

Differences and Variations among *Anguilla japonica*, *Muraenesox cinereus* and *Conger myriaster* from the Yellow Sea

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ABSTRACT : Genomic DNAs were extracted from the muscle of twenty-one specimens of three eel species collected in *Anguilla japonica* (AJ), *Muraenesox cinereus* (MC) and *Conger myriaster* (CM) from the Yellow Sea, respectively. In the present study, 7 oligonucleotides primers generated 191 specific loci in the AJ species, 226 in the (MC) species and 181 in the CM species, respectively. The primer BION-02 generated the most loci (a total of 83), with an average of 11.86 in the AJ species. The specific loci generated by oligonucleotides primers exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. With regard to average bandsharing value (BS) results, individuals from *Conger myriaster* species (0.808) exhibited higher bandsharing values than did individuals from *Muraenesox cinereus* species (0.729) ($P<0.05$). The longest genetic distance (0.430) displaying significant molecular difference was also between individual no. 01 within *Anguilla japonica* eel species and individual no. 04 within *Anguilla japonica* species. In this study, the dendrogram resulted from reliable seven oligonucleotides primers, indicating three genetic clusters composed of group I (ANGUILLA 01~ANGUILLA 07), group II (MURAENESOX 08~MURAENESOX 14) and group III (CONGER 15~CONGER 21). The existence of species differentiation and DNA polymorphisms among three eel species were detected by PCR analysis. As mentioned above, a dendrogram revealed close relationships between individual identities within three eel species. High levels of a significant genetic distance among three eel species showed this PCR approach is one of the most suitable tools for individuals and/or species biological DNA studies.

Key words : *Anguilla japonica*, *Conger myriaster*, Eel species, Genetic distance, *Muraenesox cinereus*

INTRODUCTION

Three eel species such as *Anguilla japonica* (AJ), *Muraenesox cinereus* (MC) and *Conger myriaster* (CM), belonging to the order Anguilliformes, are the most popular marine products in Korea because of their taste and nutritional value, and Koreans consume them in large quantities. Eel, ecologically important warm water fish species widely distributed on the coast of the Yellow Sea, southern sea

and the several sea areas under the natural ecosystem. As the eel culture industry grows, so doe's interest into the genetics of this finfish species. However, in spite of their economic and scientific consequences, a little information currently exists regarding the genetic levels only of eel species in Korea and/or the other countries (Min & Yang, 1993; Jing & Li, 1999; Kimura et al., 2004; Oh, 2011). In this study, to explicate the genetic distances and differences among geographical eel species, the author accomplished a

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clustering analysis of three eel species collected from the Yellow Sea.

MATERIALS AND METHODS

PCR analysis was performed on DNA samples extracted from a total of 21 individuals using seven oligonucleotides primers. Muscle tissues were obtained separately from individuals from *Anguilla japonica*, *Muraenesox cinereus* and *Conger myriaster*, respectively. Eel muscle was collected in sterile tubes, instantaneously placed in liquid nitrogen, and stored at -40°C until the genomic DNA extraction. Genomic DNA was extracted and purified under the conditions described previously (Kim et al., 2006; Yoon, 2008). After several washings, lysis buffer I (155 mM NH_4Cl ; 10 mM KHCO_3 ; 1 mM EDTA) was added to the samples, and the mixture tubes were gently inverted. The concentration of the extracted genomic DNA was measured by optical density at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK). PCR was performed using two Programmable DNA Thermal Cyclers (MJ Research Inc., Waltham, MA, USA). Seven oligonucleotides primers such as BION-02 (5'-CAATCGCCGT-3'), BION-14 (5'-TGGATTGGTC-3'), BION-22 (5'-GGTACTCCAC-3'), BION-30 (5'-GCCACCTCCT-3'), BION-34 (5'-GCCGCTACTA-3'), BION-35 (5'-AGCGGCTAGG-3') and BION-40 (5'-GTTGCGATCC-3') were displayed to generate the shared loci, specific loci, unique shared loci to each species and shared loci by the three species which could be obviously scored. The 100-bp DNA ladder (Bioneer Corp., Daejeon, Korea) was used as the DNA molecular weight marker. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a photoman direct copy system. Euclidean genetic distances within- and between-species were also calculated using the hierarchical dendrogram program Systat ver.10 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The seven oligonucleotides primers were shown to generate the shared loci, specific loci, unique shared loci to each species and shared loci by the three species which could be obviously scored. In the present study, 7 oligonucleotides primers generated 191 specific loci in the AJ species, 226 in the MC species and 181 in the CM species, respectively, as illustrated in Table 1. The primer BION-02 generated the most loci (a total of 83), with an average of 11.86 in the AJ species. The oligonucleotides primer

Table 1. The number of total loci, average loci per lane, specific loci by PCR analysis using 7 oligonucleotides primers from three eel species in the Yellow Sea of Korea

Item	No. of average loci per lane			No. of specific loci		
	AJ	MC	CM	AJ	MC	CM
Primer						
BION-02	11.86 (83)	11.29 (79)	10.71 (75)	56	49	63
BION-14	8.29 (58)	9.29 (65)	9.71 (68)	37	44	19
BION-22	3.71 (26)	3.57 (25)	3 (21)	12	11	14
BION-30	7.43 (52)	10.29 (72)	9.71 (68)	17	44	26
BION-34	8.71 (61)	8.71 (61)	8.57 (60)	26	19	25
BION-35	4.71 (33)	6.57 (46)	6.43 (45)	33	39	24
BION-40	4.43 (31)	4.86 (34)	4.43 (31)	10	20	10
Total No.	49.14 (344)	54.57 (382)	52.57 (368)	191	226	181
Average no. per primer	49.14	54.57	52.57	27.29	32.29	25.86

AJ: *Anguilla japonica*, MC: *Muraenesox cinereus*, CM: *Conger myriaster*

BION-22 generated the least loci (a total of 21), with an average of 3.00 in the CM species, in comparison to the other primers used. The specific loci generated by oligonucleotides primers exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. The gDNA isolated from three eel species were amplified by PCR. Here, the seven oligonucleotide primers were used to generate the unique shared loci to each species and shared loci by the three eel species, as illustrated in Table 2. With regard to average bandsharing value (BS) results, individuals from *Conger myriaster* species (0.808) exhibited higher bandsharing values than did individuals from *Muraenesox cinereus* species (0.729) ($P < 0.05$), as displayed in Table 3. The dendrogram resulted from reliable seven oligonucleotides primers, indicating three genetic clusters composed of group I (ANGUILLA 01~ANGUILLA 07), group II (MURAENESOX

Table 2. The number of unique shared loci to each species and number of shared loci by the three species generated by PCR analysis using 7 oligonucleotides primers in Korean eel fish such as *Anguilla japonica* (AJ), *Muraenesox cinereus* (MC) and *Conger myriaster* (CM), respectively

Item	No. of unique loci to each species			No. of shared loci by the three species
	AJ	MC	CM	
	Three species (7 individuals per species)			
BION - 02	27	30	12	126
BION - 14	21	21	49	21
BION - 22	14	14	7	21
BION - 30	35	28	42	63
BION - 34	35	42	35	28
BION - 35	0	7	21	0
BION - 40	21	14	21	42
Total no.	153	156	187	301
Average no. per primer	21.86	22.29	26.71	43

Table 3. Multiple comparisons of average bandsharing values among three eel species were created according to the bandsharing values and similarity matrix

Species	<i>A. japonica</i>	<i>M. cinereus</i>	<i>C. myriaster</i>
<i>A. japonica</i>	0.755±0.065 ^b	0.661±0.053 ^a	0.672±0.042 ^a
<i>M. cinereus</i>	-	0.729±0.084 ^b	0.728±0.060 ^b
<i>C. myriaster</i>	-	-	0.808±0.037 ^c

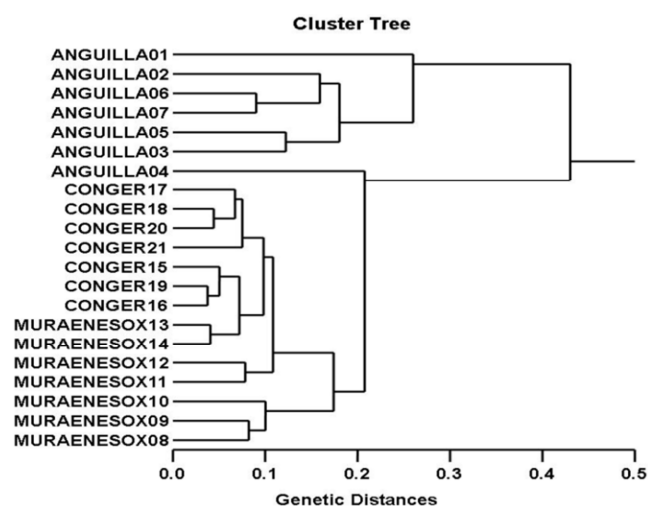


Fig. 1. Hierarchical dendrogram of genetic distances obtained from three eel species. The relatedness among different individuals of Korean eel species from *Anguilla japonica* (ANGUILLA 01 ~ ANGUILLA 07), *Muraenesox cinereus* (MURAENESOX 08 ~ MURAENESOX 14) and *Conger myriaster* (CONGER 15 ~ CONGER 21) generated according to the bandsharing values and similarity matrix.

08~MURAENESOX 14) and group III (CONGER 15~ CONGER 21), as illustrated in Fig. 1. The longest genetic distance (0.430) displaying significant molecular difference was also between individual no. 01 within *Anguilla japonica* eel species and individual no. 04 within *Anguilla japonica* species. In the present study, PCR analysis revealed a significant genetic distance among three eel species. The existence of species differentiation and DNA polymorphisms among three eel species were detected by PCR analysis. This displays that the method is one of the appropriate

tools for comparing the DNA of individuals, populations and/or species of eel. However, more researches will also be necessary to identify the discrimination between/among populations and species, using an annealing control primer system. From what has been said above, the potential of this analysis to ascertain diagnostic markers for the identification of three eel species has also been verified (Tasanakajon et al., 1998; Jing & Li, 1999; McCormack et al., 2000; Kimura et al., 2004; Kim et al., 2006; Yoon, 2008). It was reported that the species relationships revealed by the PCR approach should be consistent with previously obtained data using morphological portrait (Nebauer et al., 2000). As mentioned above, a dendrogram revealed close relationships between individual identities within three eel species. Thus, this PCR analysis revealed a significant genetic distance among the three eel species. High levels of a significant genetic distance among three eel species showed this PCR approach is one of the most suitable tools for individuals and/or species biological DNA studies. Therefore, this method can also be applied to other order of Anguilliformes and make technically-convenient the analysis of many samples in a short time.

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