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# Genetic Variations of Natural and Hatchery Populations of Korean Ayu (*Plecoglossus altivelis*) by Isozyme Markers

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Genetic variability and population structure of 11 natural ayu, *Plecoglossus altivelis* populations and one hatchery stock were assessed by starch gel electrophoretic analysis with 10 enzyme coding loci. Three loci were polymorphic (lower than 0.95 in major allele frequency) in natural populations, 2 loci in hatchery stock. The average number of alleles per locus was 1.38. Observed heterozygosities ranged from 0.0235 to 0.088 (0.055 on the average) in natural population while 0.0925 in hatchery stock. The genetic distance among natural populations measured 0.000047~0.005407 and no significant differentiation was observed among them. On the other hand, a significant genetic distance was found between natural populations and the hatchery stock with measuring 0.002032~0.008605. The results in this study suggest that the hatchery stock has diverged from natural populations, and also that careful to maintain sustainable and effective population size (parents number) should be made.

**Keywords:** Ayu, Isozyme, Population genetic, Genetic diversity, Genetic variation

## Introduction

Ayu (*Plecoglossus altivelis*) is native species to Northeast Asia, i.e., the Japanese Archipelago and the Korean Peninsula, and is also found in China (Seki et al., 1994), normally migrating amphidromously between rivers and the sea. Two ecological forms of ayu, amphidromous and landlocked exist in Japan whereas there are only amphidromous form in Korea. They are distributed ubiquitously from Gosung in the Gangwon province to Oido and the Gangjung River in the Jeju province in Korea. The ayu is amphidromous fish and are vital to the inland fisheries and aquaculture in the eastern and southern regions of Korea. This species also plays on essential role as a primary consumer in the river ecosystem of these areas. Recently, the number of ayu returning from the sea has been reduced possibly due to environmental pollution of the rivers caused by the industrial development. Such a problematic situation makes it urgent that the biological and genetic characteristics of ayu populations should be evaluated for maintaining genetic diversity of native ayu.

In the last few decades, over one million ayu larvae produced in the national hatchery have been released annually into the rivers along the east and south coasts of Korea with the purpose of enhancing the natural resources of this species. However, releasing hatchery stock into the natural waters may offer a possibility to alter the genetic properties of native stock. Genetic changes associated with artificial seed production may result from unconscious selection in artificial environment, genetic drift, foundation effect and inbreeding in small population (Taniguchi, 1986). Accordingly, we must take attention with genetic changes of genetic properties and characteristics of natural population from those of hatchery stock for the managing the genetic resources of the ayu. However, there are not so many researches on the genetic characteristics of hatchery stock, their effect on the native stock and the effectiveness of releasing hatchery stock into the natural waters.

So far, isozymes have been widely used as markers in the study of population genetics of some animal species. Taniguchi et al. (1983) likewise studied genetic variability and differentiation among amphidromous, landlocked and hatchery populations of ayu in Japan using isozymes. Seki and Taniguchi (1985) studied genetic divergence among the amphidromous populations of ayu in southwestern Japan using isozymes, and Nishida (1985) studied the genetic divergence of amphidromous ayu populations around Japan Archipelago and Ryukyu Island. Seki et al. (1988) and Sawashi et al. (1998) also stud-

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ied the genetic divergence between Korean and Japanese ayu populations using isozymes. They studied only four populations along the east coast (Seki et al., 1988) and Jeju Island's ayu (Sawashi et al., 1998) in Korea, respectively.

This study was aimed (1) to investigate the genetic structure of the natural populations of Korean ayu, (2) to evaluate genetic variability and divergence among these populations, (3) to compare the genetic characteristics of the hatchery stock with those of the natural populations in the rivers into which hatchery stock had been released.

## Materials and Methods

### Fish samples

A total of 550 individuals of ayu were collected from 11 rivers (Fig. 1) during the periods June to August 1999 and July to August 2000. Forty individuals of hatchery stock were also obtained from Uljin Marine Hatchery (in Fig. 1) in 1999. General information on the samples including number of specimen and fish sizes are shown in Table 1. Sampling sites ranged from Gosung, Gangwon province to Gwangyang, Jeonnam province. Two consecutive samples were collected from the Namdae River and the Wangpi River, as the inland fisheries resources were caught continuously for two years (1999 and 2000). Samples of the years 1999 and 2000 were named as A and B, respectively. Wild fish were caught at a single location within a few days using a pot, frozen with dry ice and stored under -20°C until use.

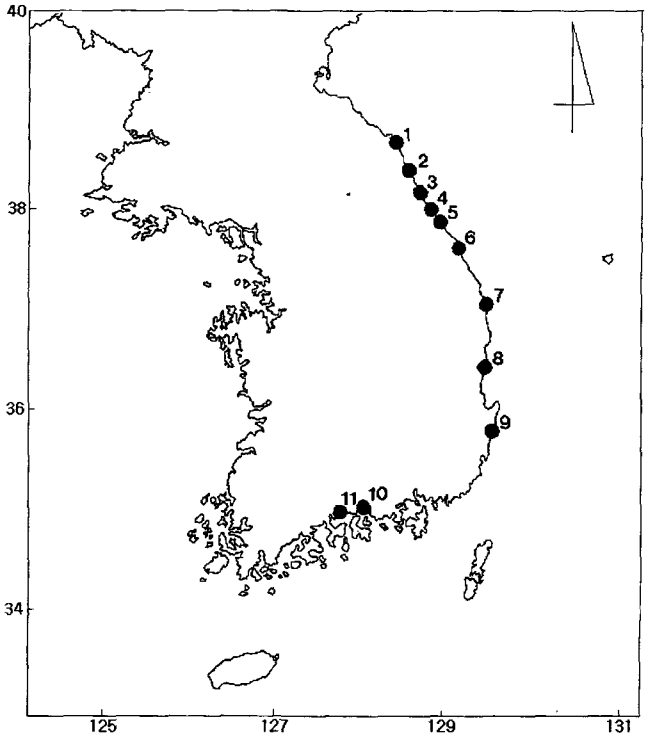


**Fig. 1.** Sampling sites of ayu specimen used in this study. Location numbers correspond to those in Table 1.

### Electrophoresis

Horizontal starch-gel electrophoresis was used for isozyme analysis. The electrophoretic technique, staining procedure, interpretation of electrophoretic banding patterns and identification of loci were based on the methods described by Seki et al. (1988) and Shaklee et al. (1990). The enzymes and proteins examined in this study were: Glucosphate isomerase (GPI, E. C. 5.3.1.9), 6-Phosphogluconate dehydrogenase (PGDH, E. C. 1.1.1.44), Phosphoglucomutase (PGM, E. C. 2.7.5.1), Malate dehydrogenase (MDH, 1.1.1.37), Malic enzyme (ME, E.C. 1.1.1.40) and Mannosephosphate isomerase (MPI, E.C. 5.3.1.8).

### Data analysis

The mean heterozygosity values and their standard errors were calculated according to Nei's formula (1978). The distribution of genetic variation and the genetic distance (D) among populations were analyzed by the method with Nei (1972). A dendrogram from the matrix of genetic distances was constructed using UPGMA (Sneath & Sokal, 1973). PHYLIP V3.5 software (Felsenstein, 1993) was used for bootstrapping, genetic distance estimation and dendrogram construction. Bootstrapped allelic frequencies (1,000 times) were used for the estimation of Nei's genetic distance (SEQBOOT and GENDIST). With these data, 1,000 dendrograms were generated using the Neighbor-Joining and UPGMA methods (NEIGHBOR). A consensus dendrogram (CONSENSE) was then constructed to estimate the confidence level of the obtained tree.

## Results

In the starch-gel electrophoresis, 6 enzymes coded by 10 loci were clearly resolved in all the ayu samples. The resolved locus with allelic distribution and frequencies of each population are shown in Table 2.

### Genetic variation of Korea population

Eighteen alleles in total were identified in 10 loci, and 12 to 15 alleles were identified in the natural populations. The

**Table 1.** Sampling sites, number of specimen and fish size in this study

| Location | No. of fish | Folk length (mm) | Body weight (g) |
|---|---|---|---|
| ① Myoungpa R. | 61 | 98.74±11.06 | 4.83±1.19 |
| ② Puk R. | 50 | 116.65±7.70 | 9.26±2.24 |
| ③ (A) Namdae R.(A) | 40 | 129.8±12.88 | 25.3±9.69 |
| ③ (B) Namdae R.(B) | 51 | 101.57±9.64 | 5.83±1.88 |
| ④ Youngok R. | 32 | 128.55±15.47 | 14.32±5.34 |
| ⑤ Nakpoong R. | 22 | 108.87±12.88 | 10.32±3.83 |
| ⑥ Kagok R. | 39 | 110.23±11.85 | 9.79±3.68 |
| ⑦ (A) Wangpi R. | 37 | 135.54±11.49 | 17.01±4.78 |
| ⑦ (B) Wangpi R. | 51 | 112.14±8.63 | 9.85±2.59 |
| ⑧ Osib R. | 52 | 143.06±11.73 | 22.26±6.26 |
| ⑨ Daejong R. | 37 | 155.0±10.81 | 57.8±12.62 |
| ⑩ Jook R. | 37 | 128.71±10.22 | 23.48±5.78 |
| ⑪ Seomjin R. | 40 | 167.1±9.47 | 63.2±9.50 |
| ⑫ Hatchery | 40 | 137.72±8.75 | 20.75±8.72 |
| **Total : 14** | 590 | | |

**Table 2.** Allelic frequencies and genetic variability for 10 loci in 14 population samples of ayu collected from Korea

| Locus | Allele | ① | ② | ③(A) | ③(B) | ④ | ⑤ | ⑥ | ⑦(A) | ⑦(B) | ⑧ | ⑨ | ⑩ | ⑪ | ⑫ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GPI-1* | *a | 0.8 | 0.71 | 0.8 | 0.706 | 0.812 | 0.727 | 0.756 | 0.716 | 0.853 | 0.625 | 0.73 | 0.743 | 0.788 | 0.6 |
| | *b | 0.2 | 0.29 | 0.2 | 0.294 | 0.188 | 0.273 | 0.244 | 0.284 | 0.147 | 0.375 | 0.27 | 0.257 | 0.212 | 0.4 |
| GPI-2* | *a | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MDH-1* | *a | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MDH-2* | *a | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MDH-3* | *a | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ME-1* | *a | 0.94 | 0.97 | 0.975 | 0.98 | 0.984 | 0.954 | 0.974 | 0.946 | 0.99 | 1 | 0.946 | 0.932 | 0.975 | 1 |
| | *b | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.041 | 0.00 | 0.00 |
| | *c | 0.06 | 0.02 | 0.025 | 0.02 | 0.016 | 0.046 | 0.026 | 0.054 | 0.01 | 0.00 | 0.054 | 0.027 | 0.025 | 0.00 |
| ME-2* | *a | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MPI* | *a | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.027 | 0.00 | 0.00 | 0.00 |
| | *b | 0.98 | 0.96 | 0.987 | 0.99 | 0.937 | 1 | 0.987 | 0.946 | 0.98 | 0.99 | 0.932 | 0.973 | 1 | 1 |
| | *c | 0.02 | 0.04 | 0.013 | 0.01 | 0.063 | 0.00 | 0.013 | 0.054 | 0.01 | 0.01 | 0.041 | 0.027 | 0.00 | 0.00 |
| PGDH* | *a | 0.00 | 0.00 | 0.012 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | *b | 0.99 | 0.94 | 0.975 | 0.98 | 0.984 | 0.91 | 0.974 | 0.987 | 1 | 1 | 1 | 0.973 | 0.975 | 0.862 |
| | *c | 0.01 | 0.06 | 0.013 | 0.02 | 0.016 | 0.09 | 0.026 | 0.013 | 0.00 | 0.00 | 0.00 | 0.027 | 0.025 | 0.138 |
| PGM* | *a | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | *b | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.014 | 0.00 | 0.00 | 0.00 |
| No. of allele | | 14 | 15 | 15 | 14 | 14 | 13 | 14 | 14 | 14 | 12 | 15 | 15 | 13 | 12 |
| Ho | | 0.054 | 0.088 | 0.035 | 0.051 | 0.0375 | 0.0645 | 0.0513 | 0.068 | 0.0235 | 0.0615 | 0.0757 | 0.054 | 0.0525 | 0.0925 |
| He | | 0.0492 | 0.068 | 0.0444 | 0.0494 | 0.0487 | 0.0649 | 0.0496 | 0.0637 | 0.031 | 0.0489 | 0.0653 | 0.0616 | 0.0432 | 0.0718 |
| Ho/He | | 1.098 | 1.294 | 0.789 | 1.033 | 0.771 | 0.995 | 1.035 | 1.068 | 0.758 | 1.259 | 1.159 | 0.877 | 1.216 | 1.288 |

average allele number is 1.4 per locus in the natural populations and an average of 1.2 alleles was identified in the hatchery stock.

Four out of the 10 detected loci; GPI-1, ME-1, MPI and PGDH were polymorphic. Genetic variability was very low except in GPI-1 where the allele *b dominated. The hatchery stock showed an especially high GPI-1 *b frequency (0.6) and an excess of heterozygotes was observed at GPI-1. Three alleles have been observed in ME-1 but most populations in this study had only two alleles, except for the samples from the Jook River. The hatchery stock was monomorphic at ME-1. In PGDH, 3 alleles were observed, but variability was low. The hatchery stock showed the highest frequency for the *c allele (0.138) among all samples.

The observed values of heterozygosity (Ho) in the natural populations, estimated as an observed number of heterozy-

gous phenotypes per locus in a given individual ranged from 0.0235 to 0.088, and mean heterozygosity was 0.055. The hatchery stock had a higher heterozygosity (0.092) than the natural populations, even though only two loci were variable. The ratio of the observed heterozygosity values to the expected ones in each population (Ho/He) was more than 1 in the populations from the Myoungpa River, the Osib River, the Daejong River, and the Seomjin River, suggesting large gene flow and interbreeding among the different river populations.

There was no clear difference in those values among the samples, except that the ratio of Ho/He were less than 1 in the samples from the Namdae River (A), the Youngok River, the Wangpi River (B) and the Jook River, suggesting simple mixing or inbreeding among the different river populations.

Reduction of genetic variability was found in the hatchery stock. The average rate of reduction was 44.4% of the polymorphic loci and 14.0% of the alleles per locus. However, the hatchery stock showed 40.5% higher observed heterozygosity (Ho) value caused by heterozygote excess in GPI-1. The hatchery stock showed a high Ho/He value of 1.288.

**Genetic divergence**

Nei's genetic distance is shown in Table 3. The populations from the Namdae River (3A) and the Seomjin River (11) had the least genetic distance among the natural populations with D value of 0.000047. The Wangpi River (7B) and the Osib River (8) had the greatest genetic distance of 0.005407. In the Puk River and the Myoungpa River, which are the nothernmost ones and located in the same water system, D was 0.001266. The Jook River and the Seomjin River, the southernmost rivers and located in the same water system, showed a genetic distance of 0.00043.

The 2-consecutive year study of the Namdae River and the Wangpi River showed that the genetic distance between samples of 1999 (A) and 2000 (B) were 0.00096 and 0.002215, respectively. This indicated that the genetic structure in the same river was not altered by the change of generations. The genetic distance for the adjacent Wangpi River and Osib River was 0.003395. However, the D value between the northernmost Puk River, and the southernmost Seomjin River showed a lower value of 0.000205. The genetic distance between the hatchery stock and the natural populations was 0.002032 to 0.008605, which indicated a significant genetic difference.

To summarize the genetic differentiation among the samples, a dendrogram was drawn based on the matrix genetic distances of Table 2. The D values were also calculated among the rivers by pooling the genotype frequencies of the samples collected in each population : The Korean ayu population clustered in 3 groups (Fig. 2). The populations from the Namdae River and the Seomjin River, which are located in the northernmost and the southernmost, respectively, were genetically indistinguishable from each other, despite geographical distance between each other. However, the populations from Jook River and the Seomjin River, though sharing similar habitats, proven to be genetically different each other. The Osib River samples were different from the other populations, while the closest to the hatchery stock with very little genetic difference. The samples of 1999 and 2000 from the Namdae River and the Wangpi River belonged to different groups.

The bootstrapped dendrogram is shown in Fig. 3. There was little discrepancy in the dendrogram between the bootstrap method and UPGMA cluster. The dendrogram by the bootstrapping also showed 3 groups. However, the sample

**Table 3.** Estimates of Nei's genetic distance between pairs of 14 populations of ayu *P. altivelis* based on allelic frequiencies at 10 loci

| Population | ① | ② | ③(A) | ③(B) | ④ | ⑤ | ⑥ | ⑦(A) | ⑦(B) | ⑧ | ⑨ | ⑩ | ⑪ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ② | 0.001266 | | | | | | | | | | | | |
| ③(A) | 0.000151 | 0.001067 | | | | | | | | | | | |
| ③(B) | 0.001215 | 0.00026 | 0.000961 | | | | | | | | | | |
| ④ | 0.000417 | 0.001341 | 0.000296 | 0.001485 | | | | | | | | | |
| ⑤ | 0.001274 | 0.000354 | 0.001116 | 0.000683 | 0.001829 | | | | | | | | |
| ⑥ | 0.000359 | 0.000396 | 0.000218 | 0.000399 | 0.000614 | 0.000553 | | | | | | | |
| ⑦(A) | 0.000847 | 0.00036 | 0.000978 | 0.000399 | 0.001111 | 0.000968 | 0.000424 | | | | | | |
| ⑦(B) | 0.000529 | 0.002467 | 0.000349 | 0.00226 | 0.000411 | 0.002532 | 0.00104 | 0.002215 | | | | | |
| ⑧ | 0.003627 | 0.001278 | 0.003332 | 0.000744 | 0.004034 | 0.002163 | 0.001952 | 0.001382 | 0.005407 | | | | |
| ⑨ | 0.000704 | 0.000629 | 0.000859 | 0.000563 | 0.000944 | 0.001274 | 0.000457 | 0.00012 | 0.001841 | 0.001732 | | | |
| ⑩ | 0.000508 | 0.000379 | 0.000527 | 0.000442 | 0.00087 | 0.000663 | 0.000203 | 0.000316 | 0.00152 | 0.001933 | 0.000393 | | |
| ⑪ | 0.000205 | 0.000878 | 0.000047 | 0.000737 | 0.00049 | 0.000822 | 0.000121 | 0.000902 | 0.00054 | 0.002928 | 0.000836 | 0.00043 | |
| ⑫ | 0.006375 | 0.002197 | 0.005758 | 0.002632 | 0.006763 | 0.002201 | 0.003955 | 0.003744 | 0.008605 | 0.002032 | 0.004575 | 0.003958 | 0.005069 |

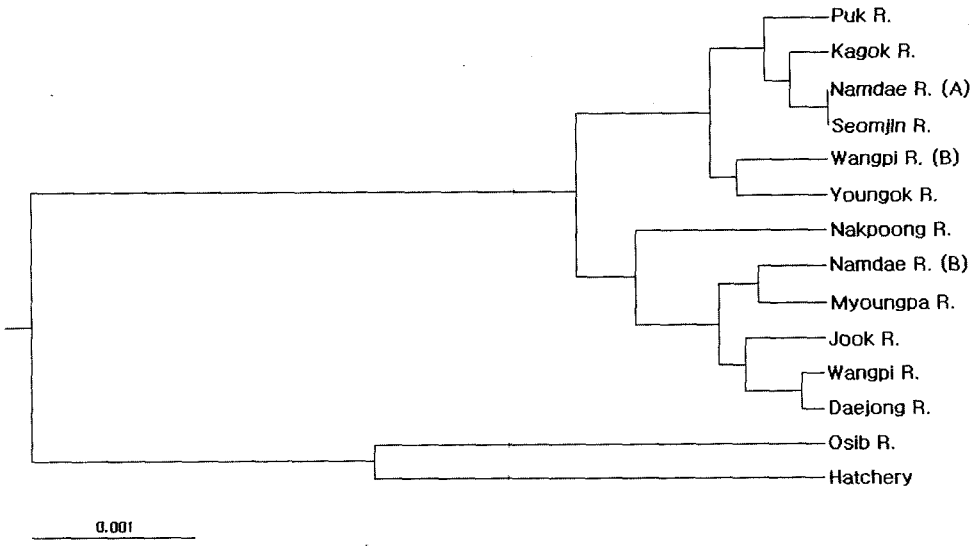


**Fig. 2.** Dendrogram based on genetic distance among populations of *Plecoglossus altivelis*. Information on the samples from each river population can be referred to Table 1.

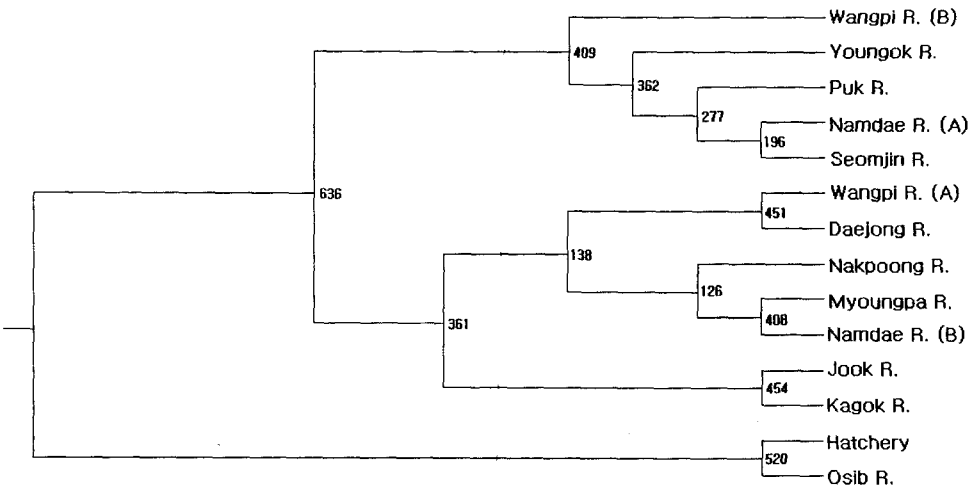


**Fig. 3.** UPGMA dendrogram based on Nei's genetic distance between pairs of 14 populations by isozyme. Values on the nodes represent the percentage of time the location at the right of the fork occured in 1,000 dendrograms generated from genetic distances calculated by bootstrapping gene frequencies.

form the Gagok River belonged to different group compared to the dendrogram in Fig. 2.

The samples from the Namdae River (A) and the Seomjin River appeared to have very close genetic properties when assessed by significance test using bootstrap method, suggesting the probability that both of the real populations would be similar to the observed ones. The dendrogram by the bootstrap method also showed a high probability (55%) that the real genetic relationship would be similar to the observed genetic relationship, indicating the significance of the observed data. Because higher bootstrap probabilities were obtained with UPGMA dendrograms, only these could be used for genetic structure analysis.

## Discussion

In this study, the allelic mean of Korean ayu was 1.4 and the heterozygosity revealed the genetic variability ranged from 0.0235 to 0.088 (mean 0.055). Populations of the Namdae River and the Seomjin River (D=0.000047), displayed the closest genetic distance, although these rivers are geographically very distant from each other. The genetic distance between the populations of the Wangpi River and the Osib River (D=0.005407) was the farthest, though they are

geographically very close to each other. In addition, the populations in the south coast of Korea, which hatched at the same time, lived almost in the same area until the juvenile stage, and finally migrated upstream in the Jook River in Sacheon and the Seomjin River in Gurye had a D value of 0.00043, suggesting both were very close to each other. These results indicate that there is no significant relationship between genetic and geographical distance, and also that Korean ayu would be same stock or the same population having same genetic structure without any significant genetic divergence. Furthermore, no divergence in each population could be detected using the isozyme markers possibly because of a short divergence period and/or limited number of isozymes tested. To resolve the genetic population structure of ayu in more detail, a more sensitive genetic analysis would be required.

Assuming that the electrophoretic loci studied here are not under the influence of selective forces, the observation of a very small amount of genetic difference among the populations could be explained by two possible models. First, the population of ayu throughout the Korean Peninsula could be a single panmictic population. Secondly, a high rate of migration (gene flow) among natural populations during sea life is sufficient to prevent a genetic differentiation. A high rate of gene flow was also observed in several other marine fishes such as Pacific herring (Grant, 1984), Atlantic herring (Ryman et al., 1984), Walleye pollock (Grant and Utter, 1980) and Pacific halibut (Grant et al., 1984), whose large populations coupled with a high rate of gene flow have prevented substantial genetic divergence among populations over wide geographic areas.

Seki et al. (1988) reported the D values of individual amphidromous ayu populations in the Japan Archipelago ranging from 0.0001~0.0012 (mean 0.0005) and those of individual landlocked ayu populations in Lake Biwa ranging from 0.0003~0.0006 (mean 0.0005). The D values of 4 populations in Korea also have been reported ranging from 0.0001~0.0005 (mean 0.0005), which are similar to the results based on this study. Thus a great genetic divergence in Korean natural ayu was not observed. The amphidromous ayu of Korea and Japan were also compared by Seki et al. (1988). The D values were 0.0007~0.0027 (mean 0.0012) indicating a genetic divergence is gradually in progress in both geographically distant populations. The D values of 0.0160~0.0273 (mean 0.0236) between Korean ayu and landlocked ayu in Lake Biwa of Japan were also reported. The D values between amphidromous ayu and landlocked ayu in Lake Biwa were 0.0132~0.0215 (mean 0.0615), and those between Korean ayu and Ryukyu ayu of Japan were 0.2649~0.2690 (mean 0.2670). The D values between amphidromous ayu in Japan Archipelago and Ryukyu ayu was 0.2689~0.2754 (mean 0.2734), suggesting that these 2 populations were divided into 2 subspecies.

Recently, artificial seeds of more than one million ayu larvae have been released annually, mainly into the Wangpi River and the Osib River, which consequently causes concern about the possible effect of hatchery stock on native stock in a harmful manner. In this study, the hatchery stock showed D values of 0.002032~0.008605 which were different from those between natural populations. The genetic similarity between hatchery and Osib River sample is due to the fact that ayu individuals collected in the Osib River were used as parental brood fish to produce artificial seed in the hatchery. This study also suggests that Osib River population was genetically altered by the effect of released hatchery stock. However, more research is required to prove this assumption and to set up appropriate countermoves.

In the present study, reduction of genetic variability was found in the hatchery stock. The ratio of reduction was 44.4% of the polymorphic loci and 14.0% of the alleles per locus. Similar finding has been made in cutthroat trout, 57% reduction in the polymorphic loci, a 29% reduction in the allelic mean per locus, and a 21% reduction in the average heterozygosity per individual (Leary et al., 1985). Reduction of genetic variation was also found in a hatchery population of the sea breams. The ratio of reduction was 40.4% of the polymorphic loci, 12.2% of the alleles per locus and 18.0% of the observed heterozygosity (Sugama et al., 1988). A limited number of founders and the effects of genetic drift on the maintenance of the hatchery stock may attribute to such reduction of genetic variation. The variation and fixation of allelic frequencies were well explained with genetic drift and the bottle neck effect, which often occurs when the number of parents is small, as in the cases of red sea bream and black sea bream (Taniguchi and Okada, 1980; Taniguchi et al., 1983). The same phenomenon has already been observed in ayu (Taniguchi et al., 1983). However, it is not likely that genetic drift may be main factor responsible for such phenomenon because the number of parents for ayu production in hatchery is much larger than that for marine fishes.

The decrease of genetic variation in artificial seed conversely the increase of an inbreeding coefficiency may cause

a reduced adaptability of the population especially when they are released into natural waters. Accordingly, it may be suggested that preventing various genetic alterations, especially a decline in the adaptability of a hatchery stock, and protecting the wild populations from the effect of artificial manipulation is a task of paramount importance for breeding programs.

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