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Population Genetic Structure of Japanese Anchovy (*Engraulis japonicus*) in Korean waters Based on Mitochondrial 12S Ribosomal RNA Gene Sequences

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We used portions of mitochondrial 12S ribosomal RNA gene sequences (339 bp) to investigate the phylogenetic and population genetic characteristics of the Japanese anchovy, *Engraulis japonicus*, in Korean waters. A total of 35 mtDNA haplotypes were obtained from the samples collected in 3 locations (the southern area of the Yellow Sea, the western coast of Jejudo, and the eastern area of the South Sea) in Korean waters. One haplotype, AN8T103, obtained from the southern area of the Yellow Sea, was formed according to an independent phylogenetic individual in the PAUP analysis, which was separated from the others by a 0.2-4.1% sequence divergence. This distinct haplotype appeared to be one that was carried by immigrants from another study area, but further study is necessary. Genetic divergence, except for AN8T103, was moderate to substantial (0.2-3.8%) and nucleotide diversity within populations was 0.015 for Yellow Sea, 0.013 for Jejudo, and 0.015 for South Sea, respectively. The female gene flow was substantial or high (*Nm*=25.5-36.4), and the genetic distances between regions were not statistically significant ($P>0.01$). These results indicated that the Japanese anchovy populations occurring in Korean waters were consisted of individuals randomly dispersed over geographic areas.

Key words – Genetic distance, genetic diversity, Japanese anchovy, Korean waters

Changes in the population structure might result from several factors, such as genetic change in response to environmental constraints, migration from other geographic areas, and natural selection pressure[15]. Population genetic tool has been used to study genetic variability that has contributed to recent episodes of spatial-temporal patterns of heterogeneity between and among marine populations[8]. Recent advances in DNA amplification and sequencing have been employed to define the nature and extent of allelic variation in fishery stock. The Japanese anchovy, *Engraulis japonicus* (Temminck & Schlegel), is a small pelagic fish widely distributed off the coast of Korea, Japan, and China, and is associated with one of the commercially important fishery resources in Korea[10,11]. Different stock structures between anchovy populations in different habitats have been identified using biochemical markers or molecular studies[4,5,7,14,18,21], mostly in the case of the European anchovy (*Engraulis encrasicolus* Linnaeus). However, studies concerning the population genetic structure of the Japanese anchovy are limited to the relationships between strains and geographic areas[9]. In this sense, an understanding of the genetic diversity and population structure of the Japanese anchovy is vital for the efficient assessment and management of fisheries resources.

Because mitochondrial DNA (mtDNA) provides a clonally inherited marker that traces maternal lineage and much variability in gene deletion or insertion throughout the evolution[2,22], it is known to have become popular in the fields of molecular phylogenesis, ecology, population genetics, and conservation, in which detection of polymorphism for natural populations is necessary. Despite the fact that population characteristics of European anchovy using the mtDNA have been much studied and debated[3,5,14,21], a genetic understanding of Japanese anchovy somewhat remains undetermined[10,11]. The goals of this study were as follows: (1) to detect the possible existence of a genetic subdivision and (2) to investigate the extent of mtDNA divergence between geographic locations. A test of population genetic structure allows us to determine if the Japanese anchovy forms a large genetic group or not. Although geographically close

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populations form roughly clusters, we can also determine whether most of the genetic variance is really distributed within a locality, instead of between localities. More specifically, we question the followings: Does the Japanese anchovy belong to a single phylogenetic group? What are the extent and nature of genetic variation among geographic populations?

Materials And Methods

Specimen

Japanese anchovies, *Engraulis japonicus*, were sampled from 3 localities in Korean waters during the period of on March 2002 (Fig. 1). We used a total of 85 specimen collected at 3 localities (the Southern area of Yellow Sea, the western coast of Jejudo, and the eastern area of South Sea). Samples were frozen at -70℃ until analyzed.

Genomic DNA

Total DNA was extracted from their fin by the method of Ashida *et al.*[1]. Potential forward and reverse primers were selected manually and using On-line Primer design program (http://www-genome.wi.mit.edu/cgi/primer), from aligned mitochondrial 12S LSU (Large Subunit) ribosomal RNA region sequencesof *Engraulis japonicus*. The primer sequences are as follows: Ancho 5F, 5'-ATTATGTAGCCC-AAAACGCC-3' and Ancho 2R, 5'-GGGAATAGAGTG-CCCCTTTG-3'. Specific PCR (Polymerase Chain Reaction) were performed under the following conditions in 25 μl reaction volumes: 20 pmol of each primer; 2.5 mM dNTPs; 1.25 unit *Taq* DNA polymerase (FastStar *Taq* DNA polymerase, Rhoche Co.); 10 PCR reaction buffer (Rhoche Co.); 5-40 ng total genomic DNA. The thermocycling profile included an initial denaturation step of 94℃ for 4 min, followed by 35 cycles of 1 min at 94℃, primer annealing for 30 sec at 55℃, and extension for 5 min at 72 ℃. Products from specific PCR amplification reactions were analyzing using 2% agarose run at 50 V for 50 min, and visualized after staining in 0.5 μg ml^{-1} ethidium bromide. The PCR product was purified using PCR Purification kit (NucleoSpinR Extract) by following manufacturer's instruction. Purified DNA fragment was stored at -20℃ until use. The DNAs using a Perkin-Elmer Applied Biosystems (ABI) 377A automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems, Warrington, United Kingdom) were carried out following the manufacture's protocol. For the sequencing reaction, 30 ng of purified PCR products, 2.5 pmol of primer, and 1 μl of Big Dye terminator were mixed and adjusted to a final volume of 7 μl with dH_2O. The reaction was run with 5% dimethyl sulfoxide for 30 cycles of 15 sec at 95℃, 5 sec at 50℃, and 4 min at 60℃. Both strands were sequenced as a crosscheck. Sequence data were aligned using the multiple alignment program Clustal W[17]. When homologous sequences differed by≥ one nucleotide, the sequences were considered as different haplotypes. Haplotype designations (AN1, AN2, AN3, and so forth) were applied to new sequences as they were discovered.

Phylogenetic analysis

To understand the possible genetic relationships among haplotypes PAUP* ver. 4.0b[(Phylogenetic Analysis Using Parsimony),19] was used. The analysis was performed using an equal weighting of transitions and transversions by heuristic search, and bootstrapping (1,000 iterations) tested reliability. To obtain phylogenetic trees, the data set was iterated 1,000 times using the subprogram SEQBOOT. Individual trees from each iterated data set were obtained using the subprogram DNAMLK with the option of Kimura's 2-parameter method[12], which attempts to correct observed dissimilarities for multiple substitutions in sequences evolving with a transition bias. A consensus tree representing reliability at each branch in the tree was obtained using the subprogram CONSENSE.

Genetic distance

Genetic distance, coefficient of coancestry, and migration

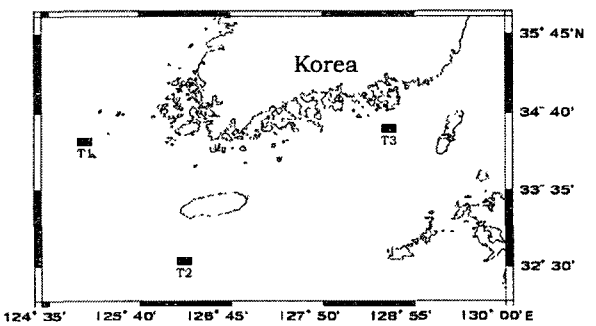

Fig. 1. Sampling locations of the Japanese anchovy, *Engraulis japonicus*, in Korean waters (the southern area of Yellow Sea, the western coast of Jejudo, and the eastern area of South Sea) on March 2002.

rate were calculated from mtDNA sequences and subroutines in the Arlequin ver. 2.0[16]. Population pairwise genetic distance (F_{ST}) was estimated by the Kimura 2-parameters method[12].

GenBank accession number

The determined rDNA sequences were deposited at the NCBI (National Center for Biotechnology Information) data library. Their accession numbers are indicated in Table 1.

Results

Haplotypes

The primer combination Ancho 5F (forward)-Ancho 2R (reverse) was 100% successful in amplifying Japanese anchovy DNA at an annealing temperature of 55℃, and a PCR product of the expected size (339 bp) was observed (Fig. 2). A total of 35 haplotypes (AN1-AN35) was obtained from a partial sequence of the 12S rRNA gene from 86 individuals of the Japanese anchovies, *E. japonicus*, collected from the 3 localities in Korean waters. The individual haplotype and GenBank accession numbers are listed in Table 1. The southern area of the Yellow Sea locality yielded 17 different haplotypes among 29 individuals, and these were similar numbers of haplotypes at locality 2 (the western coastof Jejudo) and locality 3 (the eastern area of the South Sea). Among these haplotypes, 10 individuals (AN5, AN6, AN7, AN8, AN13, AN20, AN24, AN27, AN29, and AN32) were observed at only locality 1 and accounted for 55% of the Yellow Sea sample, which was the largest number of haplotypes among localities. Three haplotypes (AN4, AN12, and AN35) were found in the 29 Japanese anchovies collected from the western coast of Jejudo as single individuals, and AN23 and AN25 were obtained only in the South Sea. 3 haplotypes (AN2, AN9, and AN33) were found in locality 1 and 2. AN1, AN3 AN10 and AN16 were commonly observed in locality 1 and 3. However, 9 individuals (AN15, AN11, AN17, AN18, AN19, AN28, AN30, AN31, and AN34) were commonly found between locality 2 and locality 3, excluding locality 1. The most frequent haplotype in locality 1 was AN1 and AN2 (4 among 17 individuals). In the case of locality 2, the most frequent haplotypes were AN9, AN22, and AN35 (3 among 18 individuals), and AN18, AN23, and AN34 were the most frequent haplotypes in locality 3 (3 among 18 individuals).

Sequence divergence

The sequence alignment revealed 25 variable nucleotides

Table 1. A list of sampling regions, animal numbers, mitochondrial 12S rRNA haplotypes, and GenBank accession numbers

Collecting locality (No. of individuals)	Collection date	Animal number	Haplotype	
			12S Large rRNA	GenBank accession number
1. The southern area of Yellow Sea (T1,29)	2002.03.16	T101	AN 1	AY707907
		T102	AN 2	AY669843
		T103	AN 8	AY669844
		T104	AN 1	AY669843
		T105	AN32	AY669842
		T106	AN 6	AY669841
		T107	AN 3	AY669840
		T108	AN 2	AY669843
		T109	AN16	AY669839
		T110	AN 1	AY669843
		T111	AN20	AY669838
		T112	AN27	AY669837
		T113	AN20	AY669838
		T114	AN 5	AY669836
		T115	AN 2	AY669843
		T116	AN33	AY669835
		T117	AN 3	AY669840
		T118	AN29	AY669834
		T119	AN 9	AY669833
		T120	AN 9	AY669833
		T121	AN16	AY669839
		T122	AN 7	AY669832
		T123	AN 7	AY669832
		T124	AN24	AY669831
		T125	AN10	AY669830
		T126	AN 2	AY669843
		T127	AN 1	AY669843
		T128	AN13	AY669829
		T129	AN29	AY669834

Table 1. Continued

Collecting locality (No. of individuals)	Collection date	Animal number	Haplotype	
			12S Large rRNA	GenBank accession number
2. The western part of Jejudo (T2,29)	2002.03.21	T201	AN 4	AY669828
		T202	AN 9	AY669833
		T203	AN17	AY669827
		T204	AN33	AY669835
		T205	AN34	AY669826
		T206	AN35	AY669825
		T207	AN 9	AY669833
		T208	AN 2	AY669843
		T209	AN22	AY669824
		T210	AN15	AY669818
		T211	AN30	AY669822
		T212	AN19	AY669821
		T213	AN18	AY669820
		T214	AN 9	AY669833
		T215	AN22	AY669824
		T216	AN35	AY669825
		T217	AN12	AY669819
		T218	AN15	AY669818
		T219	AN22	AY669824
		T220	AN28	AY669817
		T221	AN31	AY669816
		T222	AN 2	AY669843
		T223	AN35	AY669825
		T224	AN19	AY669821
		T225	AN26	AY669815
		T226	AN 4	AY669828
		T227	AN11	AY669814
		T228	AN 9	AY669833
		T229	AN14	AY669813
3. The eastern part of South Sea (T3,28)	2002.03.25	T301	AN 3	AY669840
		T302	AN30	AY669822
		T303	AN18	AY669820
		T304	AN17	AY669827
		T305	AN21	AY707904
		T306	AN23	AY707906
		T307	AN23	AY707906
		T308	AN25	AY707905
		T309	AN28	AY669817
		T310	AN34	AY669826
		T311	AN30	AY669822
		T312	AN10	AY669830
		T313	AN18	AY669820
		T314	AN34	AY669826
		T315	AN19	AY669821
		T316	AN22	AY669824
		T317	AN15	AY669818
		T318	AN11	AY669814
		T319	AN16	AY669839
		T320	AN26	AY669815
		T321	AN19	AY669821
		T322	AN31	AY669816
		T323	AN23	AY707906
		T324	AN34	AY669826
		T325	AN21	AY707904
		T326	AN18	AY669820
		T327	AN 1	AY669843
		T328	AN11	AY669814

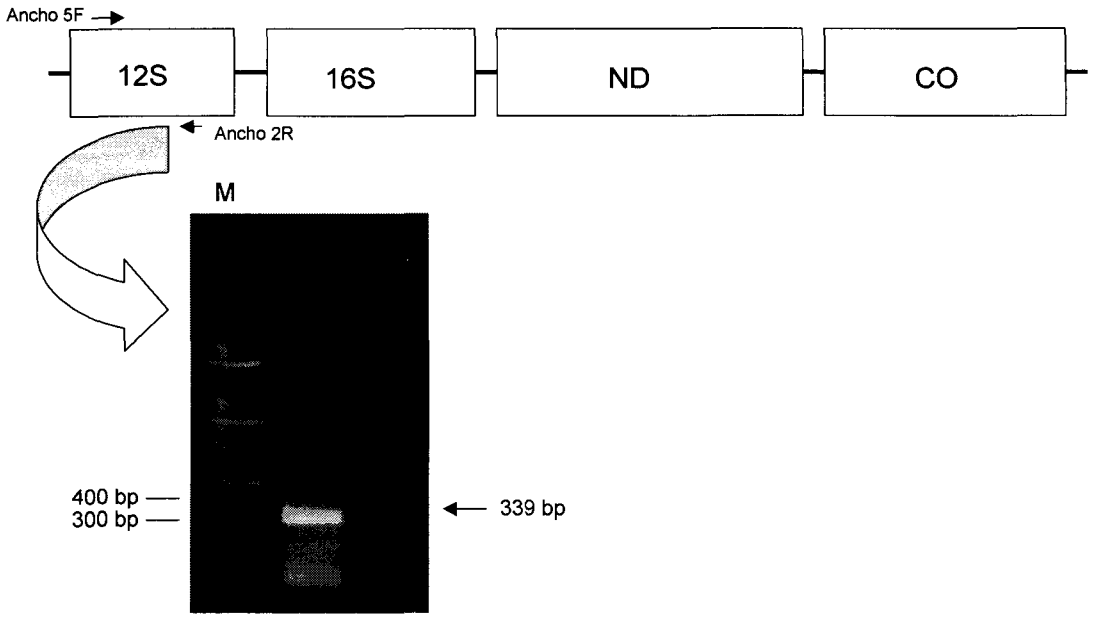

Fig. 2. Amplification product obtained with the primers Ancho 5F and Ancho 2R isolates of Japanese anchovy, *Engraulis japonicus*. 100 bp DNA ladder was used as molecular size marker in this study. 12S and 16S indicate genes of the 12S and 16S rRNA. ND indicates the gene of encoding dehydrogenase subunit. CO indicates the gene of encoding cytochrome oxidase subunit.

corresponding to positions 110-140, and most of them were transitional substitutions (TC, AG, Fig. 3). The sequence divergence among the 35 total haplotypes in pairwise comparisons, ranged from 0-4.1% (1-14 bp), and the largest sequence divergence was observed when AN8 from locality 1 was compared with AN35 from locality 2 (Table 2). Next, a pairwise comparison between AN8 and AN16 and AN14/AN16 and AN35 showed a divergence of 3.8% (13 bp). Within locality, highly sequence divergent waters were found in the Yellow Sea and off Jejudo, where the maximum sequence divergence among 18-19 haplotypes was 3.8% (13 bp), followed by the South Sea (sequence divergence of 3.3%, 11 bp).

PAUP analysis

A PAUP analysis was performed to investigate the phylogenetic relationships among haplotypes (Fig. 4). The 35 total haplotypes obtained in this study, clearly were not subdivided into independent groups: only one group consisted of a large number of haplotypes (34 haplotypes among 35 individuals), of which the overall sequence divergence were moderate and continuous (Table 2, 0.2-3.8%), and supported by bootstrap analysis. On the other hand, the AN8 haploptype independently formed a relatively strong sub-group (>50% of frequency).

Genetic diversity

Within-locality genetic diversity was estimated according to haplotype diversity (H) and nucleotide diversity (π) (Table 3). The South Sea and the Jejudo coast yielded a higher number of haplotypes (11) and wider haplotype diversity (0.39) than the Yellow Sea, though the difference was not significant. Although the within-locality H was relatively low overall, the nucleotide diversity among haplotypes was markedly high, ranging from 1.3-1.5 (maximum=1.5 in both the Yellow Sea and the South Sea). The high nucleotide diversity indices for the Yellow Sea appeared to be due to the existence of the AN8 haplotype found in 9 individuals, although lower haplotype diversity was observed.

Gene flow

The genetic distance (F_{ST}), coancestry coefficients (D), and per-generation migration rates (Nm) were shown in Table 4. The greatest genetic distance (F_{ST}=0.019) was found in a comparison between the most geographically remote localities, the Yellow Sea and the South Sea. However, the Jejudo coast showed a 0.013 genetic distance

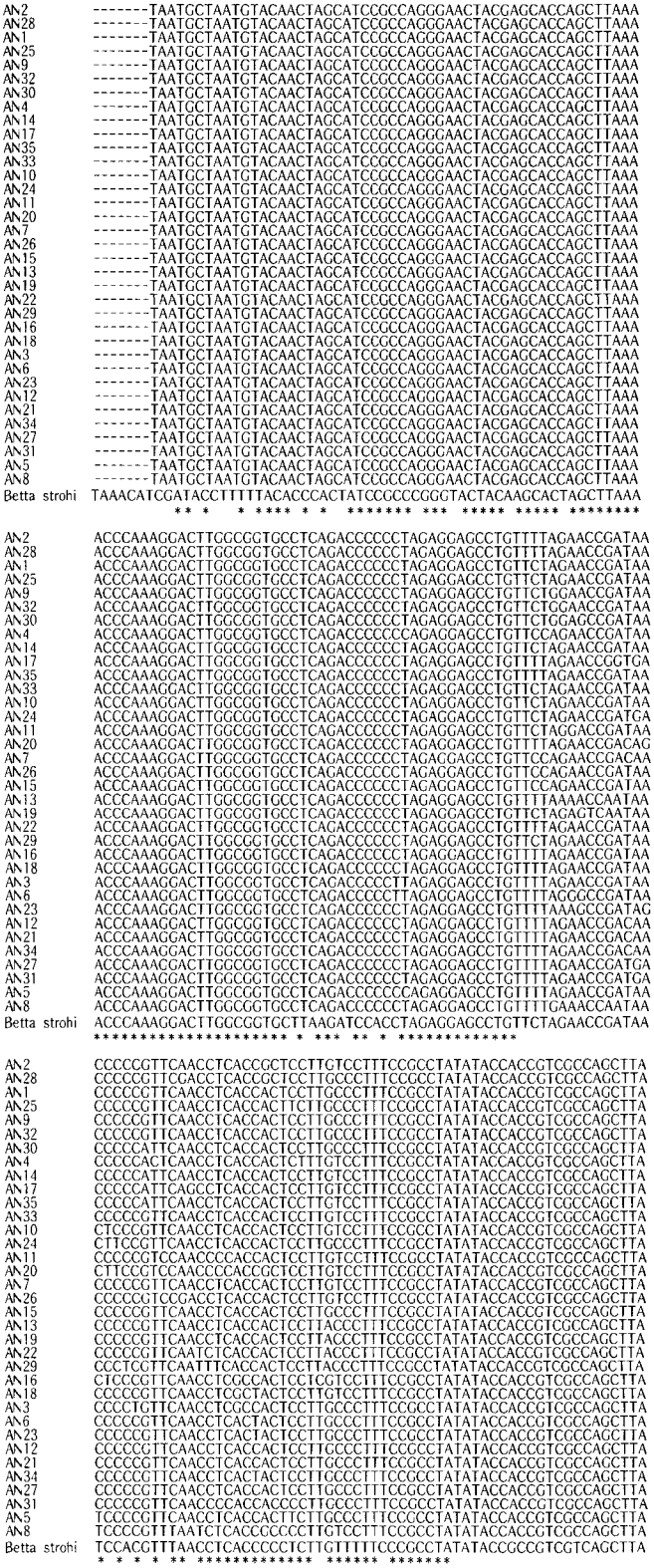

Fig. 3. Partial DNA sequences (339 bp) for a region of the mitochondrial 12S rRNA gene in 35 haplotypes collected from Japanese anchovy, *Engraulis japonicus*. CLUSTAL W generated the alignment. A hypen represents a gap and a period represents a base identical to that of the top sequence. An asterisk represents an identical sequence on vertical lines. Only positions that differ from haplotype AN1 are indicated. Sequences have been deposited in GenBank (accesson numbers AY669813-AY707907).

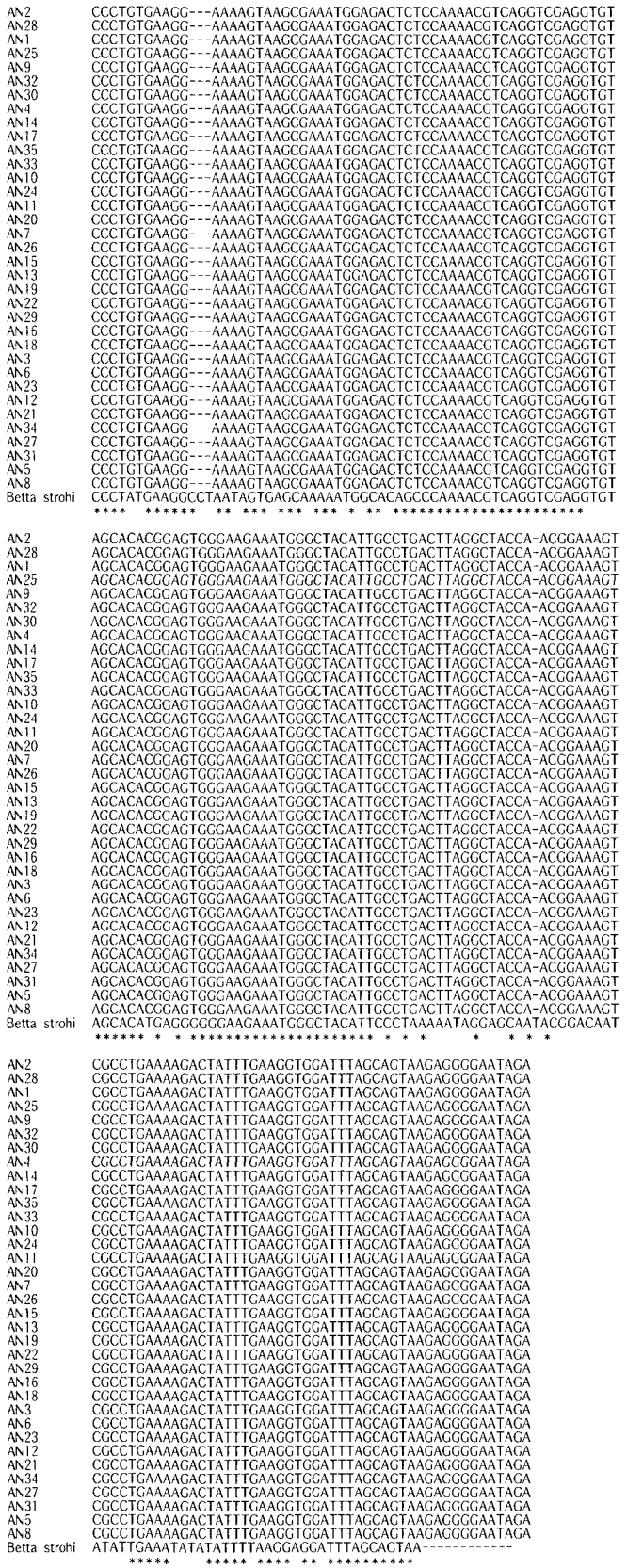

Fig. 3. Continued

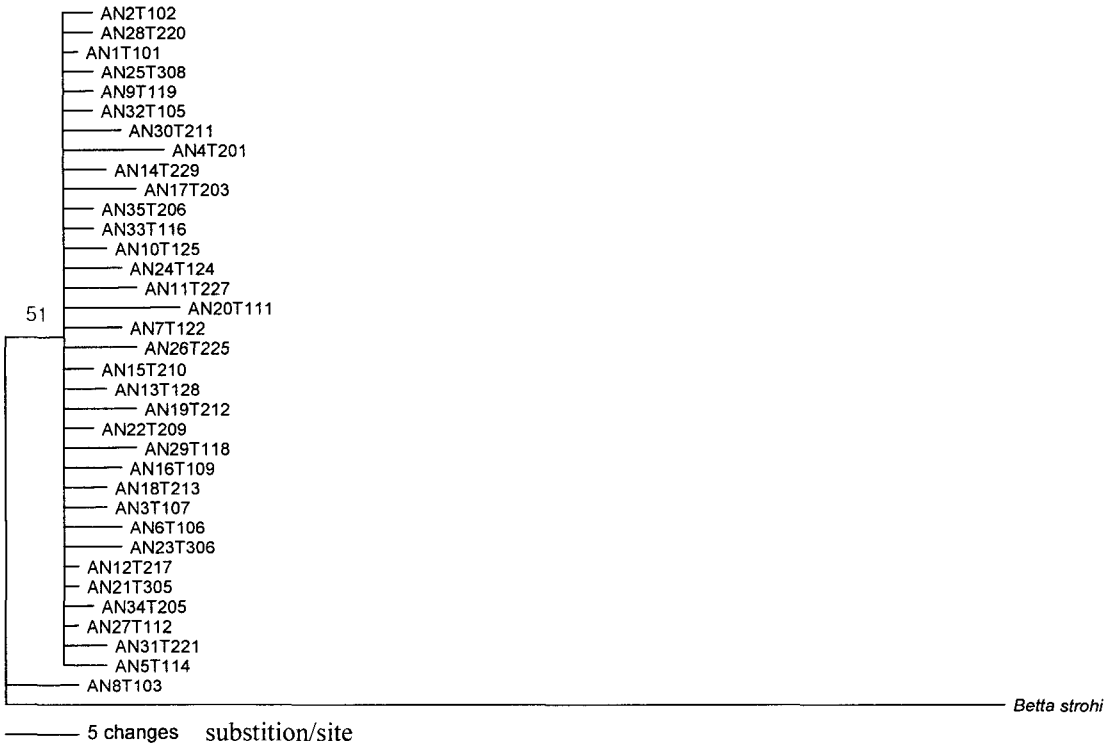

Fig. 4. PAUP analysis of mitochondrial 12S rRNA gene using mtDNA sequence of *Betta strohi*, as the outgroup. The tree shown is an unordered tree obtained with the option of frequency > 50% by majority-rule consensus of 100 equally parsimonious trees from the heuristic search. The numbers shown on the branches represent bootstrap values for 1,000 replicates. Tree length is 187 steps, Consistency Index is 0.556, Homoplasy index is 0.444, and Retention index is 0.046.

in a comparison with the southern area of the Yellow Sea, and 0.015 compared with the eastern part of the South Sea. Consequently, the increase in genetic isolation appears in proportion to the increase in geographic distance, though neither the statistical significance of the pairwise F_{ST} (P>0.05) nor and the distances among all localities was estimated in all cases. Pairwise comparisons of coefficients of coancestry (0-1, where D=0 is identical, shared ancestry) ranged from 0.015 to 0.019. The estimates of D were also consistent with the F_{ST} estimates. The lowest pairwise D was obtained for the South Sea and the Yellow Sea (D= 0.019), whereas that for the coast of Jejudo and the Yellow Sea showed the highest coancestry coefficient (D=0.013). These are very low and similar estimates overall, compared with other animals. The analysis of the per-generation migration rate estimates (Nm) showed that a gene flow occurred among the Yellow Sea and the Jejudo coast and the South Sea (Nm=25.5-36.4). The 36.4 Nm estimate was obtained between the Yellow Sea and the Jejudo coast, and the 25.5 Nm estimate was obtained between the South Sea and the Yellow Sea. Regardless, these estimates are relatively low: they represent what appears to be a high gene flow among the Japanese anchovy populations.

Discussion

In effect, it is suggested that marine organisms show lower levels of population structure and higher levels of genetic relatedness over a relatively wide geographic area than do some amphibians[2]. As expected, our 12S rRNA analysis of the Japanese anchovy occurring in Korean waters should probably show two salient characteristics of population genetic structure: one is the extremely low genetic relatedness among localities based on the subsequent F_{ST} analysis (Table 4), and the other is the large monophyletic population ranging over a wide geographic area (Fig. 4). Consequently, Japanese anchovy populations are not genetically isolated, but form a large and closely related genetic group consisting of the Yellow Sea, Jejudo coast and South Sea populations, regardless of geographic barrier. Tudela *et al.*[21] reported a considerable genetic homogeneity in the European anchovy using enzyme electrophoresis, although this is a less sensitive

Table 2. Pairwise comparisons among 35 haplotypes obtained from the partial sequences of mitochondrial 12S large ribosomal RNA

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	-	0.006	0.009	0.012	0.012	0.012	0.017	0.020	0.009	0.015	0.006	0.006	0.009	0.017	0.015	0.017	0.012	0.015	0.012	0.015
2	2	-	0.009	0.012	0.012	0.012	0.017	0.026	0.015	0.020	0.012	0.012	0.015	0.017	0.020	0.023	0.017	0.015	0.012	0.015
3	3	3	-	0.003	0.003	0.003	0.009	0.017	0.006	0.017	0.009	0.003	0.006	0.009	0.012	0.026	0.009	0.012	0.003	0.012
4	4	4	1	-	0.006	0.006	0.012	0.020	0.009	0.020	0.012	0.006	0.009	0.012	0.015	0.029	0.012	0.015	0.006	0.015
5	4	4	1	2	-	0.000	0.006	0.020	0.009	0.020	0.012	0.006	0.009	0.012	0.015	0.029	0.012	0.015	0.006	0.015
6	4	4	1	2	0	-	0.006	0.020	0.009	0.020	0.012	0.006	0.009	0.012	0.015	0.029	0.012	0.015	0.006	0.015
7	6	6	3	4	2	2	-	0.020	0.009	0.020	0.012	0.012	0.015	0.017	0.020	0.035	0.017	0.020	0.012	0.020
8	7	9	6	7	7	7	7	-	0.012	0.023	0.015	0.015	0.017	0.026	0.023	0.038	0.015	0.017	0.015	0.029
9	3	5	2	3	3	3	3	4	-	0.012	0.003	0.003	0.006	0.015	0.012	0.026	0.009	0.012	0.009	0.017
10	5	7	6	7	7	7	7	8	4	-	0.009	0.015	0.017	0.020	0.023	0.032	0.020	0.023	0.020	0.023
11	2	4	3	4	4	4	4	5	1	3	-	0.006	0.009	0.017	0.015	0.023	0.012	0.015	0.012	0.015
12	2	4	1	2	2	2	4	5	1	5	2	-	0.003	0.012	0.009	0.023	0.006	0.009	0.006	0.015
13	3	5	2	3	3	3	5	6	1	5	2	1	-	0.012	0.009	0.023	0.006	0.009	0.006	0.05
14	6	6	3	4	4	4	6	9	5	7	6	4	3	-	0.020	0.023	0.017	0.020	0.012	0.020
15	5	7	4	5	5	5	7	8	4	8	5	3	4	7	-	0.020	0.015	0.012	0.015	0.023
16	6	8	9	10	10	10	12	13	9	11	8	8	7	8	7	-	0.023	0.026	0.029	0.032
17	4	6	3	4	4	4	6	5	3	7	4	2	3	6	5	8	-	0.009	0.006	0.020
18	5	5	4	5	5	5	7	6	4	8	5	3	4	7	4	9	3	-	0.009	0.023
19	4	4	1	2	2	2	4	5	3	7	4	2	3	4	5	10	2	3	-	0.015
20	5	5	4	5	5	5	7	10	6	8	5	5	6	7	8	11	7	8	5	-

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	0.020	0.012	0.020	0.012	0.009	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.020	0.210
2	0.020	0.012	0.020	0.017	0.015	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.026	0.216
3	0.012	0.009	0.012	0.015	0.012	0.012	0.015	0.015	0.006	0.006	0.009	0.006	0.012	0.012	0.029	0.210
4	0.015	0.012	0.015	0.017	0.015	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.009	0.032	0.207
5	0.015	0.012	0.015	0.017	0.015	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.026	0.213
6	0.015	0.012	0.015	0.017	0.015	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.026	0.213
7	0.015	0.017	0.020	0.023	0.020	0.020	0.017	0.017	0.015	0.015	0.017	0.015	0.020	0.020	0.032	0.219
8	0.029	0.026	0.029	0.026	0.023	0.029	0.032	0.032	0.023	0.023	0.026	0.023	0.029	0.023	0.041	0.222
9	0.017	0.015	0.017	0.015	0.012	0.017	0.020	0.020	0.012	0.012	0.015	0.012	0.017	0.017	0.029	0.210
10	0.029	0.020	0.029	0.020	0.017	0.023	0.026	0.026	0.017	0.017	0.020	0.012	0.017	0.023	0.035	0.222
11	0.020	0.012	0.020	0.012	0.009	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.026	0.213
12	0.015	0.012	0.015	0.012	0.009	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.026	0.207
13	0.017	0.015	0.017	0.009	0.012	0.017	0.020	0.020	0.012	0.012	0.015	0.012	0.017	0.017	0.029	0.210
14	0.020	0.017	0.020	0.017	0.020	0.020	0.023	0.023	0.015	0.015	0.017	0.009	0.015	0.020	0.038	0.219
15	0.023	0.020	0.023	0.020	0.017	0.023	0.020	0.026	0.017	0.017	0.020	0.017	0.017	0.023	0.035	0.216
16	0.038	0.029	0.038	0.023	0.026	0.032	0.035	0.029	0.020	0.020	0.023	0.026	0.026	0.032	0.038	0.227
17	0.020	0.017	0.020	0.017	0.015	0.020	0.023	0.023	0.009	0.009	0.012	0.015	0.020	0.020	0.032	0.213
18	0.023	0.020	0.023	0.020	0.017	0.023	0.026	0.026	0.017	0.017	0.020	0.017	0.023	0.023	0.035	0.216
19	0.015	0.012	0.015	0.017	0.015	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.032	0.213
20	0.012	0.009	0.017	0.020	0.017	0.017	0.020	0.015	0.012	0.012	0.015	0.012	0.017	0.017	0.023	0.222

Table 2. Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
21	7	7	4	5	5	5	5	10	6	10	7	5	6	7	8	13	7	8	5	4
22	4	4	3	4	4	4	6	9	5	7	4	4	5	6	7	10	6	7	4	3
23	7	7	4	5	5	5	7	10	6	10	7	5	6	7	8	13	7	8	5	6
24	4	6	5	6	6	6	8	9	5	7	4	4	3	6	7	8	6	7	6	7
25	3	5	4	5	5	5	7	8	4	6	3	3	4	7	6	9	5	6	5	6
26	5	5	4	5	5	5	7	10	6	8	5	5	6	7	8	11	7	8	5	6
27	6	6	5	6	6	6	6	11	7	9	6	6	7	8	7	12	8	9	6	7
28	6	6	5	6	6	6	6	11	7	9	6	6	7	8	9	10	8	9	6	5
29	3	3	2	3	3	3	5	8	4	6	3	3	4	5	6	7	3	6	3	4
30	3	3	2	3	3	3	5	8	4	6	3	3	4	5	6	7	3	6	3	4
31	4	4	3	4	4	4	6	9	5	7	4	4	5	6	7	8	4	7	4	5
32	3	3	2	3	3	3	5	8	4	4	3	3	4	3	6	9	5	6	3	4
33	5	5	4	5	5	5	7	10	6	6	5	5	6	5	6	9	7	8	5	6
34	5	5	4	3	5	5	7	8	6	8	5	5	6	7	8	11	7	8	5	6
35	7	9	10	11	9	9	11	14	10	12	9	9	10	13	12	13	11	12	11	8
36	72	74	72	71	73	73	75	76	72	76	73	71	72	75	74	78	73	74	73	76

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
21	-	0.015	0.017	0.026	0.023	0.023	0.020	0.020	0.017	0.017	0.020	0.017	0.023	0.023	0.035	0.222
22	5	-	0.009	0.017	0.015	0.015	0.017	0.017	0.009	0.009	0.012	0.009	0.015	0.015	0.026	0.219
23	6	3	-	0.026	0.023	0.023	0.026	0.026	0.017	0.017	0.020	0.017	0.023	0.023	0.035	0.219
24	9	6	9	-	0.009	0.015	0.023	0.023	0.015	0.015	0.017	0.015	0.020	0.020	0.032	0.219
25	8	5	8	3	-	0.012	0.015	0.015	0.012	0.012	0.009	0.012	0.017	0.017	0.029	0.216
26	8	5	8	5	4	-	0.015	0.020	0.012	0.012	0.015	0.012	0.017	0.017	0.035	0.222
27	7	6	9	8	5	5	-	0.012	0.015	0.015	0.012	0.015	0.020	0.020	0.038	0.224
28	7	6	9	8	5	7	4	-	0.015	0.015	0.012	0.015	0.020	0.020	0.032	0.224
29	6	3	6	5	4	4	5	5	-	0.000	0.003	0.006	0.012	0.012	0.029	0.216
30	6	3	6	5	4	4	5	5	5	-	0.003	0.006	0.012	0.012	0.029	0.216
31	7	4	7	6	3	5	4	4	1	1	-	0.009	0.015	0.015	0.032	0.219
32	6	3	6	5	4	4	5	5	2	2	3	-	0.006	0.012	0.029	0.216
33	8	5	8	7	6	6	7	7	4	4	5	2	-	0.017	0.029	0.216
34	8	5	8	7	6	6	7	7	4	4	5	4	6	-	0.029	0.210
35	12	9	12	11	10	12	13	11	10	10	11	10	10	10	-	0.213
36	76	75	75	75	74	76	77	77	74	74	75	74	74	72	73	-

Numbers above the diagonal are mean distance values and numbers below the diagonal are absolute distance values. 1, AN2T102; 2, AN28T220; 3, AN1T101; 4, AN25T308; 5, AN9T119; 6, AN32T105; 7, AN30T211; 8, AN4T201; 9, AN14T229; 10, AN17T203; 11, AN35T206; 12, AN33T116; 13, AN10T125; 14, AN24T124; 15, AN11T227; 16, AN20T111; 17, AN7T122; 18, AN26T225; 19, AN15T210; 20, AN13T128; 21, AN19T212; 22, AN22T209; 23, AN29T118; 24, AN16T109; 25, AN18T213; 26, AN3T107; 27, AN6T106; 28, AN23T306; 29, AN12T217; 30, AN21T305; 31, AN34T205; 32, AN27T112; 33, AN31T221; 34, AN5T114; 35, AN8T103; 36, *Betta strohi* B169

Table 3. Within-locality diversity estimat

Locality	SS[a]	NH[b]	H[c]	π[d]
Yellow Sea	29	9	0.31	0.015737
Jejudo	29	11	0.37	0.013369
South Sea	28	11	0.39	0.015725

[a]Sample size, [b]Number of haplotypes, [c]Haplotype diversity, [d]Nucleotide diversity

Table 4. Mitochondrial 12S ribosomal RNA sequence of genetic distance, coancestry coefficients, and per-generation female migration rate in the pairs of localities

Locality	Yellow Sea	Jejudo	South Sea
Yellow Sea	-		
Jejudo	F_{ST}=0.01354	-	
	D=0.01363		
	N_m=36.44103		
South Sea	F_{ST}=0.01919	F_{ST}=0.01506	-
	D=0.01938	D=0.01518	
	N_m=25.55089	N_m=32.69450	

Distance method was utilized for that of Kimura's 2-parameter method (Kimura, 1980). Value at the first line of each column is the genetic distance (F_{ST}), at the second line is the estimate of coancestry coefficients (D), and third is per-generation female migration rate (N_m).

technique than genetic analysis. Furthermore, our results suggest that there is a minimized genetic heterogeneity among the Yellow Sea, Jejudo coast and South Sea populations, which might be governed only by silent transitional substitution based on the 12S rRNA gene sequence (Fig. 3). Because gene expression is temporally and spatially controlled in a highly regulated manner, catastrophic changes in amino acids among populations through successive generations are not expected. In gradually evolving, Japanese anchovy populations might follow a continuous pattern according to genetic drift as well as the process of natural selection. The role played by the significant association between geography and haplotype distribution is also perhaps the pacemaker of the observed almost homogeneous individuals and stable genetic structure of population. Regardless, most of our current knowledge of genetic population structure is still based on the homogeneity detected in DNA instead of the considerable geographic heterogeneity of DNA that reflects the effects of genetic drift or natural selection.

Japanese anchovies migrate to Korean coastal waters from the southern offshore area of Korea and the northern area of the East China Sea in the spring, and occur commonly in the coastal waters of Korea during the summer and autumn seasons. To effectively manage the anchovy population, more detailed migratory routes and population structures must be defined. Wintering grounds are the southwestern and southern frontal areas between coastal cold waters and offshore warm waters. The egg distribution showed that anchovy spawning occurs in the coastal and offshore regions separately during the early spring and then gradually extends to each area[10]. This means that there is a relation between the hydrographic and physical barriers to anchovy migration and the occurrence of spawning grounds. More recently, the ecological significance of the anchovy as a migratory marine fish forming large schools has led to the utilization of a species-specific genetic marker using a molecular tool to infer the origins of migration routes[6,9,17]. However, it is difficult to predict stock identification at the present level of 12S rRNA in the Japanese anchovy. Interestingly, Borsa[5] suggested that open-sea anchovy populations should be genetically different from inshore-water populations in a region. In the near future, we will sequence another nucleotide encoded-gene portion for cytochrome oxidase in order to understand the genetic characteristics and the possible existence of agenetic subdivision among micro-geographic locales instead of offshore waters. After spawning and external fertilization, Japanese anchovy larvae spend a variable period of time developing, which can include passively drifting in ocean currents. The existence of a high level of Japanese anchovy gene flow around Korean offshore waters may be associated with different water current systems in Korea[13]. In this sense, a strong intrusion of water current will create a high level of gene flow and close the genetic distance between the Yellow Sea and the Jejudo coast (Table 4). These results indicated that the Japanese anchovy populations occurring in Korean offshore waters were formed of individuals randomly dispersed individuals over geographic areas.

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초록 : 미토콘드리아 12S 리보좀 RNA 유전자배열에 의한 한국해역 멸치 개체군의 유전자 구조

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한국해역에 분포하는 멸치의 유전학적 특성을 연구하기 위하여 미토콘드리아 12S 리보좀 RNA 유전자배열(339 bp)을 분석하였다. 황해남부, 제주연안, 남해동부 등 3지역의 시료로 부터 총 35 mtDNA의 haplotype을 구하였다. 황해남부에서 채집된 멸치의 AN8T103은 PAUP 분석에서 0.2-4.1%로 분리되는 독립적인 계통을 보이므로서 다른 연구해역으로부터 유입된 개체군인 것으로 보이나 금후 추가연구가 필요하다. AN8T103을 제외한 유전자 다양성은 0.3-3.8%로서 개체군 내 염기다양성은 0.015(황해), 0.013(제주도), 0.015(남해)로 나타났다. 암컷유전자이동은 상당히 높았으며(*Nm*=25.5-36.44), 지역간 유전자거리(*FST*)는 유의한 차를 보이지 않았다(P>0.01). 이러한 결과는 한국해역에 서식하는 멸치가 지리적으로 무작위 분산된 개체군임을 암시한다.