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Molecular DNA Systematic Analyses of East Asian Mammals: Sequence Variation of Cytochrome *b* Gene and Control Region of Mitochondrial DNA of Common Otter, *Lutra lutra lutra* L. (Mammalia, Carnivora) from Korea

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Sequences of cytochrome *b* gene and control region of mitochondrial DNA from Korean common otters (*Lutra lutra lutra* L.) were examined to provide the genetic information for the conservation of this subspecies. Two haplotypes and one haplotype were revealed in cytochrome *b* gene and control region, respectively. The available sequences of European common otter (*L. l. lutra*) from GenBank were compared together with those of Korean common otter in order to determine the degree of sequence variation between them. In cytochrome *b* gene sequences, two haplotypes from Korea and two haplotypes of Europe showed differences in 12 of 1,045 sites. The Tamura-Nei nucleotide distances between two European haplotypes was 0.10% and those between two Korean haplotypes was also 0.10%, but those between Korean haplotypes and European ones ranged from 0.96% to 1.16%. In the control region, one Korean haplotype and seven European ones showed differences in seven of 300 sites; the Tamura-Nei distances among seven European haplotypes were 0.34% to 1.01%, but those between Korean haplotype and European ones ranged from 1.01% to 1.69%. Although further molecular and morphological studies with specimens from eastern Asia including Amur region and northeast China are needed, it is possible that the Korean common otter might be closer or identical to the far-eastern Asian common otter, *L. l. amurensis* Dybowski.

The common otter (*Lutra lutra* L., 1758) is distributed widely in Eurasia, however, it is listed as endangered in CITES and also in IUCN (Wozencraft, 1993). In Korea, *L. lutra* is designated as one of the Natural Monuments (Won, 1992), and the Ministry of Environment listed it as endangered.

Taxonomy of *L. lutra* has been very controversial (Corbet, 1978): Corbet did not recognize any subspecies. However, common otter in Japan (*L. l. nippon*) was elevated to the species rank, *L. nippon* (Imaizumi and Yoshiyuki, 1989). Moreover, Ellerman and Morison-Scott (1951) recognized 10 subspecies and mentioned that the distribution of subspecies *L. l. lutra* includes Europe, Russia, Mongolia, and northeast China, although common otter from Amur and Ussuri regions has been considered as a different subspecies, *L. l. amurensis* Dybowski. Common otter in northeast China is classified as a subspecies, *L. l. lutra* (Zhang et al., 1997), and that in Korea is also considered as *L. l. lutra* (Won, 1967).

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Molecular genetic studies have become widely used in the past decade for the conservation of endangered species (Hedrick, 2001). Mitochondrial DNA (mtDNA) is a marker suitable for taxonomic reconsideration of closely related species or populations of a variety of species (Wilson et al., 1985).

Mitochondrial cytochrome *b* gene sequences have been informative at various taxonomic levels in mammals including carnivores (Irwin et al., 1991; Masuda and Yoshida, 1994). In addition, mtDNA control region has been attractive to evolutionary biologists for fine scale comparative studies, because it is believed to be one of the fastest evolving segments in the animal mtDNA genome (Dillon and Wright, 1993).

We analyzed mtDNA cytochrome *b* gene and control region sequences of three Korean common otters to provide genetic information for its conservation. We also compared the sequences obtained in this study with those available for European common otter (*L. l. lutra*) in the GenBank. The degree of genetic diversity between them was determined, and a proper subspecies name of Korean common otter was suggested.

Materials and Methods

Two individuals of common otter from Yeongdong-Gun (Chungbuk, Korea) and one from Daejeon (Chungnam, Korea) were used in this study. Before their burial small bits of tissues were taken and preserved in a deep freezer. From muscle samples, total cellular DNA was extracted (Hillis et al., 1996): 500 μl of STE buffer (0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 25 μl of 10 mg/ml stock of proteinase K, and 25 μl of 20% SDS were added into a micro tube containing minced tissue. The tube was incubated at 55°C for 2 h, and DNA was extracted with equal volume of PCI and chloroform, and then DNA was precipitated with 2 volumes of ethanol. After adding RNase A (10 mg/ml), the solution was incubated at 37°C for 2 h and DNA was extracted again.

The cytochrome *b* gene was PCR-amplified using primers L14724, H15149, L15513, and H15915 designed by Irwin et al. (1991). The primers L-Pro and H-Phe were used for the control region sequences (Randi et al., 1998). PCR thermal cycle was as follows: 94°C for 5 min; 94°C for 1 min, 57°C for 1 min, 72°C for 1 min (32 cycles); 72°C for 5 min. To remove unused primers and unincorporated nucleotides, the PCR products were purified using DNA PrepMate™ kit with silica-based matrix (Bioneer Co., Korea). Sequencing of the purified PCR products were carried out using an automated DNA sequencer (Perkin Elmer 377) at Macrogen Co. (Seoul, Korea).

Almost full sequences (1,045 of 1,140 bp) of mitochondrial cytochrome *b* gene were obtained from three Korean common otters, and two different haplotypes were found (Daejeon specimen was different from Yeongdong-Gun specimens). The sequences obtained in this study were compared with corresponding sequences of European common otter (*L. l. lutra*) available from the GenBank (GenBank accession numbers AF057124 and X94923).

Almost full sequences (1,080 bp) of mtDNA control region were also acquired from three Korean common otters, but only one haplotype was found. The corresponding sequences of common otter, available from the GenBank, were seven haplotypes (300 bp) from European common otter, *L. l lutra* (accession numbers AJ006174 to AJ006178, AY425896, and AY425897), and eight haplotypes were compared. Tamura-Nei distances (Tamura and Nei, 1993) were calculated using MEGA program (version 1.01).

Results

In mitochondrial cytochrome *b* gene of *L. l. lutra*, two haplotypes from Korea and two haplotypes from Europe differ in 12 of 1,045 sites (Table 1). Tamura-Nei distance between the two European haplotypes was 0.10%, and that between the Korean ones was also 0.10%. However, those between Korean haplotypes and European ones ranged from 0.96% to 1.16%.

Table 1. Haplotype definition and Tamura-Nei distances (%) among four haplotypes of mitochondrial cytochrome *b* gene in common otter from Korea and Europe. Haplotypes Ecy1 and Ecy2 are AF057124 and X94923 from Europe in GenBank, whereas Kcy1 and Kcy2 are from Korea in this study

	1 1 1 2 2 2 4 5 8 8 9 9			
	1 6 8 3 6 9 5 1 4 6 5 8			
Type	7 1 5 6 0 9 5 5 7 0 0 2	Ecy1	Ecy2	Kcy1
Ecy1	T C G G G T A C C T C C			
Ecy2	. . . . . T	0.10		
Kcy1	C T A A A C G T T C T T	1.16	1.06	
Kcy2	C T A A A C . T T C T T	1.06	0.96	0.10

In the control region of mtDNA of *L. l. lutra*, one Korean haplotype and seven European ones differ in seven of 300 sites (Table 2). Tamura-Nei distances among seven European haplotypes were from 0.34% to 1.01%, but those between Korean haplotype and European ones ranged from 1.01% to 1.69%; clear differences were revealed in two of 300 sites between the Korean and the European common otter.

These results strongly suggested that Korean common otter is different from European common otter in mitochondrial DNA sequences.

Discussion

Because of the relaxed functional constraint, the control region may evolve faster than the average rate of substitutions in the protein coding-genes in mtDNA (Brown, 1985). The rate of base substitution in mammalian mtDNA cytochrome *b* gene is 2.1% per Myr (Irwin et al., 1991), whereas that of the control region is from 8.3% to 14.3% (Stewart and Baker, 1994). From the three individuals of Korean common otter, two haplotypes were found in the cytochrome *b* gene, whereas only one

Table 2. Haplotype definition and Tamura-Nei distances (%) among eight haplotypes of mitochondrial control region in common otter from Korea and Europe. Haplotypes Eco1 to Eco7 are AJ006174 to AJ006178, AY425896, and AY425897 of Europe in GenBank, whereas Kco1 is from Korea in this study

	1 1 2 2							
	5 7 9 0 5 3 9							
Type	9 2 5 1 3 7 6	Eco1	Eco2	Eco3	Eco4	Eco5	Eco6	Eco7
Eco1	A G G - C T C							
Eco2	. . A - . . .	0.34						
Eco3	. . . - . C .	0.34	0.67					
Eco4	. . . C . . .	0.34	0.67	0.67				
Eco5	. A . - . . .	0.34	0.67	0.67	0.67			
Eco6	. . . - T C .	0.67	1.01	0.34	1.01	1.01		
Eco7	. . . - T . .	0.34	0.67	0.67	0.67	0.67	0.34	
Kco1	G . . C . C T	1.35	1.69	1.01	1.01	1.69	1.35	1.69

haplotype was found in the control region (Tables 1, 2). It is suggested that these two regions are independent in their evolution, although further analyses with more specimens are necessary.

Mitochondrial DNA is a highly sensitive genetic marker for studies of closely related taxa or populations of a variety of species (Sunnucks, 2000). Cytochrome *b* gene variation has provided some insight on the evolutionary history and phylogenetic relationships in Caniformia, Carnivora (Ledje and Arnason, 1996), and in the comparison of cytochrome *b* gene, the taxonomic position of Japanese river otter as a distinct species was confirmed (Suzuki et al., 1996).

The cytochrome *b* gene sequence divergence between common otter and spotted-necked otter is 12.3% (Koepfli and Wayne, 1998). In mitochondrial cytochrome *b* gene sequences of *L. l. lutra* (Table 1), Tamura-Nei distance between Korean haplotypes and European ones ranged from 0.96% to 1.16%; this result strongly suggested that Korean common otter is distinct from European common otter in cytochrome *b* gene sequences.

Low level of genetic variation (nucleotide diversities within populations ranging from 0.00% to 0.17%) was revealed in European common otter, *L. l. lutra* (Cassens et al., 2000). In the control region sequences of *L. l. lutra* (Table 2), Tamura-Nei distances between Korean haplotype and European ones ranged from 1.01% to 1.69%, suggesting that Korean common otter is also diverged from European common otter in control region sequences of mtDNA.

In summary, the molecular data obtained from this study strongly indicate that the Korean common otter is clearly distinct from European *L. L. lutra*. Although further molecular and morphological studies with specimens from east Asia including Amur region and northeast China are needed, it is possible that the Korean common otter might be closer or identical to the far-eastern Asian common otter, *L. l. amurensis* Dybowski.

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