

**Diversity of Mitochondrial DNA Cytochrome b Gene in  
Roe Deer (*Capreolus pygargus tianschanicus* Satunin)  
from Jejudo Island, Korea**

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**ABSTRACT**

As one of researches to clarify the taxonomic status of roe deer from Jeju island (*C. pygargus tianschanicus*), we analyzed partial sequences of mtDNA cytochrome b gene from six roe deers at Jeju island in Korea. Maximum nucleotide Tamura & Nei's distance among three haplotypes was 0.005, and this distance was comparable to the diversity within other roe deer subspecies: it is suggested that roe deers from the mainland dispersed rarely to Jejudo Island, although further analyses are necessary to decide whether or not it was occurred by human introduction. Furthermore, nucleotide distance between cytochrome b sequences of roe deers from Jeju (*C. pygargus tianschanicus*) and the sequence of roe deer from west Siberia (*C. p. pygargus*), obtained from GenBank, was average 0.013, and it is suggested that *C. p. tianschanicus* diverged from *C. p. pygargus* of west Siberia 0.65 Myr ago.

Key words: Systematics, mtDNA cytochrome b gene, roe deer, Korea

**INTRODUCTION**

Mitochondrial (mt) DNA is a highly sensitive marker suitable for studies of closely related taxa or populations of a variety of species because of its fast rate of evolution and characteristic maternal inheritance (Wilson *et al.*, 1985), and mtDNA analysis has also helped phylogenetic studies of Artiodactyla (Gatesy *et al.*, 1992; Miyamoto *et al.*, 1993). In particular, mtDNA cytochrome b gene

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This study was supported by a grant to H. S. Koh from the Korea Science Foundation (97-0402-05-013).

has been used extensively to study variation in mammals (Irwin *et al.*, 1991). Moreover, the molecular data make it possible to estimate the time of divergence of the subspecies: it is generally estimated that the rate of nucleotide substitution in animal mitochondrial genomes is 2% per Myr based on RFLP studies (Brown *et al.*, 1979).

The taxonomy of genus *Capreolus* is not settled in species and subspecies levels. Ellerman and Morriscot (1951) summarized Eurasian roe deer into one species with three subspecies, *C. c. capreolus*, *C. c. pygargus*, and *C. c. bedfordi* including *manchuricus*, *melanotis*, and *ochracea*. Sokolov and Gromov (1990), however, concluded by morphological study that genus *Capreolus* includes 2 contemporary species (European *C. capreolus* and Siberian *C. pygargus*), and Danilkin (1996) also described roe deer into two species. Randi *et al.* (1997) confirmed that *C. capreolus* from west Europe is distinct from *C. pygargus* from east Europe and Asia in their mtDNA control region comparisons.

Sokolov and Gromov (1990) stated that the Siberian roe deer are represented by three distinctive subspecies (*C. p. pygargus*, *C. p. tianschanicus*, and *C. p. manchuricus*), but Danilkin (1996) recognized only two subspecies (*C. p. pygargus* and *C. p. tianschanicus*).

In this study, we analyzed mtDNA sequences of cytochrome b gene of six roe deers from Jeju Island in Korea (*C. p. tianschanicus*) both to investigate the level of genetic diversity among them and to describe phylogenetic relationships with other subspecies of *C. pygargus*.

## MATERIALS AND METHODS

### Sample collection

Six specimens of roe deer (*Capreolus pygargus tianschanicus*) were collected from Jeju Island in Korea, and muscles were kept in a deep-freezer.

### DNA isolation, PCR amplification, and sequencing

From muscle samples, total cellular DNA was extracted (Hillis *et al.*, 1996). 500  $\mu$ l of STE buffer (0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 25  $\mu$ l of 10 mg/ml stock of proteinase K, and 25  $\mu$ l of 20% SDS were added into a micro tube containing minced tissue. It was incubated at 55°C for 2h, and DNA was extracted with equal volume of PCI and chloroform, and then was precipitated with 2 vols. ethyl alcohol. After adding RNase A (10 mg/ml), the solution was incubated at 37°C for 2h, and DNA was extracted again.

The cytochrome b gene was PCR-amplified using primers L14724 (5'-GAT ATG AAA AAC CAT CGT TG-3') and H15149 (5'-CTC AGA ATG ATA TTT GTC CTC A-3') designed by Irwin *et al.* (1991). 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 primer and unincorporated nucleotides, amplified product was purified using DNA PrepMate™ kit with silica-based matrix (Bioneer Co.), which is very effective for the extraction of solid phase DNA and for rapid extraction of high-purified DNA.

For sequencing, the purified PCR products were analyzed with auto-sequencing machine in Korea Basic Science Institute, Dae-Jeon, Korea.

### Data analysis

Partial sequences of the mitochondrial cytochrome b gene from Jeju roe deer (*Capreolus pygargus tianschanicus*) were obtained, and it was compared with the sequences of *C. p. pygargus* from Kurgan of west Siberia and *C. c. capreolus* from west Europe obtained from GenBank (accession number AJ000025 and Y14951, respectively).

Nucleotide pair-wise distance and Tamura & Nei's distance (Tamura and Nei, 1993) were calculated and phylogenetic tree was constructed by neighbor-joining method by MEGA (version 1.01).

## RESULTS

We obtained 391 bp of sequence for a portion of mtDNA cytochrome b gene from six roe deers from Jejuo Island in Korea (*C. p. tianschanicus*), and three haplotypes were revealed, as shown in Fig. 1: two additional haplotypes of mitochondrial DNA cytochrome b sequences in two species of genus *Capreolus* are also given (CPT1, CPT2, and CPT3 are *C. p. tianschanicus* from Jeju island, Korea; CPP, *C. p. pygargus* from Kurgan of west Siberia; CCC, *C. c. capreolus* from west Europe). Sequences of roe deer from Korea are resulted from this study, and sequences of roe deers from Kurgan and west Europe were obtained from GenBank (accession number AJ000025 and Y14951, respectively).

Nucleotide Tamura & Nei's distances among five haplotypes of mtDNA cytochrome b sequences in *Capreolus* are given in Table 1. Maximum nucleotide Tamura & Nei's distance among three cytochrome b gene haplotypes of *C. p. tianschanicus* from Jejuo Island is 0.005, and the nucleotide distance between those of Jeju and Kurgan roe deer was average 0.013.

Phylogenetic tree among five haplotypes of mtDNA cytochrome b sequences in *Capreolus* by Neighbor-joining method is shown in Fig. 2. *C. p. tianschanicus* from Jejuo Island, Korea, is different from *C. p. pygargus* from Kurgan of west Siberia and *C. c. capreolus* from west Europe as well.

**Table 1.** Nucleotide Tamura & Nei's distances among five haplotypes of mtDNA cytochrome b gene in two species of *Capreolus* (haplotypes are labeled as in Fig. 1)

Haplotypes	CPT1	CPT2	CPT3	CPP
CPT2	0.005			
CPT3	0.003	0.003		
CPP	0.016	0.010	0.013	
CCC	0.040	0.034	0.037	0.034

## DISCUSSION

In the mtDNA control region sequence analyses with roe deers, 13 samples of the endemic Italian subspecies *Capreolus c. italicus* were monomorphic, but four and two haplotypes were revealed in five samples from the Kurgan region (*C. pygargus pygarhus*) and in four samples from the

CPT1 TGACCAATAT CGAAAAACT CATCCACTAA TAAAAATTGT AAATAACGCA 50  
 CPT2 .....  
 CPT3 .....  
 CPP .....  
 CCC ..... .C.....

TTCATTGATC TCCCAACCCC ATCAAACATC TCATCATGGT GAAACTTTGG 100  
 .....G.....  
 .....G..... A.....  
 .....G..... T. T ..... A.....

CTCTCTACTA GGAATCTGTC TAATTTTACA AATCCTTACA GGCCTAATTC 150  
 .....  
 .....  
 T..... C..... C.....

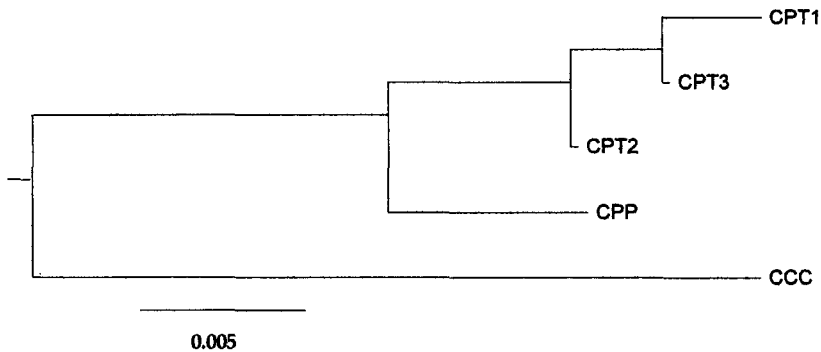
TAGCAATACA TTACACATCC GACACAATAA CAGCATTCTC CTCTGTACT 200  
 .....  
 .....  
 ..... C..... C.....

CACATCTGCC GATACGTTAA CTATGGTTGA ATTATCCGAT ACATACATGC 250  
 .....G.....  
 .....G.....  
 .....G...C..... T.....  
 .....G..... C..... T.....

AAACGGAGCA TCAATATTTT TTATTTGCTT ATTCTACAT GTAGGACGAG 300  
 .....  
 .....  
 ..... C.,C.....

GCCTATATTA TGGATCTTAC ACTTTTCTAG AGACATGAAA CATTGGAGTA 350  
 .....  
 .....  
 .....  
 .....  
 ATTCTCCTAT TCACAGTAAT AGCCACGGCA TTTGTAGGAT A 391  
 .....  
 .....  
 .....T.....

**Fig. 1.** Sequences of mitochondrial DNA cytochrome b genes among two species of genus *Capreolus*. CPT1, CPT2, and CPT3 are haplotypes of *C. pygargus tianschanicus* from Jeju Island, Korea; CPP, *C. p. pygargus* from Kurgan, west Siberia; and CCC, *C. c. capreolus* from west Europe. Total number of sequences analyzed are 391 base pairs. Sequences of roe deer from Korea are resulted from this study, and sequences of roe deer from Kurgan and west Europe are obtained from GenBank (accession numbers AJ 000025 and Y14951, respectively).



**Fig. 2.** Phylogenetic tree among five haplotypes of cytochrome b gene from two species of *Capreolus*. Neighbor-joining method was used with nucleotide Tamura & Nei's distances of mitochondrial cytochrome b sequences given in Table 1. Haplotypes are labeled as in Fig. 1.

Amur region (*C. p. tianschanicus*), respectively (Randi *et al.*, 1997). Moreover, maximum nucleotide Tamura & Nei's distance among four haplotypes of roe deers from Kurgan was 0.0089, and that among two haplotypes of roe deers from Amur was 0.0030. In these analyses of mtDNA cytochrome b gene with six samples of *C. p. tianschanicus* from Jejudo Island, three haplotypes were recognized (see Fig. 1) and maximum nucleotide distance was 0.0050 (see Table 1). The control region may evolve faster than cytochrome b gene (Brown, 1985), and it was revealed that this distance was comparable to the diversity within other roe deer subspecies.

Moreover, it is concluded that roe deers from Jejudo Island have considerable genetic variation in spite of their geographical isolation within the island. Jejudo Island is the largest continental island in the Korean peninsula and it was formed by a series of volcanic activities at the end of the Tertiary (Park, 1985). It was connected to the mainland during the Pleistocene and separated again around 10,000 years ago as other continental islands, as noted by Wilcox (1978). It is suggested that roe deers from the mainland dispersed rarely to Jejudo Island, although further analyses are necessary to decide whether or not it was occurred by human introduction.

Mitochondrial DNA cytochrome b divergence between Siberian and European roe deer is about 0.04 (Randi *et al.*, 1997). In this study nucleotide distance between cytochrome b sequences of roe deers from Jeju (*C. pygargus tianschanicus*) and that of roe deer from west Europe (*C. c. capreolus*) was 0.037 (see Table 1).

The taxonomy of *C. pygargus* in subspecies level is still in confusion. Sokolov and Gromov (1990) stated that the Siberian roe deer are represented by three distinctive subspecies [*C. p. pygargus* from west and part of east Siberia, *C. p. tianschanicus* from Tien-Shan, and *C. p. manchuricus* (= *bedfordi*) from far east Asia including Korea]. Danilkin (1996) recognized only two subspecies in *C. pygargus* (*C. p. pygargus* from west and part of east Siberia and *C. p. tianschanicus* from Tien-Shan, and east Asia including Korea).

A subspecies is an aggregate of phenotypically similar populations of a species differing taxonomically from other populations of that species (Mayr and Ashlock, 1991). And it was advocated that a classification should be the product of all available characters distributed as widely

and evenly as possible over the organisms studied (Huelsenbeck *et al.*, 1996). Fandos and Reig (1993) and Aragon *et al.* (1998) stated that morphometric characters can not be used without caution in taxonomic researches with roe deers at subspecies level.

Randi *et al.* (1997) stated that west Siberian (Kurgan region) roe deer (*C. p. pygargus*) is distinct from east Siberian (Amur region) ones (*C. p. tianschanicus*) by mtDNA control region sequences. In this study based on mtDNA cytochrome b sequences (Fig. 2), it was revealed that Korean roe deer (*C. p. tianschanicus*) is different from roe deer of Kurgan region in west Siberia (*C. p. pygargus*). It is confirmed that roe deer from Korea is *C. p. tianschanicus*, and it differed from *C. p. pygargus*.

Cytochrome b sequences diverged at a rate of 0.02 nucleotide distances/Myr in ungulates (Randi *et al.*, 1996). In this study, nucleotide distance between cytochrome b sequences of roe deers from Jeju Island and that of roe deer from west Siberia was average 0.013 (Table 1). It is suggested that roe deers from Jeju Island (*C. p. tianschanicus*) diverged from roe deers in Kurgan, west Siberia (*C. p. pygargus*) 0.65 Myr ago, although Randi *et al.* (1997) noted by mtDNA control region sequence analysis that the divergence between *C. p. tianschanicus* from Amur and *C. p. pygargus* from Kurgan occurred 0.37-0.19 Myr ago.

Barclay (1935) recognized roe deers from Korea as *C. p. ochracea*. Tate (1947) described that Manchurian roe deer (*C. c. bedfordi*; type locality of Cichuwan) included subspecies *mantschuricus* in north east China and *ochracea* in Korea. Moreover, Ellerman and Morrison-Scott (1951) stated that subspecies *mantschuricus*, *melanotis*, and *ochracea* are the synonym of *C. p. bedfordi* from Jeju (Quelpart island), the Korean peninsula, China, Mongol, and south east Siberia. Heptner *et al.* (1961) noted that roe deers from Korea, north east China and nearby Russia is *C. c. bedfordi*, and that *mantschuricus* and *ochracea* are the synonym of *C. p. bedfordi*.

Under the present classification, the subspecies name of Korean roe deer is *tianschanicus*, but it was classified as one of three subspecies (*bedfordi*, *mantschuricus*, and *ochracea*). Therefore, specimens from the Korean peninsula and other areas of east Asia, especially Tien-Shan, are needed for further molecular systematic analyses to clarify the subspecies classification of Siberia roe deer, *C. pygargus*.

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RECEIVED: 27 August 2000

ACCEPTED: 29 September 2000

한국의 제주도에 서식하고 있는 노루 (*Capreolus pygargus tianschanicus*  
Satunin)의 미토콘드리아 DNA cytochrome b 유전자의 다양성

고 흥 선 · 양 병 국 · 유 혜 숙 · 천 태 영  
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요 약

제주도산 노루 (*C. pygargus tianschanicus*)의 분류학적 위치를 규명하기 위한 연구의 일환으로, 한국의 제주도에서 채집된 6마리의 노루 표본들을 이용하여 mtDNA의 cytochrome b 유전자의 부분적인 염기서열의 분석을 하였다. 밝혀진 세 haplotype간의 nucleotide Tamura & Nei's distance는 최대 0.005로써, 노루의 다른 아종 내의 다양성과 비슷한 정도였다. 또한 제주도산 노루의 cytochrome b 염기서열들과 GenBank에서 얻은 서 시베리아 지역의 노루인 *C. p. pygargus*의 cytochrome b 염기서열간의 nucleotide distance는 평균 0.013였으며, *C. p. tianschanicus*는 65만년 전에 서 시베리아 지역의 노루인 *C. p. pygargus*에서 분화되었을 것으로 판단된다.