Heterogeneity within and between Geographical Populations of the Short-necked Clam, Ruditapes philippinarum

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Heterogeneity within and between Geographical Populations of the Short-necked Clam, *Ruditapes philippinarum*

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Summary

Starch gel electrophoresis was carried out to estimate the amount of genetic variability and the level of genetic differentiation in geographical populations of the short-necked clam, *Ruditapes philippinarum*. The observed heterozygosity ranged from 0.172 to 0.245 with a mean of 0.204, while the expected heterozygosity was from 0.204 to 0.264 with a mean of 0.248. The observed heterozygosity was lower than the expected one in the locations examined, indicating a general excess of homozygosity.

All of the 14 locations showed clear differences in gene frequencies at the four polymorphic loci and also in shell shapes, indicating that they are independent of each other. The genetic distance was positively correlated with the morphological distance based on shell shape. The populations could be divided into 5 groups from the dendrogram drawn by genetic distances. The grouping seemed to indicate a differentiation into local races. These results suggest that the population structure of the short-necked clam as a whole has a tendency to split into a number of geographical subpopulations.

An excess of homozygosity was observed in almost all subpopulations. A possible explanation of this observation is discussed in regard to the population structure.

Electrophoretic data are a valuable tool to measure the amount of genetic variablity and the degree of genetic differentiation between populations. Estimated levels of genetic varation in some of the pelecypods have been reported(1, 2). Fujio *et al.* (2) revealed that the observed heterozygosity tend to be lower than the expected heterozygosity in marine pelecypods and suggested a general trend of homozygote excess. The degree of genetic distances within and between taxa of pelecypods has so far not been treated. In fact, the interest in differentiation of population by methods of electrophoresis is quite recent (3, 4, 5). If two populations are isolated from each other by geographical or reproductive barriers, 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.

The short-necked clam, *Ruditapes philippinarum*, is distributed as colonies in suitable coastal areas throughout Japan. The short-necked clam is relatively immobile but the larvae are frequently pelagic and swim freely thereby promoting gene flow. Transplantations of the clam seeds have been sometimes carried out from the south to north coast in Japan.

In the present work, electrophoresis was used in the genetical study of the short-necked clam population; 1) to estimate the amount of genetic variability within different geographical populations, 2) to estimate the degree of genetic differentiation among these populations in relation with the shell shapes, and 3) to discuss some hypotheses to account for the excess of homozygosity.

Materials and Methods

Collections of the short-necked clam, *Ruditapes philippinarum* were made in 14 locations from Hokkaido to Aichi in Japan (Table 1). The samples were stored at −80°C until electrophoresis. As morphological characters, shell dimensions such as length, height, and width were measured. Adductor muscle was removed from the shells, homogenized with an approximately equal weight of distilled water, and the homogenates were centrifuged at 3,500 rpm for 15 minutes. The supernatants were subjected to horizontal starch gel electrophoresis. Electrophoretic procedures and staining methods are described by Fujio (6). Four enzymes, aspartate aminotransferase (AAT), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), and phosphoglucomutase (PGM) were examined.

TABLE 1. *Collection Data of Short-necked Clam (Ruditapes philippinarum)*

Location	Date	No. of Shells	Shell Size (mm)		
			Length	Height	Width
1. Akkeshi, Hokkaido (HA)	May 1984	50	48.5±4.2*	34.4±2.5*	23.3±2.1*
2. Mangokuura, Miyagi (MM)	Feb. 1983	114	41.8±4.1	29.6±2.8	20.9±2.2
3. Miyatojima, Miyagi (MiM)	Feb. 1983	118	37.9±3.2	26.7±2.3	18.7±2.2
4. Tohna, Miyagi (TM)	Feb. 1983	95	32.3±2.1	23.1±1.4	16.3±1.2
5. Isozaki, Miyagi (IM)	Feb. 1983	62	36.8±4.1	26.2±3.3	17.9±2.5
6. Hamada, Miyagi (HM)	May 1983	102	37.8±3.6	27.8±2.4	18.8±2.3
7. Daigasaki, Miyagi (DM)	May 1983	110	38.5±6.9	27.6±5.4	19.0±4.2
8. Sendai-shinko, Miyagi (SM)	Dec. 1983	95	37.6±5.1	26.2±3.3	18.1±2.7
9. Hiroura, Miyagi (HiM)	May 1983	90	34.4±4.1	24.9±3.2	17.7±2.7
10. Torinoumi A, Miyagi (TaM)	May 1983	63	35.8±4.0	26.3±3.0	19.0±2.4
11. Torinoumi B, Miyagi (TbM)	Feb. 1983	67	34.4±3.9	24.4±7.7	17.0±2.3
12. Matsukawaura, Fukushima (MF)	Apr. 1983	74	34.8±2.4	24.3±1.8	16.8±1.4
13. Hazu, Aichi (HaA)	May. 1987	57	32.8±4.6	24.1±6.5	18.6±5.6
14. Himakajima, Aichi (HiA)	May 1984	79	31.3±3.6	25.5±2.7	15.1±2.4

* Mean±SD

Loci which are shown in italics were numbered in increasing order and the alleles were designated alphabetically in order of electrophoretic mobility from the most anodal to the most cathodal locus or alleles, respectively.

Results

1. Genetic variation

Four enzymes were examined and 8 genetic loci were detected. The 4 enzyme systems have been interpreted in the following manner (Fig. 1).

AAT showed activity in the two zones which were coded by two gene loci (*Aat-1* and *Aat-2*). The *Aat-1* locus coding for the isozyme located in the more anodal migrating zone showed variation. Since the activity at *Aat-1* was not consistently scored, it was not included in the analysis. The *Aa-2* locus coding for the isozyme located in the slowest migrating zone exhibited polymorphism in all locations. A locus was considered polymporphic if the frequencies of the most common allele were no greater than 0.95. Heterozygous individuals showed three-banded phenotypes and homozygous individuals single-banded phenotypes, indicating the typical pattern of a dimeric structure of the enzyme. The different phenotypes indicated 4 alleles.

LAP produced several zones of activity which could not be scored. The more anodal migrating zone stained more intensely and developed first. One and two-banded phenotypes indicated the typical pattern of a monomer and the locus was scored. The different phenotypes indicated seven alleles and showed polymorphism in all locations.

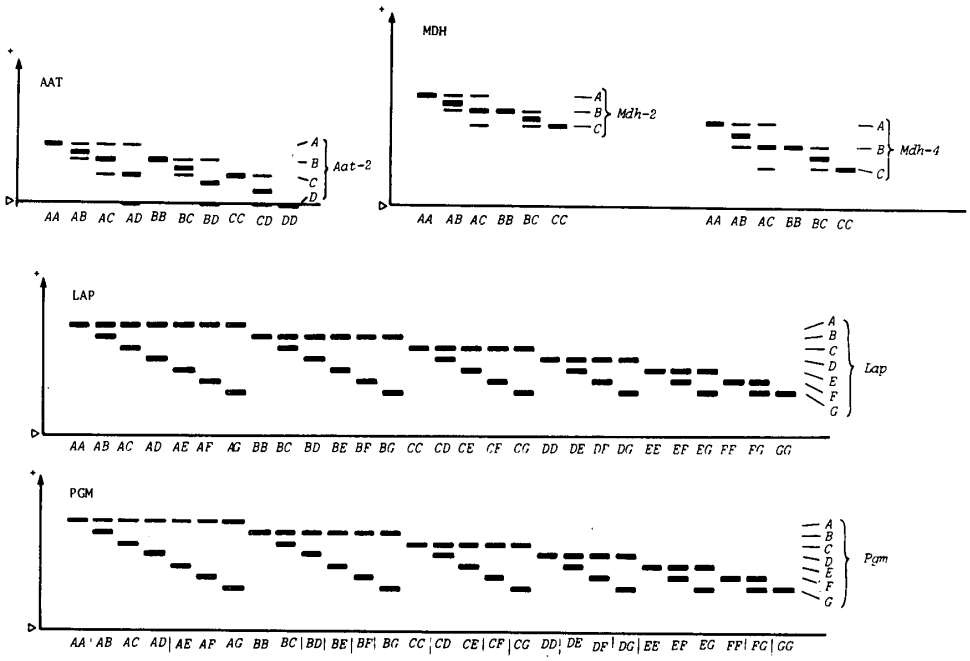

FIG. 1. The proposed phenotypic variation for four enzymes of the short-necked clam.

MDH produced two zones of activity, the expression of which showed the typical pattern of a dimer. Each of the two zones exhibited three bands with extended staining time, indicating two gene loci for each zone. The upper two bands stained weakly and developed later while the lowest band stained more intensely, developed faster, and displayed one- and three-banded phenotypes. Thus, the upper anodal migrating zone indicated tvo loci (*Mdh-1* and *Mdh-2*). The *Mdh-1* locus was monomorphic while the *Mdh-2* locus indicated polymorphism at three alleles in 11 of the 14 locations. The slower migrating zone indicated two loci (*Mdh-3* and *Mdh-4*). The *Mdh-3* locus was monomorphic while *Mdh-4* indicated variation at three alleles with low frequency in all locations.

PGM showed the typical pattern of a monomer and was coded by one gene locus. The different phenotypes indicated polymorphism in all locations at a total of seven alleles.

Table 2 gives the result of measurements of genetic variability within each location. The number of alleles per locus ranged from 2.6 to 3.6 with a mean of 3.6. The observed heterozygosity range was 0.172–0.245, the mean being 0.204, and the expected heterozygosity range was 0.204–0.264, the mean being 0.248. Variation in genetic variability was observed among locations. The value of the observed heterozygosity was lower than that expected, indicating a general 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. The index was measured by (Ho-He)/He, where Ho is observed

TABLE 2. *Genetic Variability in 14 Geographical Populations of Short-necked Clam*

Location	Proportion of polymorphic loci	No. of alleles per locus	Heterozygosity		(Ho-He)/He
			Observed (Ho)	Expected (He)	
Akkeshi, Hokkaido (AH)	0.429	2.6	0.174	0.204	−0.147
Mangokuura, Miyagi (MM)	0.571	3.3	0.207	0.260	−0.204
Miyatojima, Miyagi (MiM)	0.429	3.1	0.218	0.226	−0.035
Tohna, Miyagi (TM)	0.571	3.4	0.196	0.236	−0.169
Isozaki, Miyagi (DM)	0.571	3.1	0.231	0.257	−0.101
Hamada, Miyagi (HM)	0.571	3.1	0.216	0.248	−0.129
Daigasaki, Miyagi (IM)	0.571	3.6	0.198	0.256	−0.227
Sendai-shinko, Miyagi (SM)	0.571	3.0	0.207	0.254	−0.185
Hiroura, Miyagi (HiM)	0.571	3.3	0.189	0.260	−0.273
Torinoumi A, Miyagi (TaM)	0.429	2.7	0.204	0.253	−0.194
Torinoumi B, Miyagi TbM)	0.571	3.0	0.176	0.264	−0.333
Matsukawaura, Fukushima (MF)	0.571	2.9	0.222	0.249	−0.108
Hazu, Aichi (HaA)	0.571	2.7	0.172	0.233	−0.262
Himakajima, Aichi (HiA)	0.571	3.3	0.245	0.274	−0.106
Average	0.541	3.1	0.204	0.248	−0.177

heterozygosity and He is expected heterozygosity. The values varied from location to location, with an average of -0.177.

2. Population structure

The electrophoretic data obtained from the seven loci has been tabulated for the 14 locations in Table 3. The results of tests for significant heterogeneities between every pair of the 14 locations are given in a matrix above the diagonal in Table 4. Significant differences were seen in the frequency distribution of alleles at the four polymorphic loci between the locations. This indicated that they were independent of each other.

Genetic distance according to Nei (7) can be estimated from allele differences at loci that any two populations have in common. Such estimates were computed between the 14 geographical subpopulations. The results are given under the diagonal in Table 4. Smallest genetic distances were obtained among pairs of Mangokuura, Miyatojima, Tohna, and Daigasaki in Miyagi. Largest genetic distances were obtained between Himakajima in Aichi with Torinoumi A and B in Miyagi and Matsukawaura in Fukushima and also between Akkeshi in Hokkaido with Torinoumi A in Miyagi and Matsukawaura in Fukushdma. This indicates that larger geographical distance seems to show larger genetic distance. The average of genetic distances was 0.0377, the value being higher than 0.01 which was considered to be a local race level.

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. 2. A vertical line was drawn across the dendrogram in order to delineate a group having a distance of 0.02. Using this vertical line, the 14 subpopulations were divided into five groups. The grouping based on the 0.02 level seemed to approximate more accurately the differentiation of local races.

3. Morphological character

The morphological data obtained from the proportion of length (L), height (H), and width (W) in shell has been tabulated in Table 5. The proportion was calculated by L/(L+H+W), H/(L+H+W), and W/(L+H+W), respectively. The results of tests of significant differences between every pair of the 14 locations are given in a matrix above the diagonal in Table 6. Significant differences were seen in 74 of the 91 pairs. This indicated that the shell shapes were independent of each other. The morphological distance based on the shell shapes was calculated by $\sqrt{\Sigma(xi-yi)^2/2}$, where xi and yi represent the proportion of length, height, and width in shells of two locations, x and y. The results are given under the diagonal in Table 6. Morphological and genetic distances are plotted in Fig. 3. There was a weak positive correlation and the correlation coefficient was 0.229

TABLE 3. *Gene Frequencies in 14 Geographical*

		AH	MM	MiM	TM	IM	HM
Aat-2	N	50	102	98	90	70	103
	A	0	0.019	0	0.005	0.022	0.010
	B	0.130	0.248	0.143	0.128	0.184	0.141
	C	0.860	0.733	0.857	0.867	0.794	0.849
	D	0.010	0	0	0	0	0
Lap	N	50	115	120	90	81	99
	A	0.020	0.004	0.016	0	0.032	0
	B	0.190	0.065	0.040	0.044	0.234	0.215
	C	0.710	0.309	0.329	0.278	0.418	0.484
	D	0.080	0.100	0.111	0.039	0.146	0.113
	E	0	0.430	0.444	0.544	0.127	0.156
	F	0	0.070	0.048	0.067	0.043	0.032
	G	0	0.022	0.012	0.028	0	0
Mdh-1	N	50	115	116	94	82	103
	A	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	N	50	115	116	94	84	103
	A	0.010	0.074	0.043	0.063	0.050	0.112
	B	0.990	0.926	0.957	0.932	0.938	0.888
	C	0	0	0	0.005	0.012	0
Mdh-3	N	50	115	116	94	82	103
	A	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-4	N	50	115	116	94	82	103
	A	0.030	0.013	0.016	0.032	0.019	0.024
	B	0.960	0.987	0.976	0.963	0.981	0.967
	C	0.010	0	0.008	0.005	0	0.009
Pgm	N	50	106	109	91	74	103
	A	0	0.004	0.004	0.011	0.007	0.020
	B	0.160	0.085	0.068	0.060	0.118	0.076
	C	0.510	0.609	0.636	0.565	0.576	0.647
	D	0.280	0.024	0.032	0.038	0	0.035
	E	0.050	0.250	0.248	0.271	0.271	0.197
	F	0	0.024	0.012	0.033	0.021	0.020
	G	0	0.004	0	0.022	0.007	0.005

N : No. of shells

(n=91).

Discussion

The analyses suggest that the population structure of the short-necked clam 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 from an isolated breeding unit or a mixture due to gene flow from the other subpopulations remains. Similar population structure has been reported in

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DM	SM	HiM	TaM	TbM	MF	HaA	HiA
107	85	82	63	68	75	49	79
0.005	0	0.018	0.008	0.008	0.033	0.010	0.025
0.178	0.159	0.124	0.206	0.164	0.140	0.061	0.272
0.817	0.841	0.858	0.786	0.828	0.827	0.908	0.684
0	0	0	0	0	0	0.021	0.019
96	95	82	63	68	75	56	79
0.005	0.005	0.051	0	0.008	0	0	0.019
0.083	0.112	0.122	0.008	0.106	0.053	0.036	0.298
0.460	0.426	0.385	0.223	0.303	0.260	0.188	0.418
0.068	0.138	0.333	0.287	0.212	0.080	0.518	0.259
0.349	0.282	0.083	0.387	0.296	0.507	0.196	0.006
0.052	0.032	0.026	0.095	0.075	0.100	0.062	0
0.037	0.005	0	0	0	0	0	0
110	95	82	63	68	75	57	79
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
110	95	82	63	68	75	57	79
0.086	0.079	0.068	0.047	0.097	0.100	0.096	0.069
0.909	0.921	0.932	0.953	0.903	0.893	0.904	0.925
0.005	0	0	0	0	0.007	0	0.006
110	95	82	63	68	75	57	79
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
110	95	82	63	68	75	57	79
0.009	0.016	0.013	0.016	0.015	0.006	0	0.006
0.963	0.974	0.981	0.984	0.978	0.994	0.991	0.988
0.028	0.010	0.006	0	0.007	0	0.009	0.006
105	95	81	63	68	74	57	78
0.010	0.010	0.056	0	0	0	0	0.013
0.072	0.163	0.075	0.024	0.008	0.014	0.193	0.128
0.606	0.532	0.432	0.103	0.091	0.128	0.544	0.641
0.024	0	0.325	0.571	0.568	0.574	0.228	0.186
0.264	0.284	0.056	0.246	0.296	0.257	0.035	0.026
0.019	0.011	0.050	0.056	0.038	0.027	0	0.006
0.005	0	0.006	0	0	0	0	0

the wild oyster, *Crassostrea gigas* and the wild bay mussel, *Mytilus edulis* (3, 4). The result that the genetic similarity among the subpouplations except Hiroura (Miyagi) and Hazu (Aichi) were very closely related with geographical distance might be presumably due to limited dispersal distance of the pelagic clam larvae. Such isolations are considered responsible for forming of local races. In fact, the grouping with a distance of 0.02 indicates differentiation of local races. If the number of loci scored were sufficiently large, the value estimated for a local race is usually based on Nei (8), which is 0.01. Since in this study, the few number

TABLE 4. *Test of Significant Heterogeneities of Gene Frequencies above the Diagonal and Genetic Distances under the Diagonal, between Every Pair of Geographical Populations of Short-necked Clam*

Location	(AK)	(MM)	(MiM)	(TM)	(IM)	(HM)	(DM)	(SM)	(HiM)	(TaM)	(TbM)	(MF)	(HaA)	(HiA)
Akkeshi, Hokkaido (AH)		+4	+2	+3	+3	+3	+3	+3	+3	+2	+3	+4	+2	+4
Mangokuura, Miyagi (MM)	.0497		+1	+2	+1	+2	+2	+3	+3	+2	+3	+2	+3	+2
Miyatojima, Miyagi (MiM)	.0461	.0027		+1	+2	+1	+2	+2	+2	+2	+2	+3	+3	+3
Tohna, Miyagi (TM)	.0601	.0051	.0025		+1	+1	+1	+2	+2	+2	+2	+2	+2	+3
Isozaki, Miyagi (IM)	.0235	.0143	.0151	.0246		+2	+3	+1	+2	+2	+2	+2	+3	+3
Hamada, Miyagi (HM)	.0189	.0157	.0141	.0235	.0031		+1	+2	+2	+3	+2	+2	+3	+3
Daigasaki, Miyagi (DM)	.0349	.0031	.0024	.0062	.0085	.0071		+1	+2	+2	+2	+3	+3	+2
Sendai-shinko, Miyagi (SM)	.0291	.0072	.0061	.0116	.0047	.0060	.0027		+2	+2	+2	+3	+3	+3
Hiroura, Miyagi (HiM)	.0192	.0376	.0344	.0456	.0231	.0224	.0313	.0255		+2	+2	+2	+2	+3
Torinoumi A, Miyagi (TaM)	.0762	.0608	.0615	0.589	.0737	.0798	.0647	.0621	.0356		+1	+2	+3	+2
Torinoumi B, Miyagi (TbM)	.0612	.0621	.0617	.0598	.0638	.0680	.0601	.0560	.0310	.0040		+1	+3	+3
Matsukawaura, Fukushima (MF)	.0770	.0563	.0555	.0476	.0754	.0765	.0580	.0598	.0469	.0072	.0069		+3	+3
Hazu, Aichi (HaA)	.0523	.0401	.0350	.0459	.0368	.0370	.0398	.0331	.0139	.0504	.0549	.0665		+2
Himakajima, Aichi (HiA)	.0211	.0387	.0438	.0604	.0173	.0191	.0352	.0283	.0201	.0845	.0772	.0956	.0329	

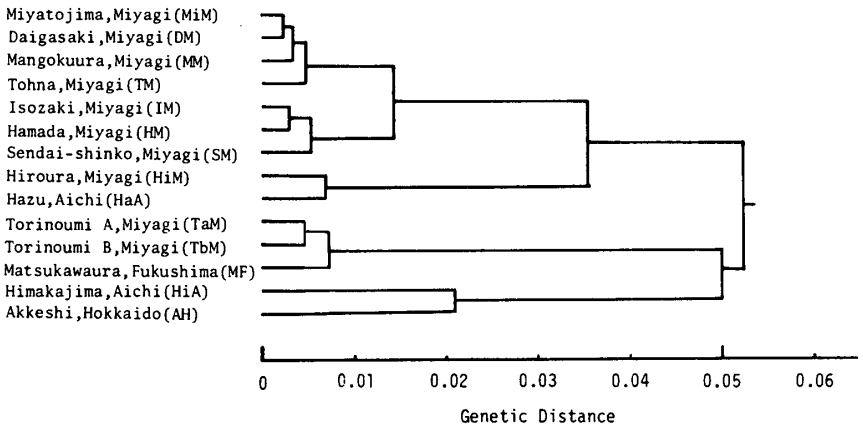

FIG. 2. Dendrogram drawn by using genetic distances in TABLE 4.

TABLE 5. *Proportion of Length (L), Hight (H) and Width (W) in Shell*

Location	No. of Shell	L/(L+H+W)	H/(L+H+W)	W/(L+H+W)
Akkeshi, Hokkaido (AH)	50	0.456±0.001	0.324±0.001	0.220±0.001
Mangokuura, Miyagi (MM)	114	0.452±0.001	0.321±0.001	0.227±0.001
Miyatojima, Miyagi (MiM)	118	0.455±0.001	0.320±0.001	0.225±0.001
Tohna, Miyagi (TM)	95	0.450±0.001	0.322±0.001	0.227±0.001
Isozaki, Miyagi (IM)	62	0.455±0.001	0.324±0.001	0.221±0.001
Hamada, Miyagi (HM)	102	0.448±0.001	0.330±0.001	0.222±0.001
Daigasaki, Miyagi (DM)	110	0.454±0.001	0.324±0.001	0.222±0.001
Sendai-shinko, Miyagi (SM)	95	0.456±0.003	0.320±0.001	0.221±0.001
Hiroura, Miyagi (HiM)	90	0.447±0.001	0.323±0.001	0.229±0.001
Torinoumi A, Miyagi (TaM)	63	0.441±0.002	0.324±0.002	0.234±0.001
Torinoumi B, Miyagi (TbM)	67	0.454±0.001	0.323±0.001	0.224±0.001
Matsukawaura, Fukushima (MF)	74	0.458±0.002	0.320±0.001	0.221±0.001
Hazu, Aichi (HA)	57	0.439±0.004	0.318±0.006	0.244±0.006
Himakajima, Aichi (HiA)	79	0.455±0.002	0.326±0.001	0.220±0.002

of loci investigated would lead to an overestimation of genetic distance levels, the genetic distance value of 0.02 was used instead.

The weak correlation between genetic and morphological distances suggests high morphological differentiation as well as genetic differentiation but seemingly independent from each other. This weak correlation may be due to the influence of environment on morphological characters, which factor might be masking any genetic effects.

Since the clam seeds were transplanted from Ariake in Kyushu to Hazu in Aichi, the Hazu population may have mixed by transplantation. Such circumstances are considered responsible for the marked genetic difference and the highest homozygote excess. The observation of the highest homozygosity excess is in agreement with the above circumstance. A similar phenomenon is considered for the Hiroura population.

TABLE 6. *Test of Significant Differences of the Proportion of Shell Length, Height, and Width above the Diagonal and Morphological Distances under the Diagonal, between Every Pair of Geographical Populations of Short-necked Clam*

Location	(AK)	(MM)	(MiM)	(TM)	(IM)	(HM)	(DM)	(SM)	(HiM)	(TaM)	(TbM)	(MF)	(HaA)	(HiA)
Akkeshi, Hokkaido (AH)		+	+	+	−	+	−	+	+	+	−	+	+	−
Mangokuura, Miyagi (MM)	.0056		−	−	+	+	+	+	+	+	−	+	+	+
Miyatojima, Miyagi (MiM)	.0046	.0020		+	+	+	+	+	+	+	−	+	+	+
Tohna, Miyagi (TM)	.0067	.0018	.0038		+	+	+	+	−	+	+	+	+	+
Isozaki, Miyagi (IM)	.0006	.0050	.0039	.0062		+	−	+	+	+	−	+	+	−
Hamada, Miyagi (HM)	.0065	.0074	.0084	.0068	.0067		+	+	+	+	+	+	+	+
Daigasaki, Miyagi (DM)	.0018	.0041	.0038	.0049	.0016	.0053		+	+	+	−	+	+	−
Sendai-shinko, Miyagi (SM)	.0032	.0050	.0033	.0065	.0028	.0090	.0038		+	+	+	−	+	+
Hiroura, Miyagi (HiM)	.0084	.0041	.0061	.0023	.0080	.0066	.0065	.0087		+	+	+	+	+
Torinoumi A, Miyagi (TaM)	.0140	.0096	.0117	.0079	.0136	.0103	.0121	.0144	.0057		+	+	−	+
Torinoumi B, Miyagi (TbM)	.0030	.0025	.0020	.0038	.0024	.0065	.0018	.0033	.0058	.0115		−	+	+
Matsukawaura, Fukushima (MF)	.0038	.0054	.0035	.0071	.0034	.0099	.0046	.0012	.0093	.0150	.0039		+	+
Hazu, Aichi (HaA)	.0208	.0154	.0172	.0142	.0203	.0185	.0190	.0203	.0127	.0084	.0179	.0206		+
Himakajima, Aichi (HiA)	.0010	.0062	.0054	.0070	.0016	.0058	.0020	.0042	.0085	.0140	.0206	.0048	.0211	

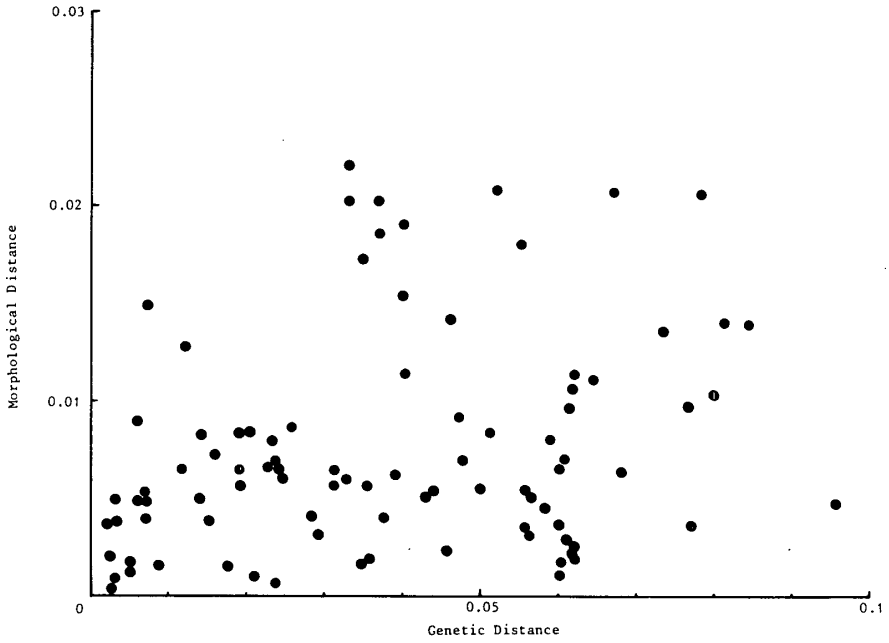

FIG. 3. Relationship between morphological and genetic distance among subpopulations of short-necked clam.

The observation of an excess of homozygosity is consistent with observations made in other pelecypods (2, 4). The simplest explanation for a general excess of homozygosity is that it results from the breeding structure of the population. If the sample is composed of progeny of subpopulations which do not exchange gametes, 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 homozygosity excess in pelecypods with pelagic larvae by Tracey *et al.*(9). The "Wahlund effect" is expected to affect the loci at which the difference of allelic frequency is significant between subpopulations. In this connection, we indicated the significant differences in the frequency distribution of alleles between the locations. Such circumstances are considered responsible for the formation of patchiness as a result of habitat.

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