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Mitochondrial DNA Polymorphism of the Japanese Anchovy (*Engraulis japonicus* Temminck & Schlegel) Collected from the Korean Offshore and Inshore Waters

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To investigate the population structure and geographic distance among anchovies (*Engraulis japonicus*) in Korea, we compared and analyzed the mitochondrial DNA control region sequences (227 bp) of anchovies from 12 localities in inshore and offshore waters. The sequence analysis of 84 individuals showed 29 haplotypes, ranging in sequence divergence by pairwise comparisons from 0.3% to 3.5% (1 bp-12 bp). E9 haplotype of anchovies were found largely in inshore waters and also in offshore waters, which was regarded as the major source in Korean waters (58.3%). However, E26, E27, E28, and E29 haplotypes were found in westsouthern (locality 10, four among 7). Phylogenetic analysis using PHYLIP was divided into two clades (clade A and B). Most of the haplotypes, excluding E26, E27, E28, and E29, were strongly supported by bootstrap analysis (>75%), whereas the relationship between clade A and B was weakly supported by bootstrap analysis (51%). High levels of genetic diversity were found; haplotype diversity (H)=0.75-1.00, and nucleotide diversity (π)=0.015-0.0244. Analysis of F_{ST} between populations in inshore waters ranged in 0.01-0.05, whereas those of offshore waters ranged in 0.01-0.58. A high gene flow occurred in inshore (Nm=22.61-34.22) and offshore (Nm=11.57-45.67) populations. The distribution of mitochondrial DNA haplotypes between westsouthern and other populations was suggestive of significantly different differentiation (F_{ST}=0.20-0.59, p<0.05; d=0.52, p=0.00; Φ=0.02-0.41, p<0.05). These results suggested that the overall anchovy population in the Korean peninsula caused considerable migration due to the mitochondrial gene flow between inshore and offshore populations to form a genetically homogenous and panmictic structure, although a heterogeneous population was found in this study.

Key words – *Engraulis japonicus*, geographic distance, population structure, gene flow, mtDNA, control region.

Introduction

The Japanese anchovy, *Engraulis japonicus* (Temminck & Schlegel), is a small pelagic, schooling fish, and plankton feeder with a wide distribution off the coast of the Korean peninsula including Japan and China, which is associated with one of the commercially important fishery resources in Korea[19,21]. In this role, several researchers have studied the vertical distribution of anchovy eggs and larvae[21,23,24], the spawning ecology of anchovies[22], and the biomass estimation of anchovies by acoustic survey[6] in Korea. Numerous studies have shown the distribution of larvae and juveniles[1], and an analysis of the spawning and reproductive characteristics of the populations[10,11] in Japan. Subsequently, the population dynamics and management of anchovy based on the recruitment of the larval fishery were carried out in China[26,41]. Consequently, anchovy stocks have been regarded as the most important fishery resource in Korea, Japan, and China. In particular, anchovies are used as a prey for higher trophic level species (i.e. piscivorous fish) and as a peacemaker to annually fluctuate their fisheries resources.

With recent advances in DNA amplification and sequencing, many sequence-based studies have been employed to define the nature and extent of allelic variation in fishery stock. 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[16]. Different stock structures between anchovy populations in different habitats have been intensively studied and debated, mostly in the case of the European anchovy, using biochemical and molecular probing methods[3-5,12,27,35,37]. Recently, the use of mitochondrial DNA (mtDNA) has a high resolution of molecular phylogenesis, population genetics, and conservation, in which detection of polymorphism for natural populations is necessary. The population genetic structure

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of European anchovy using the mtDNA has been studied[4,5,27,37], while the genetic understanding between strains and geographic areas for the Japanese anchovy has been limited[18]. Consequently, understanding of the genetic diversity and population structure of the Japanese anchovy is vital for the success of the efficient management and assessment of their resources.

We previously sequenced a portion of an mtDNA 12S ribosomal RNA gene from only 3 localities in Korean waters and reported high gene flow among populations and high haplotype diversity within populations[20]. In this study, we newly sequenced a portion of an mtDNA control region gene of anchovies collected from 12 localities in inshore and offshore Korean waters to extend our understanding of the population genetic structure of the species and provide information on the genetic variation among populations within species.

Materials and Methods

Anchovy

Japanese anchovies, *Engraulis japonicus*, were sampled from 12 localities in Korea offshore and inshore waters during the period of on March 2002 - February 2005 (Fig. 1). We used a total of 87 specimen collected at 12 localities (Inshore waters: Dolsan, Odongdo, Samcheonpo, Hakdong, Kyjedo, Taejongdae, Offshore waters: the western part of South Sea, the eastern part of East Sea, the western part of Yellow Sea, westsouthern, southwest, the western part of Jejudo). Samples were frozen at -70℃ until required.

DNA amplification

Total DNA was extracted from anchovy fins by the method of Asahida[2]. Potential forward and reverse primers were selected manually using on-line Primer program (http://www-genome.wi.mit.edu/cig/primer), from aligned mitochondrial control region sequences of *E. japonicus* (AB040676). The primer sequences are as follows: CR2F, 5'-AAGTTAAACTACCTCTGTAAT-3' and CR3R, 5'-AACACTATCAACA-3'. PCR (Polymerase Chain Reaction) reactions were performed under the following conditions in 25 μl reaction volumes: 20 pmol of each primer; 0.5 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 95℃ for 3 min, followed by 35 cycles of 15 s at 94℃, primer annealing for 15 s at 52℃, and extension for 30 s at 72℃. The final extension step was increased to 10 min. The PCR was carried out by iCycler Thermocycle (Bio-Rad). Products from specific PCR amplification reactions were analyzing using 2% agarose gel 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 (NucleoSpin® Extract) by following manufacturer's instruction. Purified DNA fragment was stored at -20℃ until use.

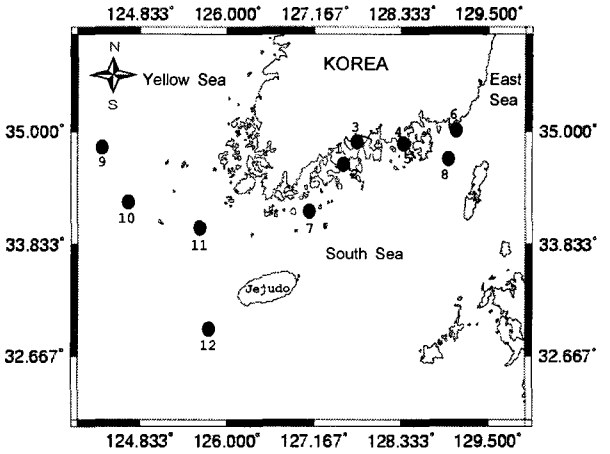

Fig. 1. Sampling locations of the anchovy, *Engraulis japonicus*, in Korea collected from March 2002 to February 2005. General locality names are as follows: Inshore waters, 1. Dolsan, Yeosu City, Chunman Province; 2. Odongdo, Yeosu City, Chunnam Province; 3. Samchenonpo, Kyungsangnam Province; 4. Hakdon, Tongyeong City, Kyungsangnam Province; 5. Kyjedo, Tongyeong City, Kyungsangnam Province; 6. Taejeongdae, Busan; Offshore waters, 7. The western part of South Sea; 8. The eastern part of East Sea; 9. The western part of Yellow Sea; 10. The westsouthern; 11. The southwest; 12. The western part of Jejudo.

Nucleotide sequence

The purified DNA was directly sequenced using an Applied Biosystem model ABI 3730XL automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems, UK). 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% DMSO for 30 cycles of 15 s at 95℃, 5 s at 50℃, and 4 min at 60℃. Both strands were sequenced as a crosscheck.

Haplotype

Sequence data were aligned using the multiple alignment program Clustal W[36]. When homologous sequences differed by ≥ one nucleotide, the sequences were considered as different haplotypes. Haplotype designations (E1, E2, E3, and so forth) were applied to new sequences as they were discovered.

Phylogenetic analysis

Phylogenetic analysis was performed by neighbor-joining (NJ) method incorporated in PHYLIP (Phylogeny Inference Package) ver. 3.5c[8] as a subprogram NEIGHBOR. To obtain the NJ tree, the data set was iterated 1,000 times using a subprogram SEQBOOT. Individual trees from each iterated data set were obtained using the subprogram DNAMLK with the option of Kimura's 2-parameter method[25], 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 diversity

To investigate the magnitude and pattern of genetic diversity within localities, genetic diversity and mean number of pairwise differences among haplotypes, gene diversity, and nucleotide diversity were calculated using Arlequin ver 1.1[33]. Mean number of differences between all pairs of haplotypes in the sample was obtained by considering the number of mutations having occurred since the divergence of any two haployptes, and the frequency of the ones involved in the calculation. Nucleotide diversity was calculated by estimating the probability that two randomly chosen homologous sequences will be different[28].

Genetic migration

Genetic distance (F_{ST}), coefficient of coancestry (D), and female migration rate (Nm) were estimated by subroutines in Arlequin ver 1.1[33]. Statistical significance of the difference between pairs of localities was tested by permutations (1,000 bootstraps [7]).

Hierarchical structure

Hierarchical genetic relationships among populations and sets of populations were assessed by Holsinger and Mason-Gamer (H-MG) method[17]. This study tested the degree of hierarchical subdivision between specified set of localities with AMOVA (Analysis of Molecular Variance) program[7] incorporated in Arlequin ver 1.1 [33]. For the present data set, this study grouped the 12 localities into two groups on the basis of rough distance. For example, Dolsan (locality 1), Odongdo (locality 2), Samcheonpo (locality 3), Hakdon (locality 4), Kyjedo (locality 5), Taejeongdae (locality 6), the western part of South Sea (locality 7), the eastern part of East Sea (locality 8), the western part of Yellow Sea (locality 9), southwest (locality 11), and the western part of Jejudo (locality 12) were grouped into one group (group A). Westsouthern (locality 10) was grouped another group (group B). After specifying these localities into two groups, this analysis provided correlation of haplotype dicersity at different levels of hierarchical subdivision in the forms of three variance components (i.e. between-regions, within-regions, and within-localities).

GenBank accession number

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

Results

DNA

The primer combination CN2F (forward)-CR3R (reverse) was successful in amplifying the anchovy genomic DNA at an annealing temperature of 52℃, and a PCR product of the expected size (227 bp) was obtained (Fig. 2). A total of 29 haplotypes (E1-E29) was obtained from a partial sequence of the mtDNA control region gene from 87 individuals of the anchovy (*E. japonicus*) collected from the 12 localities including inshore and offshore Korean waters. The individual haplotype and GenBank accession numbers are listed in Table 1. The sequence alignment showed 61 variable nucleotides (Fig. 3): one G⇔C transversion, nine A⇔T transversion, one T⇔A⇔C parallel mutations (nucleotide position 95). The rest of them were transitional substiutions (T⇔C, A⇔G, and C⇔A).

Sequence divergence

The sequence divergence among the 29 haplotypes by

Table 1. A list of sampling regions (inshore waters), animal numbers, mitochondrial control region gene haplotypes, and GenBank accession numbers

Collecting locality (no. of individuals)	Collection date	Animal number	Haplotype	GenBank number
1. Dolsan, Yeosu City, Chunnam Province (7)	2004. 9. 22	AD1	E04	DQ223772
		AD2	E05	DQ223773
		AD3	E01	DQ223774
		AD4	E19	DQ223775
		AD5	E04	DQ207811
		AD6	E05	DQ207812
		AD7	E15	DQ207813
2. Odongdo, Yeosu City, Chunnam Province (8)	2004. 9. 13	AO1	E08	DQ207814
		AO2	E02	DQ207815
		AO3	E22	DQ207816
		AO4	E01	DQ207817
		AO5	E18	DQ207818
		AO6	E25	DQ207819
		AO7	E22	DQ207820
		AO8	E24	DQ207821
3. Samcheonpo, Kyungsangnam Province (8)	2005. 2. 4	AS1	E01	DQ207822
		AS2	E05	DQ207823
		AS3	E19	DQ207824
		AS4	E18	DQ207825
		AS5	E06	DQ207826
		AS6	E08	DQ207827
		AS7	E25	DQ207828
		AS8	E06	DQ207829
4. Hakdong, Tongyeong City, Kyungsangnam Province (7)	2005. 1. 15	AH1	E03	DQ207830
		AH2	E09	DQ207831
		AH3	E13	DQ207832
		AH4	E05	DQ207833
		AH5	E22	DQ207834
		AH6	E23	DQ207835
		AH7	E08	DQ207836
5. Kyjedo, Tongyeong City, Kyungsangnam Province (8)	2004. 9. 24	AK1	E07	DQ207837
		AK2	E09	DQ207838
		AK3	E01	DQ207839
		AK4	E25	DQ207840
		AK5	E01	DQ219876
		AK6	E11	DQ219877
		AK7	E14	DQ219878
		AK8	E22	DQ219879
6. Taejongdae, Busan (8)	2005. 1. 27	AT1	E10	DQ219880
		AT2	E16	DQ219881
		AT3	E12	DQ219882
		AT4	E18	DQ219883
		AT5	E23	DQ219884
		AT6	E05	DQ219885
		AT7	E17	DQ219886
		AT8	E09	DQ219887
7. The western part of South Sea (7)	2005. 2. 27	JAA1	E21	DQ219888
		JAA2	E09	DQ219889
		JAA3	E01	DQ219890
		JAA4	E04	DQ219891
		JAA5	E14	DQ219892
		JAA6	E19	DQ219893
		JAA7	E24	DQ219894

Table 1. Continued

8. The eastern part of East Sea (6)	2005. 2. 21	JAB1	E08	DQ219895
		JAB2	E23	DQ219896
		JAB3	E15	DQ219897
		JAB4	E25	DQ219898
		JAB5	E07	DQ219899
		JAB6	E02	DQ219900
9. The western part of Yellow Sea (7)	2002. 3. 17	JAC1	E05	DQ219901
		JAC2	E12	DQ219902
		JAC3	E22	DQ219903
		JAC4	E24	DQ219904
		JAC5	E15	DQ219905
		JAC6	E09	DQ219906
		JAC7	E19	DQ219907
10. The westsouthern (7)	2002. 3. 19	JAD1	E27	DQ219908
		JAD2	E2	DQ219909
		JAD3	E26	DQ219910
		JAD4	E5	DQ219911
		JAD5	E28	DQ219912
		JAD6	E27	DQ219913
		JAD7	E29	DQ219914
11. The southwest (8)	2002. 3. 22	JAE1	E17	DQ219915
		JAE2	E20	DQ219916
		JAE3	E9	DQ219917
		JAE4	E8	DQ219918
		JAE5	E24	DQ219919
		JAE6	E25	DQ219920
		JAE7	E22	DQ219921
		JAE8	E6	DQ219922
12. The western part of Jejudo (6)	2002. 3. 24	JAF1	E1	DQ219923
		JAF2	E3	DQ219924
		JAF3	E24	DQ219925
		JAF4	E10	DQ219926
		JAF5	E15	DQ219927
		JAF6	E9	DQ219928

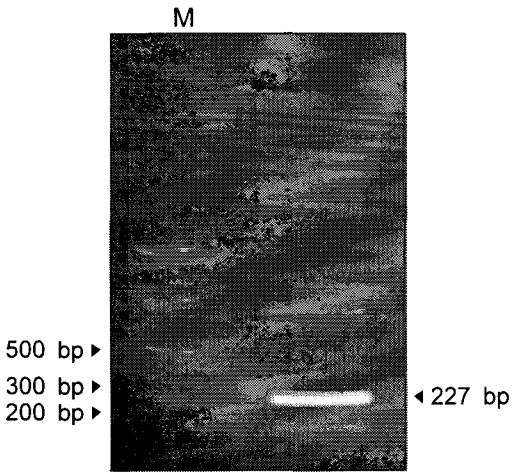

Fig. 2. Amplification product obtained with the primers CR2F and CR3R for the anchovy, *Engraulis japonicus*. 100 bp DNA ladder was used as molecular size marker in this study.

pairwise comparisons, ranged from 0.3-3.5% (1 bp-12 bp), and the largest sequence divergence was observed when E16, E18, E19, E21, E25, and E27 were compared with E26, E29, E26, E27, E28, E29, respectively (Table 2). Next, a pairwise comparison between E20/E27, E21/E26, E28, and E29, E26/E27 and E29, and E28 and E29 showed a divergence of 3.2% (11 bp). Within locality, highly sequence divergent was found in the westsouthern (locality 10), where the maximum sequence divergence among six haplotypes was 3.5% (12 bp).

Haplotypes

Distribution and relative frequency of haploytpes were shown in Table 1 and 3, respectively. Five different haplotypes among 7 individuals were observed from sample of the Dolsan (locality 1), and these were similar numbers

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E2      GGTAATGCGGCCGACGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E27     GGTAATGCGGCCAAAGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCTCTCACTTGT
E15     GGTAATGCGGCCGCCGCGCCCATATAGTGCTTGATGCCCTTAGGCAGTTCATGCACTTGT
E19     AGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E24     GGTAATGAGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E14     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTCGGCAGTTCAAGCACTTGT
E11     GGTAATGCCGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E9      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E3      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTATGCAGTTCAAGCACTTGT
E6      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGTCAGTTCAAGCACTTGT
E16     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E22     GGTAATGCGGCCGCCGCGCCCAAACAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E25     GGTAATGCGGCCGCCGCGCCCAAACAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E1      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E23     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCTCTTAGGCAGTTCAAGCACTTGT
E29     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATATATTTAGGCAGTTCAAGCACTTGT
E8      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E10     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E4      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E5      GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E21     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E13     GGTAATGCGGCCGCCGCGCCCAAATAGTTCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E20     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E18     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E28     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E12     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E17     GGTAATGCGGCCGCCGCGCCCAAATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E7      GGTAATGCGGCCGCCGCGCCCACATAGTGCTTGATGCCCTTAGGCAGTTCAAGCACTTGT
E26     GTTAATGCGGCCGCCGCGCCCAAATAGTTTTTGATGCCCTTAGGCAGTTCAAGCACTTGT
          *****   ***    ******** * ***  ****** **    ******   *******

E2      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E27     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E15     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACATTATGGTGTT
E19     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTATACATACACTATGGTGTT
E24     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E14     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E11     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E9      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E3      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E6      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATATTACATACACTATGGTGTT
E16     CCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E22     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E25     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E1      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E23     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E29     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E8      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E10     TCATGACTGCGCAGAGCATTCATGGACATATATATATTATTTTACATACACTATGGTGTT
E4      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E5      TCCTGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E21     TCCTGACTGCGCAAAGCATTCATGGACATATATGTATTATTTTCCATACACTATGGTGTT
E13     TCATGACTGCGCAGAGCATTCATGGACATATATGAATTATTTTACATACACTATGGTGTT
E20     TCATAACTGCGCAGAGCATTCATGGACATATATGAATTATTTTACATACACTATGGTGTT
E18     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E28     TCATTTCTGCGCAGAGCATTCATGGACATATACCCATTATTTTACATACACTATGGTGTT
E12     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E17     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E7      TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
E26     TCATGACTGCGCAGAGCATTCATGGACATATATGTATTATTTTACATACACTATGGTGTT
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Fig. 3. Sequence alignment of 29 mitochondrial haplotypes obtained from 227 bp of control region sequences. A hyphen 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 E1 are indicated. Sequences have been deposited in GenBank (accesson numbers DQ207800-DQ223775).

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E2      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E27     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E15     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E19     AACCCATATATGCACAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E24     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E14     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E11     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E9      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E3      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E6      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E16     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATAATATGTATAACTTT
E22     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E25     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGAATAACTTT
E1      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E23     ATCCCATATATGCATAATATTACATATTTTATGGTGTTAATACATACTATCTATAACTTT
E29     AACCCATATATGCATTTTATTACATATATTATGGTGTTAATACATACTATGTATCTCTTT
E8      AACCCATCTATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E10     AACCCATCTATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E4      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E5      AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E21     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E13     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E20     AACCCATATATTCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E18     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E28     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTACAACTTT
E12     AACCCATATATGCATAATATTACATATATTATGATGTTAATACATACTAAGTATAACTTT
E17     AACCCATATATGCATAATATTACATATATTATGGTGTTAATACATACTACGTATAACTTT
E7      AACCTATATATGCATAATATTACATATATTATGGTGTTAATACATACTATGTATAACTTT
E26     AATATATATATGCATAATATTACTTATATTATGGTGTTAATACATACTCCGTATAACTTT
        *     ** ***  **    ****** *** ***** * ************ *     *    ****

E2      ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E27     ACACTATCTATGTATAAGTAAATACATTAAGGTATAATATACTGAAT
E15     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E19     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E24     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E14     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E11     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E9      ACAATATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E3      ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E6      ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E16     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E22     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E25     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E1      ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E23     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E29     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E8      ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E10     ACACTATCTATGTATAATTAAATACCTTAAGGTATAATATACTGAAT
E4      ACACTATCTATGTATAAATAAATACCTTAAGGTATAATATACTGAAT
E5      ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E21     ACACTATCTATATATAAGTAAATACCTTAAGGTATAATATACTGAAT
E13     ACACTATCTATGTATAAGTAAATACCTTAATGTATAATATACTGAAT
E20     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAA
E18     ACACTATCTAAGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E28     ACACTATCTAAAAATAAGTAAATACCTTAAGGTATAATATACTGAAT
E12     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E17     ACACTATCTATGTATAAGTAAATACCTTAAGGTATAATATACTGAAT
E7      ACACTATCTATGTAAAAGTAAATACCTTAAGGTATAATATACTGAAT
E26     ACACTATCTATGTATAAGTAAATACCTTAAGGTATTCTATACTGAAT
        *** ******    * ** ******* **** **** *********
```

Fig. 3. Continued

of haplotypes at other sampling sites, ranging 5-7 haplotypes among 6-8 individuals. This indicates to account for ≥90% of each sample. The most frequent E9 haplotype was found largely in the inshore waters (Hakdong; locality 4, Kyjedo; locality 5, and Taejeongdae; locality 6), but it also occurred in the offshore waters (the western part of South Sea; locality 7, the western part of Yellow Sea; locality 9, southwest; locality 11, the western part of Jejudo; locality 12), showing a wide geographic distribution in Korea peninsula. Nine individuals (E11, E13, E16, E20, E21, E26, E27, E28, and E29) were found only in one locality (Kyjudo; locality 5, Hakdong; locality 4, Taejeongdae; locality 6, southwest; locality 11, westsouthern; locality 10, respectively) as a single individual, indicating regional restriction and rarity among haplotypes. In particular, E26, E27, E28, and E29 haplotypes were found only westsouthern (locality 10). The most frequent haplotypes in Dolsan (locality 1), Odongdo (locality 2), Samcheonpo (locality 3), Kyjedo (locality 5) were E4/E5, E22, E6, and E1, respectively. These haplotype distributions can be summarized as the coexistence of regional restriction in the most haplotypes with even far-reaching haplotypes, excluding westsouthern (locality 10).

Phylogenetic relationship

Phylogenetic analysis was performed to infer the genetic relationships among haplotypes using PHYLIP. In the PHYLIP analysis, haplotypes formed two major groups

Table 2. Pairwise comparisons among 29 haplotypes obtained from the partial sequences of mitochondrial control region gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	–	0.003	0.003	0.003	0.006	0.006	0.009	0.003	0.003	0.012	0.003	0.006	0.009	0.003	0.009
2	1	–	0.003	0.006	0.009	0.003	0.009	0.012	0.003	0.006	0.009	0.003	0.006	0.006	0.003
3	1	1	–	0.012	0.015	0.003	0.009	0.006	0.012	0.015	0.006	0.003	0.003	0.006	0.003
4	1	2	4	–	0.006	0.006	0.009	0.012	0.003	0.003	0.006	0.006	0.009	0.006	0.003
5	2	3	5	2	–	0.006	0.003	0.006	0.009	0.003	0.003	0.006	0.009	0.012	0.006
6	2	1	1	2	2	–	0.009	0.003	0.006	0.012	0.009	0.006	0.003	0.006	0.009
7	3	3	3	3	1	3	–	0.006	0.003	0.009	0.012	0.003	0.003	0.006	0.006
8	1	4	2	4	2	1	2	–	0.003	0.006	0.009	0.012	0.006	0.006	0.009
9	1	1	4	1	3	2	1	1	–	0.006	0.003	0.006	0.003	0.009	0.012
10	4	2	5	1	1	4	3	2	2	–	0.003	0.012	0.009	0.006	0.006
11	1	3	2	2	1	3	4	3	1	1	–	0.003	0.009	0.006	0.006
12	2	1	1	2	2	2	1	4	2	4	1	–	0.009	0.012	0.006
13	3	2	1	3	3	1	1	2	1	3	3	3	–	0.006	0.006
14	1	2	3	2	4	2	2	2	3	2	2	4	2	–	0.009
15	3	1	2	1	2	3	2	3	4	2	2	2	2	3	–

	16	17	18	19	20	21	22	23	24	25	26	27	28	29
16	–	0.012	0.006	0.009	0.003	0.006	0.006	0.006	0.009	0.012	0.035	0.023	0.029	0.026
17	4	–	0.009	0.006	0.003	0.006	0.006	0.003	0.003	0.006	0.026	0.029	0.023	0.029
18	2	3	–	0.006	0.009	0.006	0.006	0.003	0.006	0.006	0.029	0.020	0.029	0.035
19	3	2	2	–	0.006	0.009	0.003	0.003	0.012	0.006	0.020	0.035	0.026	0.035
20	1	1	3	2	–	0.009	0.006	0.003	0.003	0.006	0.026	0.032	0.020	0.029
21	2	2	2	3	3	–	0.006	0.009	0.006	0.006	0.032	0.026	0.032	0.032
22	2	2	2	1	2	2	–	0.006	0.006	0.012	0.029	0.023	0.035	0.026
23	2	1	1	1	1	3	2	–	0.003	0.006	0.026	0.020	0.023	0.023
24	3	1	2	4	1	2	2	1	–	0.009	0.026	0.029	0.023	0.020
25	4	2	2	2	2	2	4	2	3	–	0.035	0.035	0.026	0.023
26	12	9	10	7	9	11	10	9	9	12	–	0.032	0.029	0.032
27	8	10	7	12	11	9	8	7	10	12	11	–	0.035	0.035
28	10	8	10	9	7	11	12	8	8	9	10	12	–	0.032
29	9	10	12	12	10	11	9	8	7	8	11	12	11	–

Numbers above the diagonal are mean distance values and numbers below the diagonal are absolute distance values. 1, E1; 2, E2; 3, E3; 4, E4; 5, E5; 6, E6; 7, E7; 8, E8; 9, E9; 10, E10; 11, E11; 12, E12; 13, E13; 14, E14; 15, E15; 16, E16; 17, E17; 18, E18; 19, E19; 20, E20; 21, E21; 22, E22; 23, E23; 24, E24; 25, E25; 26, E26, 27, E27; 28, E28; 29, E29

Table 3. Relative frequencies of mitochondrial control region gene haplotypes through the populations

Haplotype	L1 (7)	L2 (8)	L3 (8)	L4 (7)	L5 (8)	L6 (8)	L7 (7)	L8 (6)	L9 (7)	L10 (7)	L11 (8)	L12 (6)
E01	0.14	0.12	0.12	0	0.25	0	0.14	0	0	0	0	0.16
E02	0	0.12	0	0	0	0	0	0.16	0	0.14	0	0
E03	0	0	0	0.14	0	0	0	0	0	0	0	0.16
E04	0.28	0	0	0	0	0	0.14	0	0	0	0	0
E05	0.28	0	0.12	0.14	0	0.12	0	0	0.14	0.14	0	0
E06	0	0	0.25	0	0	0	0	0	0	0	0.12	0
E07	0	0	0	0	0.12	0	0	0.16	0	0	0	0
E08	0	0.12	0.12	0.14	0	0	0	0.16	0	0	0.12	0
E09	0	0	0	0.14	0.12	0.12	0.14	0	0.14	0	0.12	0.16
E10	0	0	0	0	0	0.12	0	0	0	0	0	0.16
E11	0	0	0	0	0.12	0	0	0	0	0	0	0
E12	0	0	0	0	0	0.12	0	0	0.14	0	0	0
E13	0	0	0	0.14	0	0	0	0	0	0	0	0
E14	0	0	0	0	0.12	0	0.14	0	0	0	0	0
E15	0.14	0	0	0	0	0	0	0.16	0.14	0	0	0.16
E16	0	0	0	0	0	0.12	0	0	0	0	0	0
E17	0	0	0	0	0	0.12	0	0	0	0	0.12	0
E18	0	0.12	0.12	0	0	0.12	0	0	0	0	0	0
E19	0.14	0	0.12	0	0	0	0.14	0	0.14	0	0	0
E20	0	0	0	0	0	0	0	0	0	0	0.12	0
E21	0	0	0	0	0	0	0.14	0	0	0	0	0
E22	0	0.25	0	0.14	0.12	0	0	0	0.14	0	0.12	0
E23	0	0	0	0.14	0	0.12	0	0.16	0	0	0	0
E24	0	0.12	0	0	0	0	0.14	0	0.14	0	0.12	0.16
E25	0	0.12	0.12	0	0.12	0	0	0.16	0	0	0.12	0
E26	0	0	0	0	0	0	0	0	0	0.14	0	0
E27	0	0	0	0	0	0	0	0	0	0.28	0	0
E28	0	0	0	0	0	0	0	0	0	0.14	0	0
E29	0	0	0	0	0	0	0	0	0	0.14	0	0

L1, locality 1: Dolsan; L2, locality 2: Odongdo; L3, locality 3: Samcheonpo; L4, locality 4: Hakdong; L5, locality 5: Kyjedo; L6, locality 6: Taejongdae L7, locality 7: The western part of South Sea; L8, locality 8: The eastern part of East Sea; L9, locality 9: The western part of Yellow Sea; L10, locality 10: The westsouthern; L11, locality 11: The southwest; L12, locality 12: The western part of Jejudo. Numbers in parentheses indicate sample size of each population.

(Fig. 4). One group (Clade A) consisting of haplotypes E27, E28, E29, and E26 formed a monophyletic group, which differed by 10-12 nucleotides (see Table 2, 2.9-3.5%) among them. Haplotypes E27, E28, and E29 were nested within haplotype E26, which was somewhat weakly supported by bootstrap analysis (>55% of frequency). The other group (Clade B) consisted of a large number of haplotypes (25 haplotypes among 29 individuals), of which the overall sequence divergence were moderate (see Table 2, 1-4 nucleotides difference, 0.3-1.2%) and strongly supported by bootstrap analysis (>75%). These four haplotypes (E27, E28, E29, and E26) and Clade B were weakly supported by bootstrap analysis (51%), which was found only in locality 10 (westsouthern water). The four haplotypes found in the westsouthern (locality 10) alone formed a relatively strong monophyletic group, separated from clade B in PHYLIP analysis.

Genetic diversity

Within-locality genetic diversity was estimated in terms of haplotype diversity (H) and nucleotide diversity (π) (Table 4). Most of the localities yielded a higher number of haplotypes, ranging from 5-8. Hakdong (locality 4), Taejongdae (locality 6), the western part of South Sea (locality 7), the eastern part of East Sea (locality 8), the western part of Yellow Sea (locality 9), westsouth (locality 11), and the western part of Jejudo (locality 12) obtained the number of haplotype of 100% against sample size, indicating more higher the number of haplotype in offshore waters than that of inshore waters. The H (minimum=0, maximum=1) ranged in 0.75-1.00. Even if the localities with relatively small sample size (6-8 individuals), the estimate was remarkably high. The nucleotide diversity (π) also was markedly high, ranging from 0.015 (Hakdong; locality

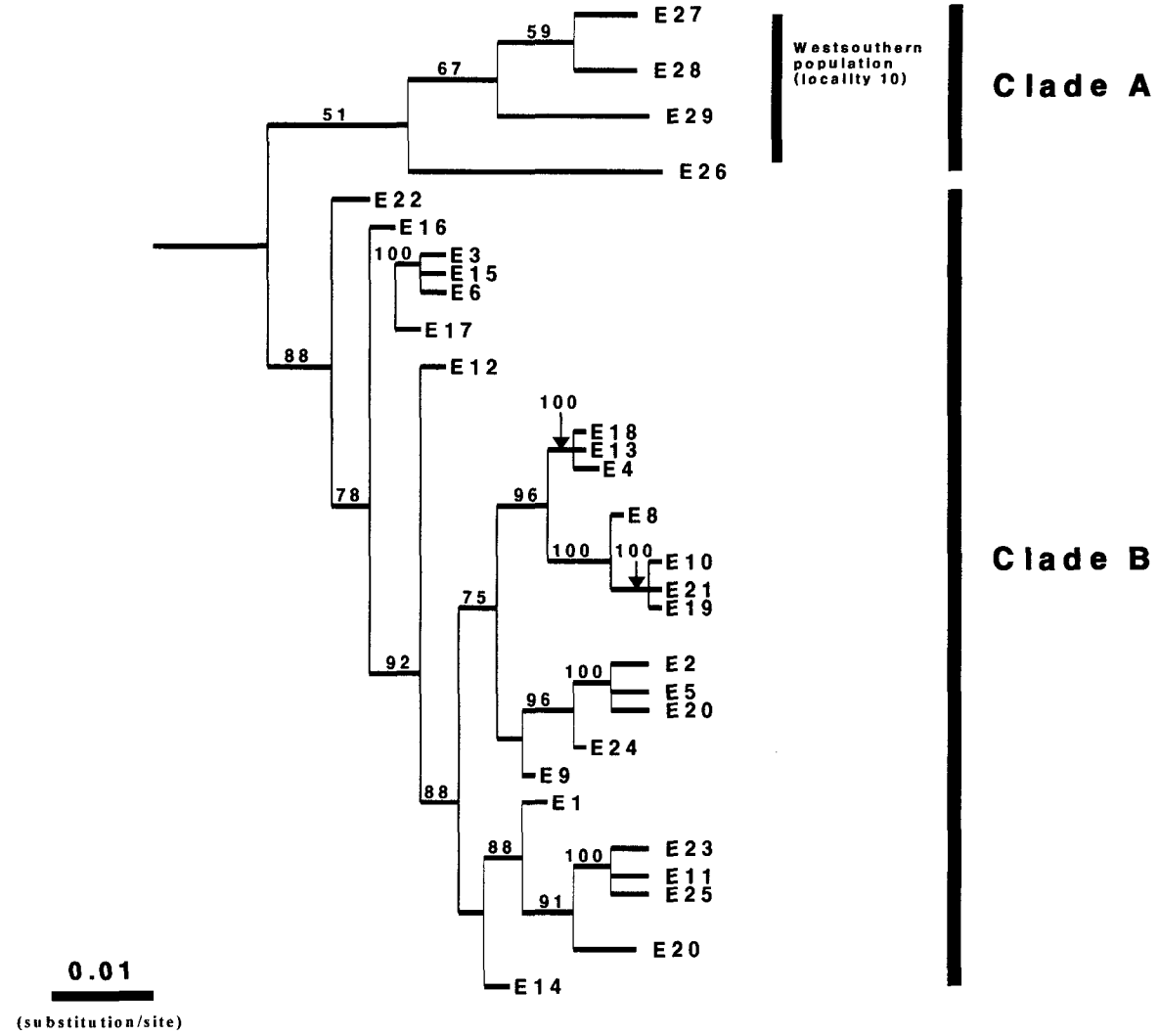

Fig. 4. PHYLIP analysis of mitochondrial control region gene sequences using mtDNA sequence alignment of 29 *Engraulis japonicus*. The phylogenetic tree constructed using subprogram NEIGHBOR incorporated in PHYLIP with the option of Kimura's 2-parameter method (1980). The tree was rooted using *Betta strohi*. The numbers shown on branches, which represent bootstrap values for 100 replications, were obtained from using the subprogram CONSENSE.

Table 4. Within-locality diversity estimates

Locality	SS[a]	NH[b]	H[c]	π[d]
1. Dolsan	7	5	0.75	0.0027
2. Odongdo	8	7	0.82	0.0020
3. Samcheonpo	8	7	0.88	0.0018
4. Hakdong	7	7	1.00	0.0015
5. Kyjedo	8	7	0.86	0.0022
6. Taejongdae	8	8	1.00	0.0019
7. The western part of South Sea	7	7	1.00	0.0079
8. The eastern part of East Sea	6	6	1.00	0.0133
9. The western part of Yellow Sea	7	7	1.00	0.0088
10. The westsouthern	7	6	0.95	0.0244
11. The southwest	8	8	1.00	0.0092
12. The western part of Jejudo	6	6	1.00	0.0123

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

4)-0.0244 (westsouthern; locality 10). The highest estimate in westsouthern (locality 10) is possibly associated with the existence of the E26, E27, E28, and E29 haplotypes found in 7 individuals.

Gene flow

The genetic distance (F_{ST}), coancestry coefficients (D), and per-generation migration rates (Nm) were shown in Table 5. Analysis of F_{ST} between populations in inshore waters ranged in 0.015-0.053 [maximum: comparison between Samcheonpo (locality 3) and Odongdo (locality 2)], whereas those of offshore waters ranged in 0.015-0.584 [maximum: comparison between westsouthern (locality 10) and the western part of Yellow Sea (locality 9)]. The esti-

Table 5. Mitochondrial control region gene sequence of genetic distance (F_{ST}), coancestry coefficients (D), and per generation female migration rate (Nm) of each locality

Locality	1	2	3	4	5	6	7	8	9	10	11	12
1	–											
2	F_{ST} = 0.0157 D = 0.0254 Nm = 24.58	–										
3	F_{ST} = 0.0224 D = 0.0322 Nm = 22.61	F_{ST} = 0.0534 D = 0.0620 Nm = 30.23	–									
4	F_{ST} = 0.0257 D = 0.0307 Nm = 29.97	F_{ST} = 0.0224 D = 0.0297 Nm = 30.29	F_{ST} = 0.0159 D = 0.0199 Nm = 25.38	–								
5	F_{ST} = 0.0341 D = 0.0379 Nm = 37.44	F_{ST} = 0.0157 D = 0.0309 Nm = 33.67	F_{ST} = 0.0243 D = 0.0366 Nm = 26.28	F_{ST} = 0.0345 D = 0.0442 Nm = 40.97	–							
6	F_{ST} = 0.0266 D = 0.0407 Nm = 33.45	F_{ST} = 0.0442 D = 0.0634 Nm = 29.12	F_{ST} = 0.0321 D = 0.0355 Nm = 33.12	F_{ST} = 0.0255 D = 0.0299 Nm = 26.42	F_{ST} = 0.0311 D = 0.0423 Nm = 34.22	–						
7	F_{ST} = 0.0153 D = 0.0159 Nm = 28.74	F_{ST} = 0.0221 D = 0.0337 Nm = 40.19	F_{ST} = 0.0459 D = 0.0625 Nm = 33.66	F_{ST} = 0.0141 D = 0.0178 Nm = 38.89	F_{ST} = 0.0542 D = 0.0634 Nm = 39.26	F_{ST} = 0.0634 D = 0.0753 Nm = 29.67	–					
8	F_{ST} = 0.0301 D = 0.0378 Nm = 37.21	F_{ST} = 0.0123 D = 0.0157 Nm = 35.12	F_{ST} = 0.0524 D = 0.0633 Nm = 40.93	F_{ST} = 0.0245 D = 0.0355 Nm = 42.66	F_{ST} = 0.0214 D = 0.0309 Nm = 36.47	F_{ST} = 0.0225 D = 0.03657 Nm = 32.55	F_{ST} = 0.0512 D = 0.0632 Nm = 40.69	–				
9	F_{ST} = 0.0229 D = 0.0338 Nm = 39.45	F_{ST} = 0.0214 D = 0.0309 Nm = 34.17	F_{ST} = 0.0245 D = 0.0355 Nm = 38.64	F_{ST} = 0.0156 D = 0.0305 Nm = 33.68	F_{ST} = 0.0339 D = 0.0429 Nm = 40.51	F_{ST} = 0.0226 D = 0.0337 Nm = 39.82	F_{ST} = 0.0332 D = 0.0429 Nm = 44.24	F_{ST} = 0.0108 D = 0.0233 Nm = 29.57	–			
10	F_{ST}=0.4242* D = 0.4297 Nm = 15.39	F_{ST}=0.5971** D = 0.6381 Nm = 20.15	F_{ST}=0.2042* D = 0.4062 Nm = 22.67	F_{ST}=0.4578* D = 0.5892 Nm = 25.92	F_{ST}=0.4310* D = 0.5973 Nm = 20.64	F_{ST}=0.3314* D = 0.4891 Nm = 16.97	F_{ST}=0.3243* D = 0.4223 Nm = 20.62	F_{ST}=0.2472* D = 0.3972 Nm = 22.39	F_{ST}=0.5854** D = 0.6257 Nm = 16.33	–		
11	F_{ST}=0.0188 D = 0.0245 Nm = 44.21	F_{ST}=0.0355 D = 0.0521 Nm = 39.26	F_{ST}=0.0351 D = 0.0408 Nm = 35.64	F_{ST}=0.0441 D = 0.0559 Nm = 30.95	F_{ST}=0.0108 D = 0.0239 Nm = 37.46	F_{ST}=0.0175 D = 0.0257 Nm = 36.21	F_{ST}=0.0224 D = 0.0356 Nm = 38.20	F_{ST}=0.0478 D = 0.0552 Nm = 45.67	F_{ST}=0.0159 D = 0.0235 Nm = 29.57	F_{ST}=0.3472* D = 0.4453 Nm = 11.57	–	
12	F_{ST}=0.0204 D = 0.0307 Nm = 36.55	F_{ST}=0.0201 D = 0.0300 Nm = 32.69	F_{ST}=0.0108 D = 0.0233 Nm = 33.67	F_{ST}=0.0230 D = 0.0357 Nm = 36.92	F_{ST}=0.0124 D = 0.0238 Nm = 33.59	F_{ST}=0.0355 D = 0.0497 Nm = 35.78	F_{ST}=0.0157 D = 0.2042 Nm = 32.51	F_{ST}=0.0105 D = 0.0238 Nm = 46.28	F_{ST}=0.0427 D = 0.0563 Nm = 32.27	F_{ST}=0.5143** F_{ST}=0.0307 D = 0.5177 D = 0.0395 Nm = 15.32 Nm = 44.61	–	

1. Dolsan; 2, Odongdo; 3, Samcheonpo; 4, Hakdong; 5, Kyjedo; 6, Taejongdae; 7, The western part of South Sea; 8, The eastern part of East Sea; 9, The western part of Yellow Sea; 10, westsouthern; 11, southwest; 12, The western part of Jejudo. *p<0.05; **p<0.01.

mate (F_{ST}) of the populations between inshore and offshore waters was ranged in 0.015-0.597 [maximum: comparison between westsouthern (locality 10) and Odongdo (locality 2)]. Interestingly, the estimate obtained in a comparison between westsouthern (locality 10) and all of the populations was statistically significant, suggesting overall genetic differentiation between localities. Pairwise comparisons of coefficients of coancestry (0-1, where D=0 is identical, shared ancestry) ranged in 0.015-0.638, which were also consistent with the F_{ST} estimates. The highest pairwise D was obtained for westsouthern (locality 10) and Odongdo (locality 2, D=0.638), whereas the western part of South Sea (locality 7) and Dolsan (locality 1) showed the lowest coancestry coefficient (D=0.015). Consequently, overall pairwise D excluding for westsouthern (locality 10) was >0.07, indicating shared ancestry of the populations involved. The analysis of the per-generation migration rate estimates (Nm) showed that a high gene flow occurred in inshore (Nm=22.61-34.22) and also offshore (Nm=11.57-45.67) populations. The highest 44.21 Nm estimate was obtained between southwest (locality 11) and the eastern part of East Sea (locality 8), whereas the lowest (Nm=11.57) was obtained between southwest (locality 11) and westsouthern (locality 10). In particular, westsouthern (locality 10) found relatively low rate of migration (Nm<26)) compared with other populations. Regardless, they represent what appears to be a high gene flow among the anchovy populations.

Genetic structure

The hierarchical relationship among the populations analyzed by M&M method (1996) was shown in Fig. 5. Most localities were grouped together with neighboring ones, excluding for the subgroup consisting of westsouthern (locality 10). The deeper node, which includes each sub-group [e.g. Odongdo (locality 2) and Kyjedo (locality 5), and Hakdong (locality 4) and Samchenonpo (locality 3)] showed no statistical significance at all. On the basis of the deepest node, genetic distance (d) in some nodes [Odongdo (locality 2) and Kyjedo (locality 5), Hakdong (locality 4) and Samcheonpo (locality 3), southwest (locality 11 and the western part of Yellow Sea (locality 9), and the eastern part of East Sea (locality 8) and the western part of South Sea (locality 7)] was negative, suggesting that the anchovy found in those waters appear to form one large and close genetic group regardless of their geographic barrier. Statistically significant genetic structure showed that westsouthern (locality) was completely separated from the group consisted of localities 2, 5, 4, 3, 11, 9, 8, 7, 6, and 12 (p=0.000)

Hierarchical level

Genetic variance and fixation index (Φ) of each hierarchical level was shown in Table 6. The within-locality genetic variance and fixation index was 93.29, and 0.027, respectively. These estimate was statistically significant (p<0.01), suggesting that most of genetic diversity in the anchovy exist within locality rather than at hierarchical

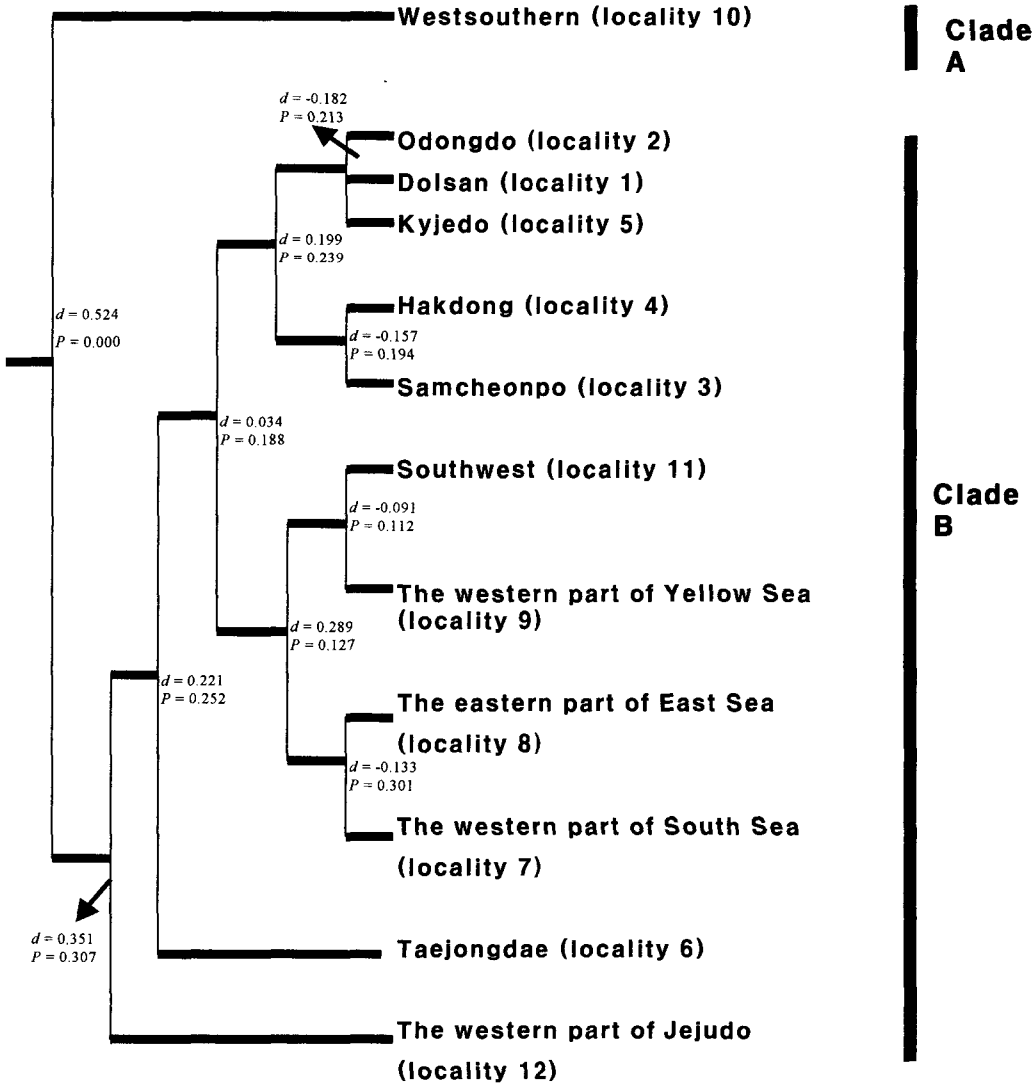

Fig. 5. Hierarchical genetic relationships among localities analyzed using Holsinger and Mason-Gamer method (1996). The value at each node is the genetic distance (d) between its two daughter nodes and the p value is the significance of differentiation based on 10,000 random resampling.

Table 6. Hierarchical analysis of variance

Source of variation	Group A vs Group B			
	d.f	%	Φ	p
Between regions	3	49.57	0.418	*
Among localities within regions	9	2.67	0.117	*
Within localities	55	93.29	0.027	**

d.f: degree of freedom; %: percentage of variation; Φ: fixation index; p: significant of percentage variation and fixation indices estimated from permuation tests (1,000 permutations); $^{*}p<0.05$; and $^{**}p<0.01$.

Group A includes 1. Dolsan, 2. Odongdo, 3. Samcheonpo, 4. Hakdong, 5. Kyjedo, 6. Taejongdae, 7. The western part of South Sea, 8. The eastern part of East Sea, 9. The western part of Yellow Sea, 11. The southwest, and 12. The western part of Jejudo. Group B includes 10. The westsouthern.

level and mostly compose of heterogeneous individuals. Also, the estimate of genetic variance and fixation index between regions was 49.57 and 0.418, respectively, which was significantly different ($p<0.05$) like the component of within-localities. This indicates that there is maximized genetic heterogeneity among regions, consistent with that obtained from F_{ST} analysis and H-MG method (see Table 4). Furthermore, genetic variance and fixation index among localities within regions were 2.67 and 0.117, respectively. These estimates were significantly different ($p<0.05$), suggesting that there is genetic distance among localities, consistent with that obtained from F_{ST} analysis (see Table 4).

Discussion

The estimation of the genetic distance among populations of the genus *Engraulis* using starch gel electrophoresis of proteins has reported a genetic homogeneity (F_{ST} in parentheses): *E. capensis* Gilchrist (0.005)[12]; *E. mordax* Girard (0.032)[15]; *E. encrasicolus* Linnaeus (0.003-0.026) [37]. Furthermore, numerous studies concluded that genetic differentiation among populations seemed to be somewhat low[3,14,34]. However, our current molecular data is much more sensitive than the enzyme electrophoresis technique; in particular, the mtDNA control region gene used in this study provided much greater resolution in the process of identifying heterozygosity within populations and geographic heterogeneity among populations of the anchovy (*E. japonicus*) than that found in a previous study that used an mtDNA 12S ribosomal RNA gene[20]. Although the number of samples available was relatively small, the significant difference in genetic distance between westsouthern (locality 10) and all of the populations (localities 1-9, 11, and 12) was suggestive of geographic subdivision, as reflected in the F_{ST} value of 0.20-0.58 (see Table 5). This value is as much as ten times higher comparing between the populations, excluding westsouthern anchovies (locality 10) (see Table 5). Wright[39] reported that G_{ST} values of less than 0.05 were considered as indicative of the absence of genetic differentiation. Our results (homogeneity of haplotype frequencies, F_{ST} value of range in 0.01-0.05) supported the absence of genetic structuring and found no significant association between geography and haplotype distribution among the populations in the anchovy (*E. jaonicus*), excluding westsouthern anchovies (locality 10). This was associated with the existence of a high level of gene flow of the anchovy (*E. japonicus*) around Korean waters (Table 5).

As a result of the mtDNA analysis in this study, several haplotypes (E26, E27, E28, and E29) were found in a single locality (four among 7), indicating geographic restriction in their distribution. However, E9 haplotype was represented as a major one in Korean waters based on the frequency of a total of 58.3% and the distribution (several localities in inshore and offshore waters, Table 3). Possibly due to these extensively distributed haplotypes, a high genetic relatedness among localities was shown in the subsequent F_{ST} analysis (Table 5), and one large connected population over a wide geographic range was observed in the analysis of genetic structure (Fig. 5). Even if several distinct spawning grounds adjacent to Korea and China were found, possibly most of the populations (localities 7, 8, 9, and 11) migrated to more passive southern coastal waters for spawning or feeding from the western, eastern, and southern offshore area of Korea than to western and eastern inshore waters in the analysis of geographic population structure (d=0.03, p=0.188, see Fig. 5). The adult populations of Taejongdae (locality 6) and the western part of Jejudo (locality 12) were assumed to have a more northward migration than a southward migration (d=0.22, d=0.35, respectively, see Fig. 5). However, westsouthern (locality 10) population was severely restricted in its migration toward southern coastal waters for spawning or even feeding based on the data of geographical population structure (d=0.52, p=0.00, see Fig. 5 and Table 6). Probably, this independent population appears to migrate more to the coastal waters of China than to the coastal waters of Korea.

Yu *et al.*[40] proposed three models for the adult population structure of the anchovy (*E. japonicus*): first, demographically independent; second, connection by gene flow; third, a panmictic population structure. Overall, our current populations should be close to a panmictic structure among 3 models under the influence of larval migration or adult migration in the analysis of geographic and population differentiations (Table 5, and Fig. 5). Consequently, our results overall support the general observation that the genetics of marine populations including the anchovy, with a pelagic larval stage, has often been characterized by low genetic variation among populations and has a high genetic relatedness due to increased potential for the larvae dispersal and the migration of adults in the spawning or feeding grounds[12,29,30,31,32,37].

Population genetic studies have showed evidence of unusually high levels of genetic heterogeneity in pelagic fish[13,15,35]. Likewise, significant heterogeneity in mtDNA in anchovy populations has been reported [4,27]. In the present study, we also found a significantly different population based on the estimation of geographical distance (Table 5), population structure (Fig. 5), and genetic variance (Table 6). Generally, a high level of gene flow is associated with maintaining homogeneity within a group across geographically distant regions. However, westsouthern (locality 10), which is between all localities, indicates that effective dispersal is relatively limited due to the presence of barriers against gene flow. Several researchers have suggested that genetically distinct populations are associated with geographic heterogeneity: oceanographic discontinuity [3], freshwater discharge[9,38], and different reproduction[10,37]. Limited migration events caused by geographic and physical barriers are sufficient to provide the existence of temporally or spatially isolated populations over time. Indeed, heterogeneous population in this study is not associated with heterogeneous environments (i.e. geographic scale and hydrographic restriction) that are limited to effective gene flow or dispersal between populations. After spawning and external fertilization, the anchovy (*E. japonicus*) larvae spend a variable period of time developing, which can include passively drifting in ocean currents. A strong influence of water current will create a high level of gene flow, whereas it will cause the population to more toward a heterogeneous composition. Consequently, the occurrence of a heterogeneous population for our current study may be associated with a continuous pattern according to genetic drift, which usually occurs in the high population densities and large geographic ranges of many marine species[3,16].

In conclusion, this study confirmed the previous finding that the anchovy populations occurring in Korean offshore waters were formed of individuals randomly dispersed over geographic areas and that the gene flows between populations were relatively high. In particular, we found a genetically distinct population that was different compositions of haplotypes and statistically different based on the estimation of geographical distance, population structure, and variance analysis, although no clear reason could be given for the differences.

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초록 : 한국근해 및 외해역에 채집된 멸치의 미토콘드리아 DNA 다양성

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멸치의 유전적 집단구조 및 지리적 거리를 조사하기 위하여 한국근해 및 외해역 12개 정점에서 채집된 멸치의 미토콘드리아 DNA control 부위를 대상으로 염기서열을 상호 비교 및 분석했다. 염기서열 분석결과 89개체 중 29 haplotype이 나타났고, 상호 염기치환율은 0-3.5% 차이를 보였다. E9 haplotype이 근해 및 외해역에서 가장 넓게 분포하고 있는 것으로 나타났다 (58.3%). 반면에, E26, E27, E28, E29 haplotype 들은 서남해역 (정점 10)에서만 보였다. PHYLIP 프로그램을 이용한 유전적 관계에서도 두개의 clade로 분리되었다. E26, E27, E28, E29 haplotype을 제외한 나머지 haplotype 들은 상호 잘 유지되는 것으로 나타났다 (bootstrap 75% 이상). 그러나 clade A와 B bootstrap은 매우 약하게 나타났다 (51%). haplotype 간의 상호분석 결과 다양도는 0.75-1.00, 염기다양도는 0.015-0.0244로 보였다.