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journal or publication title	Tohoku journal of agricultural research
volume	34
number	3/4
page range	73-84
year	1984-03-30
URL	http://hdl.handle.net/10097/29845

Tohoku Journal of Agricultural Research
Vol. 34 No. 3-4, March 1984
Printed in Japan

Heterogeneity within and between Geographical Populations of the Bay Mussel, *Mytilus edulis*

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(Received, December 10, 1983)

Summary

Starch gel electrophoresis was carried out to estimate the levels of genetic variations and the genetic distances for the geographical populations of the bay mussel, *Mytilus edulis*. The observed heterozygosity ranged from 0.119 to 0.172, while the expected heterozygosity was from 0.131 to 0.221. The observed heterozygosity tended to be lower than that expceted in the localities examined, indicating a general excess of homozygosity.

All of the 15 localities showed clear differences of gene frequencies at the six polymorphic loci, indicating that they are independent of each other. The populations could be divided into 3 groups by the dendrogram drawn by genetic distances. The grouping seemed to indicate a differentiation of local races. These results suggest that the population structure of the bay mussel as a whole has a tendency to split a number of geographical subpopulations.

An excess of homozygosity was observed at many loci in most geographical subpopulations. Possible explanations for this observation are discussed in regard to the population structure.

Electrophoretic data have been well used for estimating levels of genetic variation in natural populations (1,2), and for measuring genetic similarities and distances within and between taxa (3). Estimated levels of genetic variation in some of the pelecypods are reported (4-9). Fujio *et al.* (9) revealed that the observed heterozygosity tended to be lower than that expected in marine molluscs and suggested a general excess of homozygosity. The degree of genetic similarities and distances within and between taxa of pelecypods has so far not been treated. In fact, the interest in divergence of populations by methods of electrophoresis is quite recent (7,8,9).

There are many colonies of bay mussels in suitable coastal areas through out Japan. The bay mussel is immobile but the larvae are frequently pelagic and swim freely thereby promoting gene flow. In the present work, the elcetrophoresis was used in the genetical study of bay mussel population ; (1) to estimate

the level of genetic variations within different geographical populations, (2) to determine the genetic distances among these populations, and (3) to discuss some hypotheses in an attempt to account for an excess of homozygosity.

Materials and Methods

For this work, collections of the bay mussel *Mytilus edulis* were made in 15 locations from Hokkaido to Hiroshima in Japan from March 1980 through September 1982 (Fig. 1). The tissue extraction and starch gel electrophoresis were carried out by the method described by Yamanaka and Fujio (11). The 9 enzymes, aspartate aminotransferase (AAT), α-glycerophosphate dehydrogenase (αGPD), catalase (CAT), glucosephosphate isomerase (GPI), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), superoxide dismutase (SOD), and xanthine dehydrogenase (XDH) were examined.

Results

1. Genetic variation

Nine enzymes were examined and the 12 genetic loci were detected. The Loci were numbered consecutively from the most anodal to the most cathodal and alleles were designated alphabetically. For example, *Aat-2*^{A} refers to an allele of

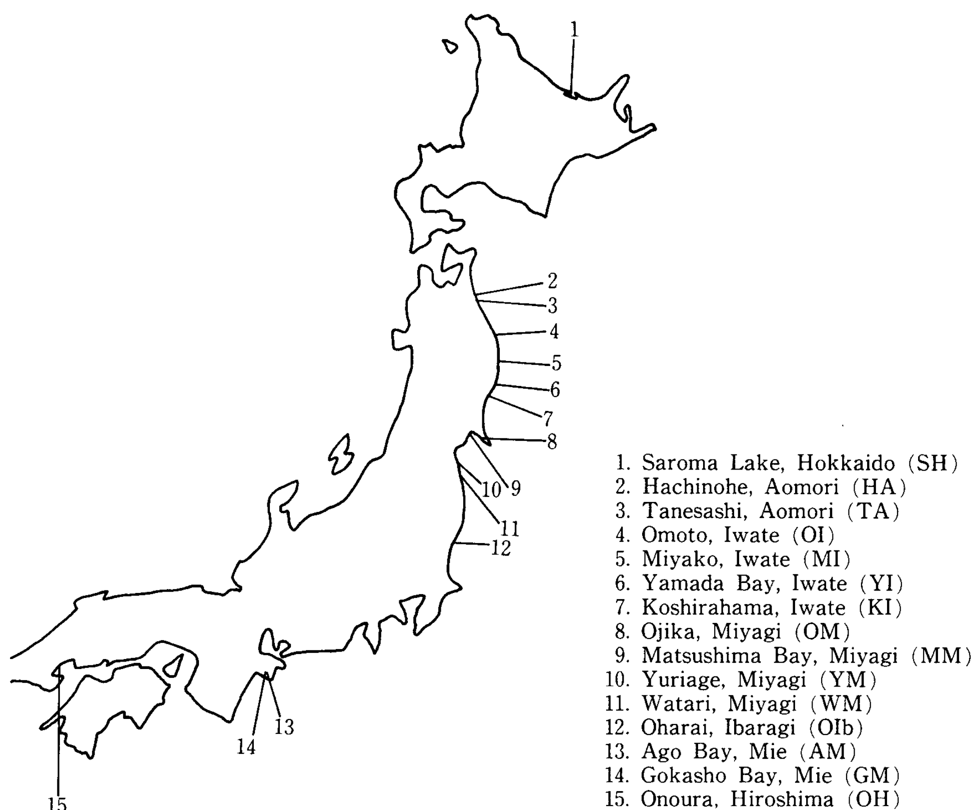

FIG. 1. Locations from which samples of the bay mussel were collected.

the second *Aat* locus whose protein product migrates to the most anodal position. Of the 12 loci examind, six loci were polymorphic at least in one lacation A locus was considered polymorphic if the frequencies of the most common allele were no greater than 0.95. The 9 enzyme systems have been interpreted in the following manner.

AAT showed activity in both digestive caecum and adductor muscle. AAT activity 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 was monomorphic. The *Aat-2* locus coded for the enzyme located in the slowest migrating zone exhibited polymorphism. Heterozygous individuals showed three-banded phenotype and homozygous individuals single-banded phenotype, indicating the typical pattern of a dimeric structure of the enzyme. The different phenotypes indicated five alleles (Fig. 2A).

The αGPD activity in both digestive caecum and muscle tissue appeared in the two zones which were controlled by two gene loci (*αGpd-1* and *αGpd-2*). They were monomorphic in all localites examined.

CAT was examined in muscle samples and appeared as a single locus. Different phenotypes were observed in the samples from Saroma lake in Hokkaido and Ago bay in Mie. Heterozygotes showed two-banded phenotype and homozygotes single-banded phenotype, indicating the typical pattern of monomer (Fig. 2B).

GPI was examined in muscle samples and resolved with the expceted one-and three-banded phenotypes for this enzyme. They were polymorphic in all localities. The different phenotypes indicated five alleles (Fig. 2C).

IDH was examined in muscle samples and resolved with the typical pattern of a dimer. Polymorphism was observed in all localities and the different phenotypes indicated four alleles (Fig. 2D).

LAP was tested in both digestive caecum and muscle samples and produced several zones of activity some of which could not be scored. In the more anodal migrating zone which was stained more intensily and developed first, one and two phenotypes were resolved and the locus was scored. The different phenotypes indicated polymorphism in all localities and five alleles (Fig. 2E).

PGM was examined in adductor muscle and showed the typical pattern of a monomer. The enzyme was coded by one gene locus. The different phenotypes at this locus indicated five alleles (Fig. 2F).

SOD and XDH were examined in adductor muscle and showed patterns which were controlled by two and one gene loci, respectively. They were monomorphic in all locations.

Table 1 gives the result of measurements of genetic variability within each locality. The proportion of polymorphic loci was 0.416 in all localities. The observed heterozygosity was in the range 0.119-0.172, the average being 0.149 and

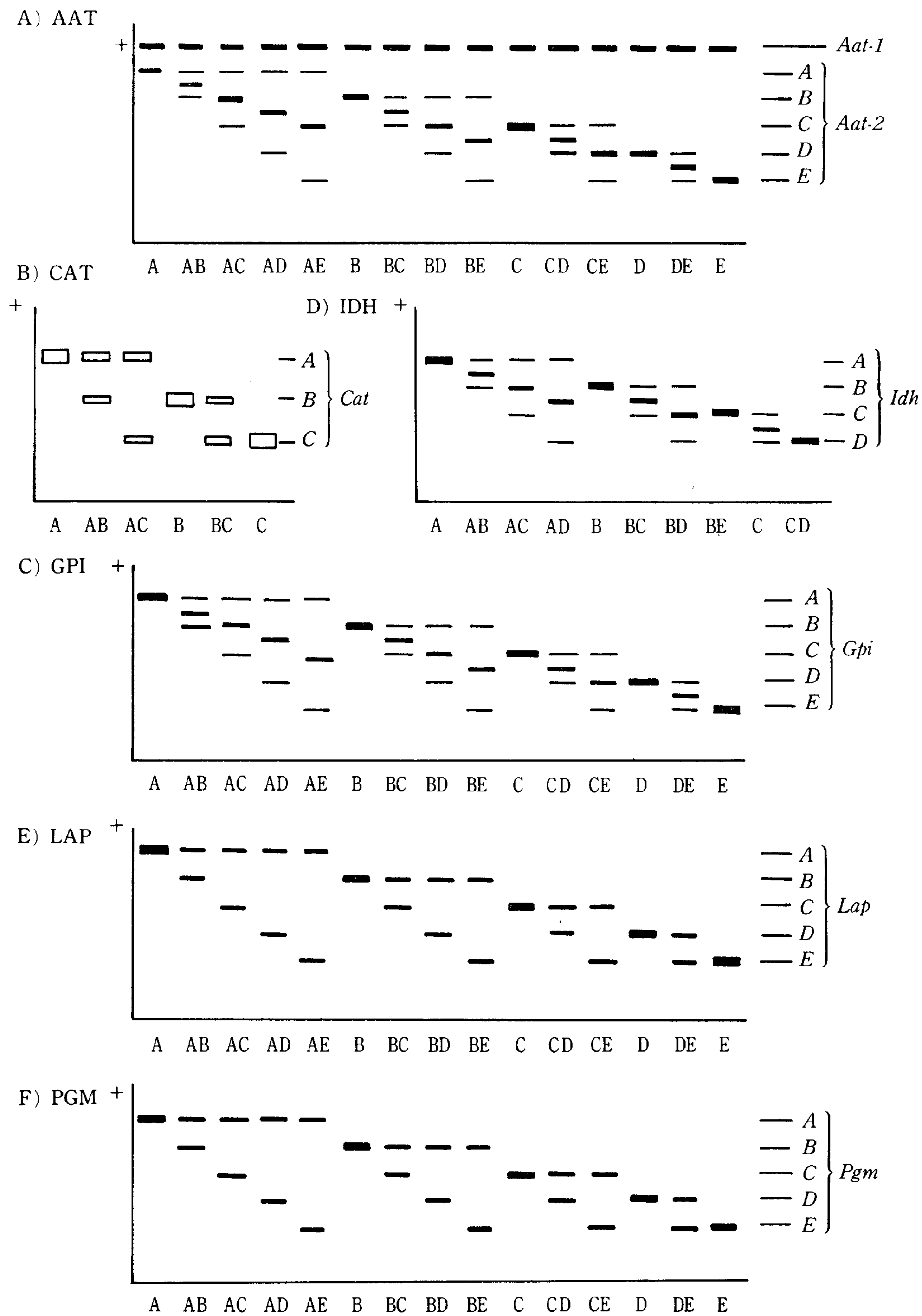

FIG. 2. The proposed phenotypic variation for six polymorphic enzymes of the bay mussel.

the expected heterozygosity was in the range 0.131-0.221, the average being 0.169. The value of the observed heterozygosity tended to be lower than that expected, indicating a general excess of homozygosity. A variation was observed in genetic variability among localities. The number of alleles per locus was in the range

TABLE 1. *Summary of Genetic Variability in 15 Geogrophical Populations of Mytilus Edulis*

Location	No. of shells	Proportion of polymorphic loci	Heterozygosity		No. of alleles per locus
			Observed	Expected	
Saroma Lake, Hokkaido (SH)	39	0.416	0.195	0.215	2.5
Hachinohe, Aomori (HA)	39	0.416	0.145	0.175	2.4
Tanesashi, Aomori (TA)	35	0.416	0.119	0.155	2.3
Omoto, Iwate (OI)	38	0.416	0.153	0.160	2.3
Miyako, Iwate (MI)	122	0.416	0.147	0.166	2.4
Yamada Bay, Iwate (YI)	128	0.416	0.146	0.161	2.5
Koshirahama, Iwate (KI)	126	0.416	0.159	0.168	2.4
Ojika, Miyagi (OM)	130	0.416	0.142	0.221	2.4
Matsushima, Miyagi (MM)	157	0.416	0.146	0.186	2.4
Yuriage, Miyagi (YM)	70	0.416	0.172	0.179	2.5
Watari, Miyagi (WM)	74	0.416	0.152	0.156	2.4
Oharai, Ibaragi (OIb)	23	0.416	0.126	0.132	2.2
Ago Bay, Mie (AM)	48	0.416	0.137	0.151	2.3
Gokasho Bay, Mie (GM)	55	0.416	0.162	0.172	2.3
Onoura, Hiroshima (OH)	40	0.416	0.137	0.131	2.1
Average		0.416	0.149	0.169	2.4

2.1-2.5, the average being 2.36.

2. Excess of Homozygosity

Since the emphasis in this paraghaph is on excess of homozygosity, it is convenient to use an index that assumes negative values when there is an excess of homozygosity and positive values when there is a deficiency. We use the symbol D to denote deviation from expected heterozygosity for a given locus. The D was measured by (Ho-He)/He, where Ho is observed heterozygosity and He is expected heterozygosity. The observed heterozygosity is calculated by direct count of the heterozygotes observed. The expected heterozygosity is defined as $1\text{-}qi^2$, where qi is the frequency of the ith allele for a given locus. Table 2 lists the D values for the polymorphic loci except for the *Cat* locus in each locality. The excess of homozygosity varied from locus to locus within localities and locus-specific excess also varied between localities. However, the excess of homozygosity tends to be significant for *Pgm*, *Lap*, and *Gpi* loci in most of the localities. Although the mean values of D for five loci varied from locality to locality, there is an overall excess of homozygotes in all but 2 of the 15 localities examined.

TABLE 2. *Deviations from Expected Heterozygosity for Each Locus*

Location	Locus					Mean
	Aat-2	*Gpi*	*Idh*	*Lap*	*Pgm*	
Saroma Lake, Hokkaido (SH)	+0.026	−0.051	0	−0.188	−0.134	−0.069
Hachinohe, Aomori (HA)	+0.087	−0.429	+0.269	−0.082	−0.271	−0.085
Tanesashi, Aomori (TA)	+0.094	−0.375	+0.111	−0.355	−0.275	−0.160
Omoto, Iwate (OI)	+0.154	+0.132	−0.012	−0.158	−0.136	−0.004
Miyako, Iwate (MI)	+0.044	−0.078	−0.167	−0.095	−0.216	−0.102
Yamada Bay, Iwate (YI)	+0.069	−0.157	+0.003	−0.186	−0.088	−0.072
Koshirahama, Iwate (KI)	+0.012	−0.030	−0.167	−0.102	−0.012	−0.060
Ojika, Miyagi (OM)	−0.170	−0.534	−0.437	−0.342	−0.279	−0.352
Matsushima, Miyagi (MM)	−0.181	−0.268	−0.329	−0.211	−0.154	−0.229
Yuriage, Miyagi (YM)	+0.048	−0.015	+0.118	−0.029	−0.148	−0.005
Watari, Miyagi (WM)	+0.088	+0.044	+0.053	−0.103	−0.073	+0.002
Oharai, Ibaragi (OIb)	−0.643	+0.177	+0.148	−0.133	−0.028	−0.092
Ago Bay, Mie (AM)	+0.177	−0.045	−0.173	−0.255	−0.067	−0.067
Gokasho Bay, Mie (GM)	−0.016	+0.066	−0.008	−0.139	−0.097	−0.039
Onoura, Hiroshima (OH)	+0.111	+0.160	+0.111	−0.034	+0.068	+0.083
Mean	+0.079	−0.160	+0.034	−0.177	−0.187	−0.074

(The value presents D which is defined in the text.)

3. *Population structure*

The electrophoretic data obtained from the twelve gene loci has been tabulated for the 15 localities sampled in Table 3. A geographical cline of gene frequencies was not clearly observed at the six polymorphic loci. The *Cat* locus, one of the six ploymorphic loci, exhibited only polymorphism in the Saroma Lake in Hokkaido but not in the other localtites. A matrix above the diagonal in Table 4 gives the results of tests of significant heterogeneities between every pair of the 15 localities examined. Significant differences were seen in the frequency distribution of alleles at the six loci between the localities except a few pair of localities. This indicated that they were independent of each other.

Genetic distcnce can be estimated from the number of loci that any two populations have in common according to Nei (3). Such estimates were computed between the 15 geographical subpopulations. The results are given under the diagonal in Table 4. The genetic distances ranged from 0 to 0.042, the average being 0.009 ± 0.001. Larger genetic distances were obtained between the Saroma Lake in Hokkaido and the 14 localities in Honshu. The mean genetic distance within Honshu was 0.006 ± 0.000, any value lower than 0.01 was considered to be a local race. However, the mean genetic distance between Hokkaido and Honshu

was 0.032 ± 0.001, the value being higher than 0.01.

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 delineate a group having a distance of 0.01. Using this vertical line, the 15 subpopulations are divided into three groups. The grouping seemed to indicate the differentiation of local races.

Discussion

The analyses suggest that the population structure of the bay mussel, *Mytilus edulis*, as a whole has a remarkable tendency to split into a number of geographical subpopulations, although the question of whether each of the subpopulations sampled formed an isolated breeding unit or a mixture due to gene flow from the other subpopulations remained. Similar population structure has been reported in the wild oytser, *Crassostrea gigas* (12). The result that the subpopulations except Saroma Lake (Hokkaido) and Ojika (Miyagi) were very closely related with genetic similarity over a geographical distance might be presumably due to the great dispersal distance of the pelagic mussel larvae.

The genetic distance between Hokkaido and Honshu supports the hypothesis that geographical isolation may have been caused by the Tsugaru Straight. Similar distance is obtained between the Hokkaido and Honshu populations of *Patinopecten yessoensis* (10). Since the Ojika population was collected from about a 9 m depth on oyster rafts in the sea where they were not formed naturally, the Ojika population may have colonized by a migration from several origins. Such circumstances are considered responsible for the marked genetic differences and the highest homozygote excess.

The observation of an excess of homozygosity is in agreement with observations made in other pelecypods (9). Several studies have revealed that frequencies of homozygotes were often higher than those expected under Hardy-Weinberg's equilibrium in pelecypodes. An excess of homozygosity in the most extensively studied locus which is coded for the leucine aminopeptidase is observed in several populations of *Mytilus edulis* (13-16). The high negative value of D for this locus supports the above observations. Similar phenomenon was observed in other pelecypodes, such as *Mytilus carifornianus* (17), *Crassostrea virginica* (18), *Crassostrea gigas* (6,19), and *Pinctata fucata* (20). Such observations made by various studies surveying different enzyme loci in different organisms were for aspartate aminotransferase of *Mytilus edulis* (21) and *Crassostrea gigas* (19), for acetaldehyde oxidase of *Crassostrea virginica* (22), for adenylate kinase of *Crassostrea gigas* (6), for esterase of *Crassostrea virginica* (18), for glucosephosphate isomerase of *Mytilus californianus* (17), *Crassostrea virginica* (18) and *Crassostrea gigas* (6,19), for isocitrate dehydrogenase of *Crassostrea gigas*

TABLE 3. *Gene Frequencies in 15*

Locus	Allele	SH (39)	HA (39)	TA (35)	OI (38)	MI (122)	YI (128)
Aat-1		1.000	1.000	1.000	1.000	1.000	1.000
Aat-2	*A*	0	0.103	0.014	0.119	0.056	0.043
	B	0.035	0.077	0.069	0.026	0.085	0.077
	C	0.017	0.026	0.014	0.013	0.026	0.004
	D	0.931	0.749	0.903	0.842	0.833	0.876
	E	0.017	0	0	0	0	0
Gpd-1		1.000	1.000	1.000	1.000	1.000	1.000
Gpd-2		1.000	1.000	1.000	1.000	1.000	1.000
Cat	*A*	0.293	0	0	0	0	0
	B	0.646	1.000	1.000	1.000	1.000	1.000
	C	0.061	0	0	0	0	0
Gpi	*A*	0.024	0.013	0	0.013	0.040	0.031
	B	0.122	0.128	0.181	0.105	0.168	0.104
	C	0.402	0.846	0.750	0.842	0.765	0.811
	D	0.220	0.013	0.069	0.013	0.027	0.050
	E	0.232	0	0	0.027	0	0.004
Idh	*A*	0.012	0	0	0	0	0
	B	0	0.013	0.029	0.039	0.028	0.054
	C	0.976	0.859	0.871	0.882	0.842	0.827
	D	0.012	0.128	0.100	0.079	0.130	0.119
Lap	*A*	0.037	0.038	0.076	0	0.008	0.027
	B	0.329	0.385	0.197	0.474	0.460	0.331
	C	0.561	0.564	0.697	0.526	0.520	0.573
	D	0.073	0.013	0.030	0	0.012	0.058
	E	0	0	0	0	0	0.011
Pgm	*A*	0.024	0.038	0.030	0.013	0.012	0.017
	B	0.415	0.218	0.228	0.230	0.188	0.158
	C	0.439	0.539	0.591	0.473	0.660	0.654
	D	0.122	0.154	0.121	0.257	0.136	0.163
	E	0	0.051	0.030	0.027	0.004	0.008
Sod-1		1.000	1.000	1.000	1.000	1.000	1.000
Sod-2		1.000	1.000	1.000	1.000	1.000	1.000
Xdh		1.000	1.000	1.000	1.000	1.000	1.000

(19), for malate dehydrogenase of *Mytilus californianus* (17), for phosphoglucomutase of *Crassostrea virginica* (18) and *Crassostrea gigas* (19), for sorbitol dehydrogenase of *Crassostrea gigas* (6), for superoxide dismutase of *Mytilus demissus* (23), and for xanthine dehydrogenase (22).

The simplest explanation for a homozygote excess so widely spread over the

Geographical Populations of Mytilus Edulis

KI (126)	OM (130)	MM (157)	YM (70)	WM (74)	OIb (23)	AM (48)	GM (55)	OH (40)
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.053	0.058	0.058	0.087	0.031	0.061	0	0.081	0
0.085	0.058	0.091	0.080	0.069	0	0.143	0.081	0.125
0.016	0.032	0.022	0.007	0	0	0	0	0
0.846	0.852	0.829	0.826	0.900	0.935	0.857	0.838	0.875
0	0	0	0	0	0	0	0	0
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0.008	0	0
1.000	1.000	1.000	1.000	1.000	1.000	0.992	1.000	1.000
0	0	0	0	0	0	0	0	0
0.043	0	0	0.100	0.014	0	0.008	0.042	0.022
0.094	0.126	0.143	0.086	0.152	0.146	0.150	0.166	0.112
0.789	0.761	0.739	0.778	0.784	0.833	0.825	0.750	0.851
0.062	0.113	0.118	0.029	0.036	0.021	0.017	0.025	0.015
0.011	0	0	0.007	0.014	0	0	0.017	0
0	0	0	0	0	0	0	0	0
0.027	0.142	0.015	0.043	0.022	0.021	0.008	0	0
0.836	0.530	0.869	0.864	0.840	0.854	0.875	0.875	0.891
0.137	0.308	0.116	0.093	0.138	0.125	0.117	0.125	0.109
0.039	0.093	0.057	0.035	0.022	0.042	0.021	0	0.010
0.283	0.272	0.272	0.415	0.203	0.250	0.531	0.375	0.281
0.646	0.470	0.570	0.494	0.732	0.646	0.427	0.562	0.646
0.032	0.096	0.075	0.049	0.029	0.062	0.021	0.063	0.063
0	0.069	0.026	0.007	0.014	0	0	0	0
0.044	0.066	0.043	0.023	0.038	0.028	0.032	0.038	0.019
0.186	0.301	0.268	0.227	0.259	0.194	0.226	0.406	0.039
0.583	0.358	0.468	0.515	0.532	0.750	0.678	0.472	0.750
0.167	0.219	0.163	0.212	0.139	0.028	0.048	0.075	0.192
0.020	0.056	0.058	0.023	0.032	0	0.016	0.009	0
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

genome is that it results from the breeding structure of the population. If the sample is comprised of progeny of subpopulations which do not exchange gamates, or exchange only a small amount of gametes, then the homozygotes in the sample will be more than expected from Hardy-Weinberg's equilibrium. This result known as the "Wahlund effect" has been proposed as the general explanation of

TABLE 4. *Test of Significant Heterogeneities of Gene Frequencies above the Pair of Geogrophical Populations of Mytilus edulis*

Location	SH	HA	TA	OI	MI
Saroma Lake, Hokkaido (SH)		+5	+4	+6	+6
Hachinohe, Aomori (HA)	0.033		+2	0	0
Tanesashi, Aomori (TA)	0.027	0.005		+3	+2
Omoto, Iwate (OI)	0.031	0.002	0.008		+2
Miyako, Iwate (MI)	0.031	0.002	0.006	0.003	
Yamada Bay, Iwate (YI)	0.031	0.002	0.003	0.004	0.001
Koshirahama, Iwate (KI)	0.029	0.002	0.001	0.004	0.003
Ojika, Miyagi (OM)	0042	0.013	0.015	0.014	0.016
Matsushima, Miyagi (MM)	0.024	0.002	0.002	0.004	0.005
Yuriage, Miyagi (YM)	0.028	0.001	0.006	0.001	0.002
Watari, Miyagi (WM)	0.028	0.004	0.000	0.008	0.007
Oharai, Ibaragi (OIb)	0.034	0.006	0.002	0.010	0.005
Ago Bay, Mie (AM)	0.034	0.004	0.010	0.006	0.001
Gokasho Bay, Mie (GM)	0.024	0.003	0.005	0.005	0.005
Onoura, Hiroshima (OH)	0.039	0.006	0.005	0.009	0.004

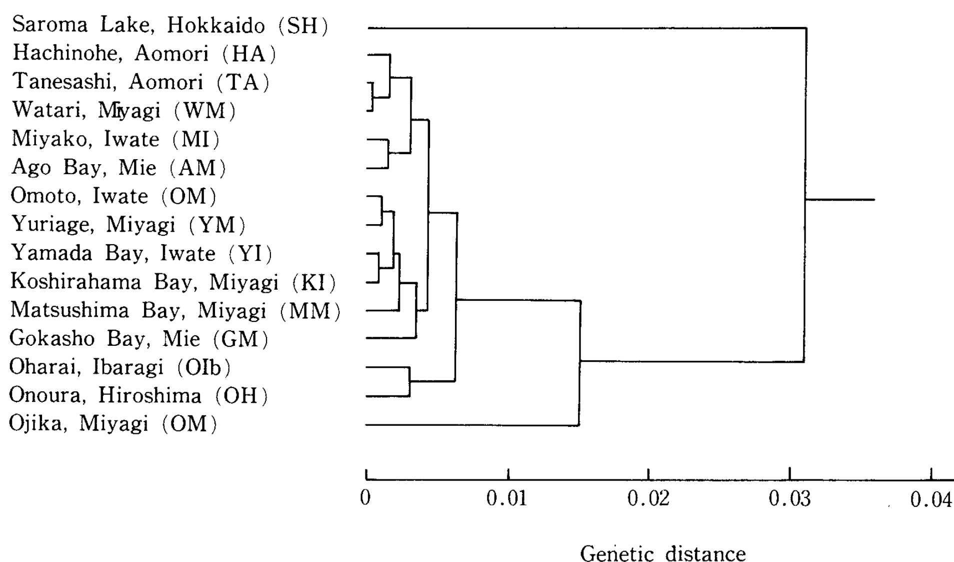

FIG. 3. Dendrogram drawn by using genetic distances in TABLE 4.

homozygosity excess in pelecypodes with pelagic larvae by Tracey *et al.* (17). The "Wahlund effect" is expected to effect the loci at which the difference of allelic frequency is significant between subpopulations. Since most of pelceypodes are relatively immobile animals but the larvae are pelagic and swim freely, we feel that two or more parental subpopulations produced progeny with different Hardy-Weinberg's proportion at the sample site. In this connection, we indicated

Diagonal, and Genetic Distances under the Diagonal, between Every

YI	KI	OM	MM	YM	WM	OIb	AM	GM	OH
+5	+5	+6	+6	+4	+6	+4	+6	+4	+4
+3	+1	+4	+2	+1	+1	+2	+2	+1	+2
+4	+2	+3	+2	+3	0	+1	+1	+4	+1
+3	+3	+4	+3	+2	+3	+2	+2	+2	+3
+3	+3	+4	+3	+2	+3	+4	+3	+4	+5
	0	+4	+3	+2	+2	+3	+5	+3	+4
0.000		+5	+3	+2	+1	+3	+4	+4	+5
0.013	0.012		+3	+4	+5	+5	+5	+5	+5
0.003	0.002	0.010		+2	+3	+4	+4	+5	+5
0.002	0.002	0.012	0.003		+2	+3	+3	+3	+3
0.003	0.001	0.013	0.002	0.006		+2	+3	+3	+2
0.002	0.003	0.020	0.007	0.008	0.004		+2	+2	+2
0.004	0.007	0.020	0.008	0.004	0.012	0.008		+3	+2
0.006	0.004	0.013	0.002	0.003	0.004	0.008	0.006		+3
0.002	0.003	0.023	0.008	0.007	0.006	0.003	0.008	0.012	

that the population structure of the bay mussel as a whole has a remarkable tendency to split into a number of geographical subpopulations. Such circumstances are considered responsible for the formation of patchness as a result of habitat.

Next, the general excess of homozygosity suggests that there may be substantial inbreeding in the population. If this is the case, this result is expected to effect all loci in the same direction. High proportion of the loci showing a homozygote excess do not exclude inbreeding as the cause of homozygosity excess. The remarkable tendency to split into a number of geographical subpopulations is also considered responsible for the inbreeding structure of the population. Thus, it might be concluded that the homozygosity excess reflected the population sudivision and/or inbreeding structure.

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