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Julie M. MACARANAS, Masamichi NAKAJIMA and Yoshihisa FUJIO

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

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Summary

Genetic differentiation among 10 cultured populations of Japanese char, *Salvelinus leucomaenis*, was examined based on allelic frequencies at 16 genetic loci. Genetic distance values and cluster analysis distinguish two population groups, indicating the probable origin of the hatchery stocks. Genetic variability was compared between groups and among other salmonid species. Moreover, the level of genetic differentiation in Japanese char was compared with those of other salmonid populations.

Japan is the southernmost habitat of the chars (1). Artificial propagation of Japanese chars has been successful in the *leucomaenis* ("amemasu" or "ezo-iwana") and *pluvius* ("nikko-iwana") forms of *Salvelinus leucomaenis*. Hatcheries have been established from which seed have been dispersed to other hatcheries and maintained as separate stocks.

As a biological resource, the Japanese char remains to be uncharacterized and the origin of the present hatchery stocks remain undocumented. Morphologically, chars are very plastic and color variants are common. As a result, numerous species of *Salvelinus* have been described, many of which are probably synonymous (2). Yoshiyasu (3) used hemoglobins to differentiate the Japanese chars inhabiting the southwestern part of Japan and observed a marked degree of uniformity in patterns that were different from "oshorokoma", *Salvelinus malma*, a species distributed in Hokkaido. Based on these results, he concluded that the Japanese chars are composed of only two species, *S. malma* and *S. leucomaenis*, the former consisting of 2 subspecies and the latter having several forms. It is apparent that morphological characters and structural proteins such as hemoglobin are not sufficient markers for differentiating taxonomic units below the species level. The application of isozyme markers for measuring genetic variability from allelic variants at distinct loci has proven to be more reliable in the delineation of population structure in different species. The existence of genetically distinct

populations has been documented in several salmonid species as revealed by biochemical markers (4, 5, 6, 7). Moreover, gene frequencies obtained from these biochemical markers permitted quantitative estimates of the magnitude and relative importance of genetic differences at various levels of organization in 4 salmonid species (8).

It is the purpose of this paper to present observed genetic differences in cultured populations of Japanese char (*Salvelinus leucomaenis*) and discuss findings in relation to their origin and population structure based on biochemical markers.

Materials and Methods

Gene frequency data at 16 detectable loci in 8 cultured Japanese char (*Salvelinus leucomaenis*) populations were taken from Fujio et al. (9). Two additional cultured populations were sampled and analyzed using the same procedure. The genetic interpretation of electrophoretic banding patterns were also described wherein gene duplication displaying a disomic mode of inheritance was observed in 16 isozyme systems.

Pair-wise genetic distance values (D) among the 10 cultured populations were calculated from Nei (10) and a dendrogram was constructed from the matrix of the distances using the UPGMA method of Sokal and Sneath (11). Average heterozygosity (H) was obtained from the heterozygosity per locus using the following formula: $h=1-\Sigma x_i^2$ where x_i denotes the frequency of the ith allele, and then averaging the values over all loci. The equation was applied to all loci except *Mdh B1, B2* which was considered as one tetrasomic locus in the calculations, thus, $h=1-\Sigma x_i^4$. The expressions at these two loci indicated patterns of duplicity at overlapping alleles, which was difficult to score as two disomic loci. The proportion of polymorphic loci (P) is the ratio of polymorphic loci over the total number of loci, where a locus was considered polymorphic if the frequency of the most common allele was no greater than 0.95. In the calculation of P, *Mdh B1, B2* was also considered as one locus.

Results

Table 1 presents the collection information for the 10 cultured char populations sampled. Results of the electrophoretic analyses at 16 loci are presented in Table 2 (a total of 24 isozyme systems coding for 40 loci were actually analyzed in the previous study (9), 16 of which were found to be duplicated). These were the loci consistently scored in all the populations. Null allele polymorphism at one locus of duplicate muscle-specific isocitrate dehydrogenase was observed in Miyagi-1, -2, and -3 hatchery populations. Out of the 16 loci analyzed, 11 were monomorphic. Gene frequencies at *α-Gpd-A1* and *Me-B* were not significantly different among the 10 hatchery populations. From gene frequencies at *Mdh B1,*

TABLE 1. *Collection Data on Japanese Char*

Cultured Population	Natural Origin*	Date	Number of Fish	Mean of Body Length (mm)
Kazuno	(?)	1980. 8/28	30	173.6
Iwate	Iwate (?)	1984. 8/25	31	132.9
Miyagi-1	Miyagi	1982. 7/26	42	151.1
Miyagi-2	Miyagi	1983. 5/31	54	244.4
Miyagi-3	Miyagi	1983. 5/31	91	149.6
Miyagi-4	Iwate	1984. 12/13	40	77.0
Fukushima-1	Tochigi	1980. 4/26	10	284.0
Fukushima-2	Fukushima	1980. 4/26	19	200.6
Fukushima-3	Iwate (?)	1980. 4/26	29	119.5
Nikko	Tochigi	1982. 8/ 2	30	196.0

* The founding populations are composed of native chars which were caught in the rivers located in the following prefectures.

B2, two groups were observed, one group with *B* as the major and *E* as the minor allele while the other group had *E* as the major and *B* as the minor allele. The other alleles at these loci were observed at relatively low frequencies.

Table 3 presents pair-wise genetic distance values for the 10 hatchery populations. A dendrogram constructed from this genetic distance matrix using the UPGMA method of Sokal and Sneath clearly illustrates two genetically distinct groups (see Fig. 1). A genetic distance value of 0.013 ± 0.0160 separate the two groups, which value falls within the range for local fish populations or races (12). Mean genetic distance values within groups were 0.0024 ± 0.0007 and 0.0016 ± 0.0004 for groups 1 and 2 respectively. Among the 10 hatchery populations, the minimum value of D was obtained between Miyagi-1 and -2 ($D = 0.0000$) while the maximum D was between Nikko (group 1) and Miyagi-1 (group 2) ($D = 0.0289$).

Measures of genetic variability within the populations could be gleaned from their proportion of polymorphic loci (P) and average heterozygosity (H) values (see Table 4). Between groups 1 and 2, the latter displayed greater genetic variability with mean values of $P = 0.093$ and $H = 0.062$ compared to 0.080 and 0.052 respectively for group 1. Fukushima-1 and Nikko hatchery populations displayed the least average heterozygosity values of 0.014 and 0.028 respectively.

Discussion

Assuming that the genetic change was stable throughout the process of population subdivision, the dendrogram obtained in this study could be regarded as an approximation of Japanese char phylogeny. On the other hand, if some of the split populations had undergone unstable genetic changes, the dendrogram presented here would show only the present status of genetic differentiation. The

TABLE 2. *Gene Frequencies of 10 Populations of Japanese Char*

Locus	Allele	Kazuno	Iwate	Miya-1	Miya-2	Miya-3	Miya-4	Fuku-1	Fuku-2	Fuku-3	Nikko
Mdh-B1	*A*	0	0	0	0	0	0	0	0.013	0	0
-B2	*B*	0.658	0.654	0.306	0.319	0.308	0.481	0.875	0.355	0.647	0.942
	C	0.042	0.016	0	0	0	0.044	0	0	0.078	0
	D	0.017	0	0	0	0	0	0	0.079	0	0
	E	0.283	0.339	0.694	0.681	0.692	0.475	0.125	0.553	0.275	0.058
Idh-A1	*A*	1.000	1.000	0.881	0.898	0.963	1.000	1.000	1.000	1.000	1.000
	O	0	0	0.119	0.102	0.037	0	0	0	0	0
αGpd-A1	*A*	1.000	1.000	0.976	1.000	0.995	1.000	1.000	1.000	1.000	1.000
	B	0	0	0.024	0	0.005	0	0	0	0	0
Me-B	*A*	0.967	0.984	1.000	1.000	0.995	1.000	1.000	1.000	1.000	1.000
	B	0.033	0.016	0	0	0.005	0	0	0	0	0
Adh-1		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh-2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
αGpd-A2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-A2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-A		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-A1		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-A2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-A1		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-A2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-B1		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-B2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

TABLE 3. *Matrix of Genetic Distances Among 10 Cultured Populations of Japanese Char*

	Kazuno	Iwate	Miya-1	Miya-2	Miya-3	Miya-4	Fuku-1	Fuku-2	Fuku-3	Nikko
Kazuno										
Iwate	0.0001									
Miyagi-1	0.0114	0.0096								
Miyagi-2	0.0103	0.0087	0.0000							
Miyagi-3	0.0102	0.0085	0.0004	0.0002						
Miyagi-4	0.0024	0.0017	0.0038	0.0031	0.0028					
Fukushima-1	0.0025	0.0032	0.0234	0.0220	0.0222	0.0095				
Fukushima-2	0.0059	0.0049	0.0020	0.0015	0.0010	0.0010	0.0157			
Fukushima-3	0.0001	0.0002	0.0114	0.0104	0.0103	0.0023	0.0027	0.0060		
Nikko	0.0045	0.0054	0.0289	0.0274	0.0277	0.0132	0.0002	0.0204	0.0046	

TABLE 4. *Genetic Variation in 10 Coltured Populations of Japanese Char*

		Proportion of Polymorphic Loci (P), 95% Criterion	Average Heterozygosity (H)
Group 1	Kazuno	0.067	0.058
	Iwate	0.067	0.056
	Fukushima-1	0.067	0.028
	Fukushima-3	0.067	0.055
	Nikko	0.067	0.014
	Mean	0.067	0.042
Group 2	Miyagi-1	0.133	0.068
	Miyagi-2	0.133	0.064
	Miyagi-3	0.067	0.057
	Miyagi-4	0.067	0.060
	Fukushima-2	0.067	0.059
	Mean	0.093	0.062
	Overall Mean	0.080	0.052

unstable genetic change can be brought about by founder effect, by the operation of some selective forces, or by bottleneck effect.

The dendrogram suggests that at least two groups of different natural origin were the sources of the hatchery populations. This grouping also coincides with the fact that two forms of *Salvelinus leucomaenis*, namely, *S. leucomaenis* and *S. leucomaenis pluvius* are presently the only successfully employed chars for artificial propagation in Japan. The Miyagi-1, -2, and -3 hatchery populations were derived from the same founder stock, the natural origin of which was claimed to be the rivers in the Kurikoma area of Miyagi prefecture. This char is probably

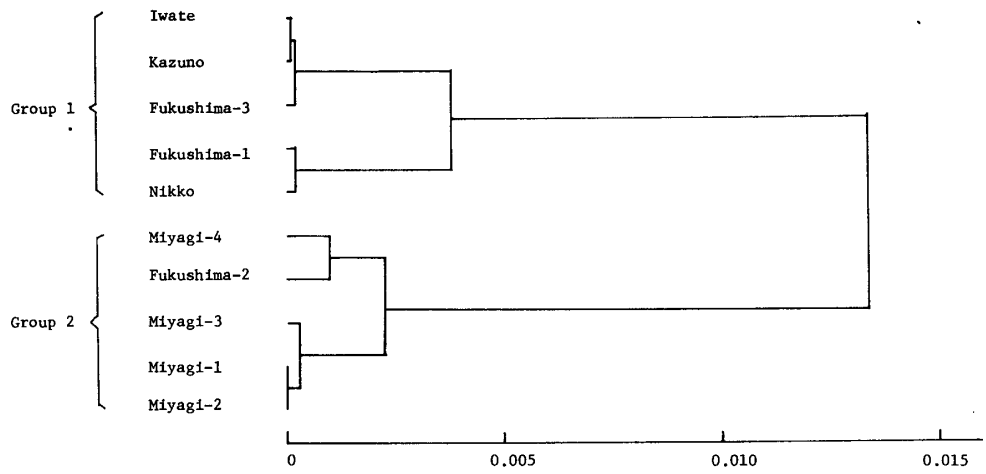

FIG. 1. Dendrogram (UPGMA, Sokal and Sneath 1963) constructed from genetic distance (D) values of Table 3.

the *S. leucomaenis* ("ezo-iwana") form. Fukushima-1 and Nikko hatchery populations were also derived from the same founder stock, the natural origin of which was claimed to be the rivers in Tochigi; this char is presumably the *S. leucomaenis pluvius* ("nikko-iwana") form. Note that in the dendrogram, the Miyagi-1, -2, and -3 populations form a cluster, and so do the Fukushima and Nikko populations. This supports the claim that these population groups were derived from common stocks. The Kazuno, Iwate, and Fukushima-3 populations also form a cluster, so do Miyagi-4 and Fukushima-2; whether they come from common founder stocks is not certain, as reflected in the collection data in Table 1. Whether hybridization between the 2 char forms has occurred in some of the hatcheries could not also be ascertained from the results of the study because no comparable data on the natural populations of each form are presently available.

The importance of using adequate numbers of parents in hatchery rearing has always been emphasized but not always followed. Higher polymorphism and heterozygosity in group 2 populations may be indicative of a higher number of originally introduced parents, resulting in the effective sampling of representative alleles of the natural population. The low heterozygosities in Fukushima-1 and Nikko populations, on the other hand, may be indicative of bottlenecking, one effect of utilizing a small effective number of parents.

In comparison with other salmonid species, genetic variation within the cultured Japanese char populations included in this study is higher than in natural populations of Arctic char ($H=0.007$), Atlantic salmon ($H=0.026$), and brown trout ($H=0.025$), well within the range observed in other salmonid species, i.e., sockeye salmon ($H=0.044$) and rainbow trout ($H=0.058$), but lower than in chum salmon ($H=0.097$) (7, 8, 13).

The results were obtained from a small number of individuals per hatchery

TABLE 5. *Genetic Differentiation Among Populations of Salmonid Species*

Genus	Species (Origin)	Number of Populations	Number of Loci	Genetic Distance (D)* Mean (Range)	Reference
Salvelinus	Char (Japan)	10	16	0.0081 (0.0000−0.0289)	Present study
	Arctic Char (Scandianvia)	9	37	0.0075 (0.0000−0.0136)	Ryman and Stahl, 1981 (7)
	Arctic Char (Northeastern America)	5	25-26	0.0359 (0.0010−0.0780)	Kornfield et al., 1981 (14)
Salmo	Cutthroat trout (Wyoming)	7	23	0.0024 (0.0000−0.0060)	Loudenslager and Kitchin, 1979 (15)
	Atlantic Salmon (Northern Sweden)	6	45	0.0036 (0.0000−0.0084)	Ryman and Stahl, 1981 (7)
	Brown trout (Sweden)	38	35	0.0155 (0.0000−0.0783)	Ryman, 1983 (8)
	Steelhead trout (North America)	5	37	0.0033 (0.0005−0.0082)	Okazaki, 1984 (16)
Oncorhynchus	Chum Salmon (Japan)	37	16	0.0040 (0.0003−0.0214)	Kijima, 1980 (17)
	Chum Salmon (Southern British Columbia)	29	10	0.0030 (0.0002−0.0128)	Beacham et al, 1985 (18)
	Sockeye Salmon (Alaska)	7	26	0.0103 (0.0000−0.0286)	Wilmot and Burger, 1985 (19)

* Range and mean values for Nei's genetic distance (D) were directly calculated from the gene frequency data of the references cited, except for cutthroat trout which were only converted from genetic identity values. When only the gene frequencies of allele 100 were mentioned, it wes assumed that only two alleles for the locus were observed.

stock as well as from a few hatchery populations. However, the clarity of diagnosis of two different population groups based on the *Mdh B1, B2* loci could not be ignored. Genetically distinct populations within other salmonid species have been similarly based on one or two diagnostic loci, i.e. transferrin and esterase in char (*Salvelinus alpinus L.*) inhabiting 4 Welsh lakes (5), lactate dehydrogenase in Scandinavian brown trout (6), and serum proteins and esterases in Atlantic salmon (4).

A measure of genetic differentiation within species populations can be estimated from the gene frequencies at several enzyme loci as proposed by Nei (10). A summary of the degree of genetic differentiation within salmonid species is given in Table 5, which includes the present study. Among the chars, isolated populations of land-locked Arctic char (*Salvelinus alpinus*) from eastern United States and Canada display the greatest degree of genetic differentiation ($D_{mean}=0.0359$). Scandinavian Arctic char and Japanese chars are characterized by the same degree of genetic differentiation ($D_{mean}=0.0075$ and 0.0081 respectively). Japanese char, *S. leucomaenis*, is characterized by at least two population groups; similarly, Scandinavian Arctic char is said to be made up of at least three sibling species that evolved allopatrically during the last period of glaciation. Among the other salmonids, the brown trout displays the highest degree of genetic differentiation on a microgeographic scale ($D_{mean}=0.0155$) which was no correlated with geographic distance. In contrast, the cutthroat trout ($D_{mean}=0.0024$), Atlantic salmon ($D_{mean}=0.0036$), and steelhead trout ($D_{mean}=0.0033$) display a narrow range of gene differentiation. Japanese chum salmon, chum salmon from Southern British Columbia, and sockeye salmon are characterized by levels of genetic differentiation comparable with Japanese and Arctic chars. From the data presented here, there is an observed tendency for land-locked species populations such as those of brown trout and chars to display higher levels of genetic differentiation than the open-sea populations such as those of steelhead trout, Atlantic salmon, and chum salmon. This may be correlated with the rate of gene flow, which is expected to be higher in open-sea populations.

It is notable that biochemical tags or isozyme labels are so far the most powerful tools in the delineation of population structure. Because of the relatively recent developments in char culture compared to other salmonid species in Japan, hatchery practices including knowledge of the available genetic resources are not well-documented. One thing, however, is clear-that there are at least two population groups of Japanese chars which can be identified from biochemical markers. A comparative analysis of natural populations of Japanese char will not only define the population structure of these fishes but will also determine the genetic changes that may possibly occur with domestication.

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