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Genetic Differentiation among Localities in the Natural Pacific Herring around Japan and Genetic Characterization of the Artificial Seeds Compared with the Natural Population

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Summary

Genetic differentiation among 9 localities in the natural Pacific herring were estimated by allozyme surveys at the 18 loci. Allelic compositions were stable for several years in the same location. Dendrogram drawn from Nei's genetic distance and heterogeneity tests indicated that the Pacific herring around Japan was largely divided into two groups, one of which was located in the northern part and the other in the southern part, and they were subdivided into 5 subpopulations.

Genetic fluctuation at the 4 polymorphic loci was observed among lots of the artificial seeds produced by the natural parents collected from one locality. It suggests an occurrence of genetic drift by a small number of parental individuals. Significant deviations of the observed heterozygotes from the expected heterozygotes at the 4 polymorphic loci were not observed in natural populations but in the artificial seeds. The genetic change from homozygote excess to heterozygote excess was observed in the artificial seeds. This is interpreted by the lower viability in homozygotes than that in heterozygotes under the artificial rearing condition in the Pacific herring.

Pacific herring, *Clupea pallasi*, is divided into two types in Japan. One of which is the oceanic type that has migrated widely and the other is the brackish type which has a narrow migration. The species migrate to the coastal water and spawn attached demersal eggs to seaweed with the heaviest concentrations in late winter to spring. Several major spawning areas have been constructed in the coastal water around Japan (1). Natural resources of the species, however, have been rapidly depleted scince the 1950's, and now small spawning groups have migrated to the coastal water around the northern part of Japan (1-3), Because of the depletion of natural resources, artificial seed production has been carried out in some areas to restore the natural resources.

Because of their restricted spawning area and egg type, subdivision of the Pacific herring population could be anticipated. Based on the ecological and morphological surveys, a number of subpopulations have been reported around Japan (1).

If the parents spawn in their birth area and isolate themselves from other spawning groups for the long term, the spawning groups will have different genetic compositions. To clarify the population structue of natural Pacific herring, genetical surveys are necessary to add to morphological and ecological surveys.

Electrophoretically detectable isozyme genes are a convenient tool for analysis of genetic composition of a population. Isozymic analysis were performed in the Pacific herring populations (4-6). In Japan, Kobayashi (4) revealed two different subpopulations in Ishikari Bay by IDH isozyme genes, and Kobayashi *et al.* (5) reported the differences of allele frequencies between oceanic and brackish type in the Pacific herring around Japan.

The aims of the present study are to estimate genetic differentiation among localities of the natural Pacific herring collected around the northern part of Japan and to characterize genetically both the natural and artificial seeds of Mangoku-ura.

Materials and Methods

The specimens of the natural Pacific herring surveyed in the present study were collected from three localities in Hokkaido and six localities in Honshu as shown in Fig. 1. The specimens collected from Lake Furen in Hokkaido, Lake Obuchi and Lake Hinuma in Honshu were categorized as the brackish type. Sampling site, date, the total survey number and their mean body length are shown in Table 1. In Miyako and Mangoku-ura the specimens were collected over three different years.

The specimens of the artificial seeds are shown in Table 2. These were supplyed by the Japan Sea-Farming Association and some of them were cultivated in Tohoku National Fisheries Research Institute. All of the artificial seeds were produced by the parent fish collected from Mangoku-ura at a different time. Lot number represent the parent groups, and the numbers of the Lot I represent the sampling numbers collected at different times from the same parental group (Lot I). The specimens were transported to our laboratory in frozen state and stored at -50 to -80°C until starch-gel electrophoresis was performed.

The procedure of starch-gel electrophoresis and staining were followed Fujio (7). A total of eleven enzymes, aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), α-glycerophosphate dehydrogenase (αGPD), glucosephosphate isomerase (GPI), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), 6-

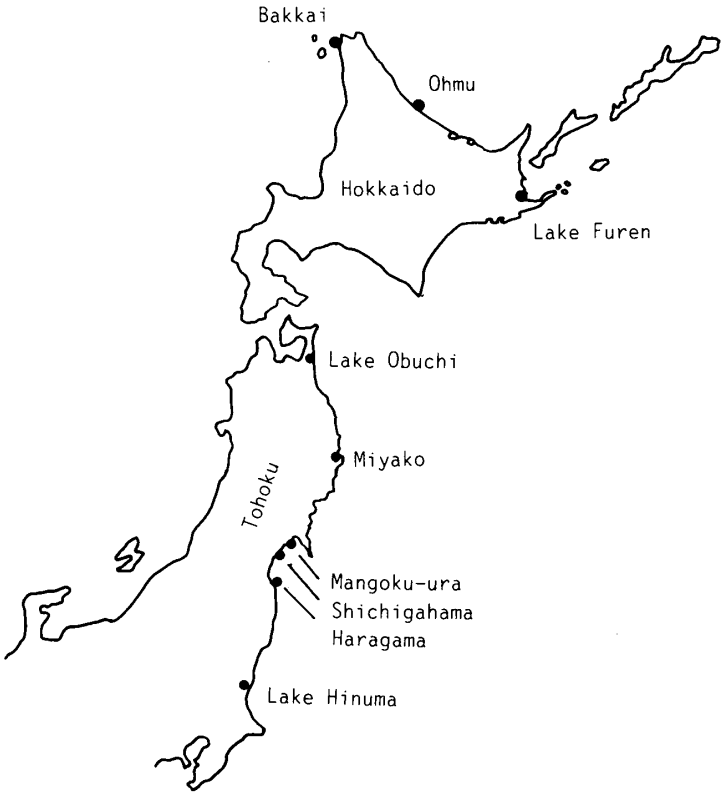

FIG. 1. Map showing the collection sites of the Pacific herring surveyed in the present study

TABLE 1. *Data of Specimens of the Natural Pacific Herring Examined in the Present Study*

Area	Location	Number of individuals	Date of collection	Mean length (mm)±SD
Hokkaido	Bakkai	50	Jun. '87	208.6±11.7
	Ohmu	52	Jun. '87	207.0± 9.8
	Lake Furen*	63	May '87	226.0± 9.7
Honshu	Lake Obuchi*	81	Apr. '86	282.4±11.4
	Miyako-1	40	May '86	232.8± 5.7
	Miyako-2	68	Apr. '87	232.8±21.0
	Miyako-3	60	Mar. '88	285.1±19.9
	Mangoku-ura-1	109	Jan.-Apr. '86	289.9± 2.9
	Mangoku-ura-2	57	Dec. '87-Jan. '88	275.5±10.4
	Mangoku-ura-3	55	Apr. '87	180.3±12.6
	Shichigahama	148	Mar. '87	184.2± 7.6
	Haragama	39	May '87	239.8±27.9
	Lake Hinuma*	24	Jan.-Feb. '86	304.1±16.9

* brackish type

TABLE 2 *Data of Specimens of Artificial Seeds Produced from the Natural Individuals Collected from Mangoku-ura in the Pacific Herring*

	Lot*	Age	Date of collection	Number of individuals	Mean length (mm)±SD
I	I-1	0+	2 May '86	178	45.0± 5.3
	I-2	0+	2 Jun. '86	99	72.6± 6.1
	I-3	0+	2 Aug. '86	36	98.0± 6.5
	I-4	1+	3 Jun. '87	57	147.8± 6.1
II	II	0+	1 May '87	139	45.7± 9.0
III	III	0+	22 May '87	179	61.3± 5.2
IV	IV	1+	15 Jul. '86	39	106.5± 9.3
V	V	1+	29 Jul. '87	88	197.9±14.0
VI	VI	1+	10 May '88	222	166.0±11.1

* Different lot number represents the production from different parental individuals, and the same lot number represents the production from same parental individuals.

phosphogluconate dehydrogenase (6PGD), sorbitol dehydrogenase (SDH) and superoxide dismutase (SOD) were surveyed.

Nomenclature of alleles and loci followed Nakajima (8). Genetic distance was calculated from the formula proposed by Nei (9). A dendrogram was constructed by the unweighted pair group method using arithmetric average (UPGMA ; 10) based on genetic distance.

Results

1. *Genetic Differentiation among Localities in the Natural Pacific Herring*

The 18 isozymic loci were estimated from 11 enzymes. Of the 18 loci, the 4 loci, namely *Gpi*, *Idh-1*, *Pgm* and *6Pgd* were polymorphic where the frequency of the most common allele was no greater than 0.95. In order to certify whether genetic differences exist or not among lots collected over different years from the same locality, homogeneity tests for allele frequencies at the 4 polymorphic loci were performed in the three Miyako lots and in the three Mangoku-ura lots. As shown in Table 3, no significant differences were observed in either of the three lots within Miyako and Mangoku-ura. It indicates that the allelic compositions are stable for several years in the same location. Then, the allelic frequencies were calculated from the total specimens in Miyako and Mangoku-ura.

Allele frequencies at the 18 loci in the 9 localities are shown in Table 4. Of the 18 loci, four loci were polymorphic and 9 loci were monomorphic in the 9 localities. At *Adh*, polymorphism was observed in Bakkai and rare variation

TABLE 3 *Allele Frequencies at the Polymorphic Loci in the Three Lots Collected from Miyako and Mangoku-ura*

Locus	Allele	Miyako				Mangoku-ura			
		1	2	3	Total	1	2	3	Total
Gpi	N	40	68	60	168	109	57	55	221
	A	0.063	0.147	0.108	0.113	0.133	0.079	0.073	0.104
	B	0.937	0.853	0.892	0.887	0.867	0.921	0.927	0.894
Idh-1	N	40	54	60	154	105	51	55	211
	A	0.025	0.028	0.042	0.032	0.057	0.069	0.027	0.052
	B	0.925	0.879	0.866	0.887	0.876	0.862	0.891	0.877
	C	0.050	0.093	0.092	0.081	0.067	0.069	0.082	0.071
Pgm	N	40	68	60	168	109	53	55	217
	A	0.537	0.441	0.575	0.512	0.468	0.566	0.545	0.512
	B	0.438	0.515	0.392	0.452	0.504	0.434	0.436	0.470
	C	0.025	0.044	0.033	0.036	0.028	0.000	0.018	0.018
6Pgd	N	40	68	60	168	109	57	55	221
	A	0.150	0.103	0.058	0.098	0.170	0.149	0.091	0.145
	B	0.850	0.897	0.942	0.902	0.830	0.851	0.909	0.855

where the frequency (q) of the most common allele was $0.95<q<1.00$ was observed in Ohmu and Lake Furen. At *αGpd-1*, polymorphism was observed in Bakkai and Ohmu and rare variation was observed in Lake Furen. Rare variation was observed at *Ldh-1* in Bakkai, at *Sdh* in Lake Furen, Lake Obuchi and Shichigahama, and at *Sod* in Ohmu. Average heterozygosities ranged from 0.081 to 0.107 in Hokkaido and Lake Obuchi and from 0.060 to 0.077 in southern Tohoku and Lake Hiuma. It indicates that genetic variability is larger in the northern localities than in the southern localities.

In order to estimate a degree of genetic differentiation, Nei's genetic distance was calculated from allele frequencies at the 18 loci. The genetic distances among the 9 localities ranged from 0.00015 to 0.00819. Based on the genetic distances the dendrogram was drawn as shown in Fig. 2. The 9 localities were divided into two groups at the distance of 0.0050, one of which included Hokkaido and Lake Obuchi which are located in the northern part, and the other included southern Tohoku and Lake Hinuma which are located in the southern part.

Homogeneity tests were performed among localities at the 18 loci. Although a significant difference at all loci was not observed between Lake Furen and Lake Obuchi at the 5% level of significance and among the four localities in southern Tohoku (Miyako, Mangoku-ura, Haragama and Shichigahama) at the 10% level of significance, the other combinations showed a significant difference at least at one

TABLE 4 *Allele Frequencies at the 18 Loci in the 9 Localities of the Natural Pacific Herring*

Locus	Allele	Localities								
		Bakkai	Ohmu	Lake Furen	Lake Obuchi	Miyako	Mangoku -ura	Shichi -gahama	Hara -gama	Lake Hinuma
Aat-1	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat-2	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh	N	50	52	63	81	168	221	148	39	24
	A	0.060	0.010	0.008	0	0	0	0	0	0
	B	0.890	0.990	0.976	1.000	1.000	1.000	1.000	1.000	1.000
	C	0.050	0	0.016	0	0	0	0	0	0
αGpd-1	N	50	52	63	81	168	221	148	39	24
	A	0.120	0.163	0.008	0	0	0	0	0	0
	B	0.860	0.799	0.992	1.000	1.000	1.000	1.000	1.000	1.000
	C	0.020	0.038	0	0	0	0	0	0	0
αGpd-2	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi	N	50	52	63	81	168	221	145	39	24
	A	0.260	0.260	0.175	0.259	0.113	0.104	0.055	0.128	0.146
	B	0.740	0.740	0.825	0.741	0.887	0.896	0.945	0.872	0.854
Idh-1	N	50	52	63	80	154	211	147	39	23
	A	0.050	0.048	0.111	0.063	0.032	0.052	0.014	0.013	0.022
	B	0.790	0.856	0.778	0.824	0.887	0.877	0.935	0.923	0.891
	C	0.160	0.096	0.111	0.113	0.081	0.071	0.051	0.064	0.087
Idh-2	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-1	N	50	52	63	81	168	221	148	39	24
	A	0.010	0	0	0	0	0	0	0	0
	B	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-2	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-3	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-1	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	N	50	52	63	81	168	221	148	39	24
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm	N	50	52	63	81	168	217	144	39	24
	A	0.490	0.731	0.706	0.654	0.512	0.512	0.504	0.474	0.479
	B	0.510	0.269	0.294	0.346	0.452	0.470	0.465	0.488	0.500
	C	0	0	0	0	0.036	0.018	0.031	0.038	0.021

TABLE 4 *Continued*

Locus	Allele	Localities								
		Bakkai	Ohmu	Lake Furen	Lake Obuchi	Miyako	Mangoku -ura	Shichi -gahama	Hara -gama	Lake Hinuma
6Pgd	N	50	52	63	81	168	221	148	39	24
	A	0.130	0.125	0.175	0.191	0.098	0.145	0.172	0.103	0.292
	B	0.870	0.875	0.825	0.809	0.902	0.855	0.828	0.897	0.708
Sdh	N	50	52	63	81	168	221	148	39	24
	A	0	0	0.016	0	0	0	0.047	0	0
	B	1.000	1.000	0.984	0.981	1.000	1.000	0.953	1.000	1.000
	C	0	0	0	0.019	0	0	0	0	0
Sod	N	50	52	63	81	168	221	148	39	24
	A	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B	0	0.010	0	0	0	0	0	0	0
Av. He		0.107	0.090	0.081	0.083	0.062	0.065	0.063	0.060	0.077

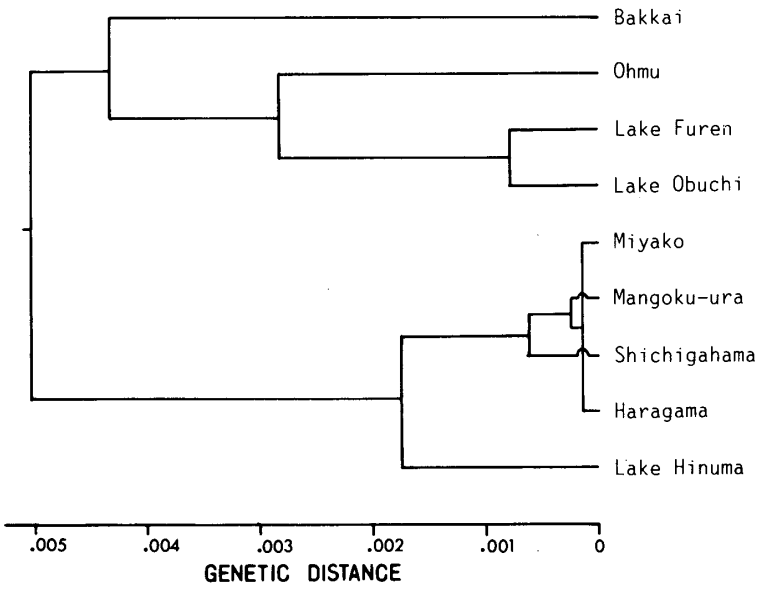

FIG. 2. Genetic relationship among 9 localities of the Pacific herring

locus. From the results of homogeneity tests and dendrogram, the 9 localities were divided into five local subpopulations.

2. Genetic Characterization of the Artificial Seeds Produced from the Natural Parents of Mangoku-ura

Allele frequencies at the 4 polymorphic loci, *Gpi*, *Idh-1*, *Pgm* and *6Pgd* were compared with the natural population of Mangoku-ura (Table 5). Homogeneity tests were performed among lots of the artificial seeds. Significant differences were observed among all the 6 lots at least at one locus and in all combinations

TABLE 5 *Phenotypic Distribution at the Four Polymorphic Loci in the Natural and Artificial Seeds of the Pacific Herring Surveyed in the Present Study*

Locus	Allele	Artificial seeds of mangoku-ura								Natural
		Released size				1+ year class				Mangoku-ura
		I-1	II	III	average	IV	V	VI	average	
Gpi		178	139	178		39	87	222		221
	q*A*	0.126	0.165	0.152	0.148	0.103	0.075	0.167	0.115	0.104
	q*B*	0.874	0.835	0.848	0.852	0.897	0.925	0.833	0.885	0.896
	Ho	0.208	0.317	0.292		0.205	0.149	0.270		0.199
	He	0.220	0.276	0.258		0.185	0.139	0.278		0.186
	(Ho-He)/He	−0.055	0.149	0.132*		0.108	0.072	−0.029		0.070
Idh-1		163	100	170		39	85	191		211
	q*B*	0.000	0.020	0.000	0.007	0.128	0.012	0.016	0.052	0.052
	q*C*	0.825	0.885	0.915	0.875	0.718	0.859	0.903	0.827	0.859
	q*D*	0.175	0.095	0.085	0.118	0.154	0.129	0.081	0.121	0.071
	Ho	0.337	0.210	0.159		0.564	0.282	0.178		0.237
	He	0.289	0.207	0.156		0.444	0.245	0.178		0.223
	(Ho-He)/He	0.166**	0.014	0.019		0.270	0.151	0.000		0.063
Pgm		170	137	177		39	87	218		217
	q*A*	0.600	0.627	0.520	0.582	0.526	0.603	0.583	0.571	0.512
	q*B*	0.350	0.318	0.452	0.373	0.436	0.368	0.383	0.396	0.470
	q*C*	0.050	0.055	0.028	0.045	0.038	0.029	0.034	0.033	0.018
	Ho	0.435	0.599	0.576		0.564	0.494	0.472		0.479
	He	0.515	0.503	0.525		0.532	0.500	0.512		0.517
	(Ho-He)/He	−0.155*	0.191*	0.097		0.060	−0.012	−0.078		−0.064
6Pgd		178	139	179		39	87	221		221
	q*A*	0.084	0.083	0.073	0.080	0.064	0.075	0.011	0.050	0.145
	q*B*	0.916	0.917	0.927	0.920	0.936	0.925	0.989	0.950	0.855
	Ho	0.146	0.165	0.145		0.128	0.126	0.023		0.244
	He	0.154	0.152	0.135		0.120	0.139	0.022		0.248
	(Ho-He)/He	−0.052	0.086	0.074		0.067	−0.094	0.045		−0.016

* and ** represent significant difference at the level of 0.05 and 0.01, respectively.

of each lot of the artificial seeds with the natural population. It indicated that each lot of the artificial seeds fluctuated in genetic composition and they differed from the natural parental population. However, the average allele frequencies of both released size lots and 1+ year class lots of the artificial seeds were similar to those of the natural population.

The deviation of the observed heterozygotes (Ho) from the expected heterozygotes (He) under Hardy-Weinberg equilibrium was measured as (Ho-He)/He. Significant deviations were observed at *Idh-1* (heterozygote excess) and *Pgm* (homozygote excess) in the Lot I-1, at *Pgm* (heterozygote excess) in the Lot II, and at *Gpi* (heterozygote excess) in the Lot III. On the other hand, no significant

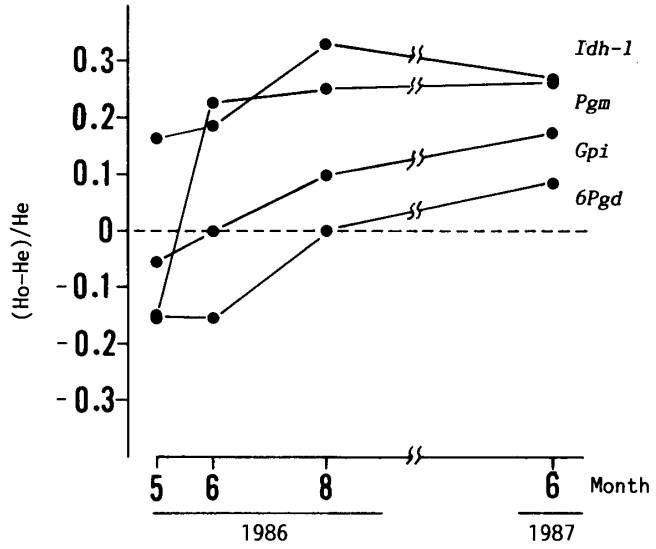

FIG. 3. Change of genetic composition at the four polymorphic loci during growth in the Lot I of the Pacific herring

deviation was observed at any loci in the three lots of 1+ year class. It suggests that the genetic composition was changed during rearing periods by reduction of a certain genotypic characteristic.

Change of genetic composition at the 4 polymorphic loci in the same lot during the rearing period is shown in Fig. 3. Homozygote excess was observed at the *Gpi*, *Pgm* and *6Pgd*, in the early period (May, 1986). After 1 month rearing (June, 1986), homozygote excess was not observed at *Gpi* and *Pgm*, and after 2 more months rearing (August, 1986), heterozygote excess was observed at the three loci. At *Idh-1*, significant heterozygote excess was observed in all periods and the level of heterozygote excess increased in proportion with the rearing periods. It indicates that the proportion of homozygotes decreased during rearing periods under the artificial rearing condition.

Discussion

The present results of heterozygosity and dendrogram showed that the Pacific herring around Japan was largely divided into two groups, one of which was located in the northern part and the other in the southern part. This suggests that the subpopulations have been constructed in a large area including several spawning localities. This suggestion is supported by the evdience of small genetic distances between Lake Furen and Lake Obuchi which are separated about 500 km and between Miyako and Haragama which are separated about 250 km. A possible explanation of genetic differentiation mentioned above is as follows; although the spawning area is isolated at the present time when the natural resources have decreased, the gene migration among spawning localities

had possibly occurred in the past period when the natural resources were large. Differentiation of the two types (oceanic and brackish) was reported based on ecological and genetical studies (1−3, 5). The present result shows that the brackish type is genetically separated from the oceanic type both in the northern and southern parts. Then, the brackish type is relic of the oceanic type in the state of large natural resources.

In comparison with the natural population, genetic fluctuation among produced lots in artificial seeds was reported in red seabream (11), Atlantic salmon (12) and masu salmon (13). The genetic fluctuation among artificial seeds was interpreted as genetic drift by a small number of parental fish.

Heterozygote excess has not been found in the natural population of the fish species including the Pacific herring (14), but has been reported in the artificial seeds of bustard halibut (15) and marbled sole (16). These reports suggest lower vaiability in homozygotes than in heterozygotes. This suggestion would be supported by the change from homozygote excess to heterozygote excess observed in the present study. Fujio and Brand (17) showed the genetic change from homozygote excess to heterozygote excess during the cultured period in the Japanese scallop population, and they suggested that the homozygotes at the isozymic loci are possibly correlated to lower viability, probably on the basis of the homozygosity of deleterious recessive alleles in the linkage with isozymic loci. Heterozygote excess observed in the present study might be similar to the above phenomenon. In conclusion, it suggests that the isozyme genes are possibly linked to deleterious recessive genes. However, a deeper study about linkage between them is required for future undertaking. At any rate, the isozyme genes are a good indicator of chrorosomes which are suitable for artificial seed production in the Pacific herring.

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