, 1455–1459 (1977)
5) Nagaya, N., Sasaki, K., and Fujino, K., *Bull. Japan. Soc. Sci. Fish.*, **44**, 1041–1045 (1978)
6) Fujio, Y., *Fish Genetics and Breeding Science*, **3**, 13–16 (1978)
7) Fujio, Y., *Japan. J. Genet.*, **52**, 117–124 (1977)
8) Milkman, R., and Beaty, L.D., *Biol. Bull.*, **139**, 430 (1970)
9) Koehn, R.K., and Mitton, J.B., *Amer. Natur.*, **106**, 47–56 (1972)
10) Boyer, J.F., *Biol. Bull.*, **147**, 535–549 (1974)
11) Koehn, R.K., Milkman, R., and Mitton, J.B., *Evolution*, **30**, 2–32 (1976)
12) Tracey, M.L., Bellet, N.F., and Graven, C.B., *Marine Biology*, **32**, 303–311 (1975)
13) Koehn, R.K., Turano, F.J., and Mitton, J.B., *Evolution*, **27**, 100–105 (1973)
14) Singh, S.M., and Zouros, E., *Evolution*, **32**, 342–353 (1978)