



Short communication

Genetic distinction of roe deer (*Capreolus pygargus* Pallas) sampled in Korea

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Receipt of Ms. 17. 01. 2001

Acceptance of Ms. 30. 05. 2001

Key words: *Capreolus pygargus*, mitochondrial DNA, molecular taxonomy

The roe deer (genus *Capreolus*, Artiodactyla, Cervidae) includes two species: the smaller European (*C. capreolus*) and the larger Siberian (*C. pygargus*) roe deer (GROVES and GRUBB 1987; SOKOLOV and GROMOV 1990; HEWISON and DANILKIN 2001), which have widespread distributions in central-western Europe, and in Asia and east Europe, respectively. They show a number of diagnostic morphologic, chromosomal and DNA traits (GROVES and GRUBB 1987; HEPTNER et al. 1989; DOUZERY and RANDI 1997; RANDI et al. 1998a). Populations of the two species occur in putative contact zones in the Caucasus, where *C. capreolus* is distributed at the southern slopes and *C. pygargus* at the northern slopes of the mountain ranges, and along the rivers Volga and Don (HEPTNER et al. 1989; DANILKIN 1996). However, most probably European and Siberian roe deer populations do not hybridize in nature where they overlap in distribution (DANILKIN 1996; HEWISON and DANILKIN 2001).

Populations of the Siberian roe deer show extensive body size and coat colour variability, and up to six subspecies were recognized (GRUBB 1993). However, recently DANILKIN (1996) and HEWISON and DANILKIN (2001) recognized only two subspecies: *C. p. pygargus*, from western and part of

eastern Siberia, and *C. p. tianschanicus*, from Tian Shan and eastern Asia, including Korea. Other authors referred to roe deer populations from Korea as a distinct subspecies, e.g., *C. p. bedfordi* (SOKOLOV and GROMOV 1990), or *C. p. ochracea* (BARCLAY 1935). The genetic basis and taxonomical significance of morphological variation among Siberian roe deer populations are still unclear. Aim of this study is to assess the genetic distinction and clarify the taxonomic status of the *C. pygargus* population from Cheju Island in Korea.

Three specimens of roe deer from Cheju Island in Korea were collected and muscle samples were stored in a deep-freezer. Total cellular DNA was extracted from muscle samples digested for 2 hours at 55 °C in 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 Proteinase K stock solution, and 25 µl of 20% SDS solution. DNA was extracted with equal volumes of phenol-chloroform and chloroform, precipitated with 2 volumes of ethylalcohol, and resuspended in 50 µl of distilled water. The solution was incubated at 37 °C for 30 min with 5 µl of 10 mg/ml RNase stock solution, and DNA was extracted again.

The entire mtDNA CR was PCR-amplified using primers L-Pro and H-Phe (DOUZERY

and RANDI 1997; RANDI et al. 1998 b) and the following thermal cycle: 94 °C for 5 min; 94 °C for 1 min, 72 °C for 1 min, 54 °C for 1 min (32 cycles); 72 °C for 5 min. PCR-amplified products were purified using the DNA PrepMate™ kit (Bioneer Co., Cheongwon-gun, Korea), and sequenced using an automated DNA sequencer (Perkin Elmer 377) at the Korea Basic Science Institute (Daejeon).

The new mtDNA CR sequences were aligned with homologous complete (DOUZERY and RANDI 1997) and partial (RANDI et al. 1998 b) sequences, using the computer program CLUSTALX (THOMPSON et al. 1997). Phylogenetic analyses were performed by maximum parsimony (MP), using PAUP* 4.0b 2a (SWOFFORD 1998), with unordered character states (uninformative nucleotide positions excluded), 10 replicates of random addition of terminal sequences and TBR branch swapping, and by neighbor-joining procedure (NJ; SAITOU and NEI 1987), with TAMURA and NEI (1993) DNA distances (TN93), using PAUP*. Majority-rule (50%) consensus trees were constructed when multiple equally parsimonious topologies resulted from MP analyses. Robustness of the phylogenies was assessed by bootstrap percentages (BP; FELSENSTEIN 1985), with 1000 random resamplings. Clades can be considered significantly supported when BP values are $\geq 70\%$.

PCR products were clean single bands of the expected molecular weight, and the sequencing allowed unambiguous nucleotide identifications. Regional organization of the CR from *Capreolus* was concordant with data from other cervid CRs (DOUZERY and RANDI 1997; RANDI et al. 1998 b; NAGATA et al. 1998; COOK et al. 1999; RANDI et al. 2001), thus suggesting that they represent true mtDNA sequences and not nuclear copies. The CR of *Capreolus* includes a central conserved region (CR-II), a left domain (CR-I, on the tRNAs Thr and Pro side), and a right domain (CR-III, on the tRNA Phe side; not shown).

A complete alignment 926 nucleotide long was obtained (the new sequences are available from the GenBank with accession nos.

AJ311188 and AJ311189). The average TN93 genetic distance between the Cheju samples and European or Siberian roe deer was 4.5%, and 2.5%, respectively. Roe deer from Cheju showed two distinct mtDNA CR haplotypes, which diverged at 0.2% of their sequences. Moreover, we have aligned partial mtDNA CR sequences, 679 nucleotide long, obtained from 23 samples of European, Siberian, and Korean roe deer. Equally weighted MP analysis of the CR alignment (with 47 parsimony-informative characters) produced 90 trees (length $L = 81$; consistency index $CI = 0.617$; retention index $RI = 0.881$). The 50% majority-rule consensus tree is equivalent to the NJ phylogenetic tree, which is shown in Fig. 1.

MP and NJ trees concordantly showed that: (1) European and Siberian roe deer sequences split into two divergent evolutionary lineages ($BP = 100\%$); (2) Siberian roe deer sequences split into three significantly different evolutionary lineages ($BP = 70\% - 100\%$), including roe deer from east Siberia (Amur Region), Korea, and west Siberia (Kurgan Region); and (3) roe deer sequences from Korean individuals joined into a monophyletic clade (supported by $BP = 91\%$), which was nested within the Siberian roe deer clade which is the sister clade of the roe deer sampled in the Kurgan Region (west Siberia).

Thus, mtDNA sequences support a genetic distinction between west Siberian and far eastern roe deer, in accordance with both SOKOLOV and GROMOV (1990) and DANILKIN (1996) taxonomical listings. The new mtDNA sequences analysed in this study strongly suggest that roe deer from Cheju island are more closely related to populations sampled from the geographically distant Kurgan Region in west Siberia (*C. p. pygargus*) than to those from the geographically close Amur region in south-east Siberia (Fig. 1). The mtDNA sequences from Cheju Island form a significantly distinct clade within *C. pygargus*, thus indicating that roe deer from Korea belong to distinct populations, and supporting SOKOLOV and GROMOV's (1990) view that roe deer from

the far east including Korea, should be kept distinct from the other Siberian roe deer populations. The Korean peninsula is a part of eastern Asia within the Palaearctic re-

gion, and it is bound on the north by north-eastern China and far eastern Russia. Cheju Island, the largest of the southern Korea islands, originated by a series of volcanic ac-

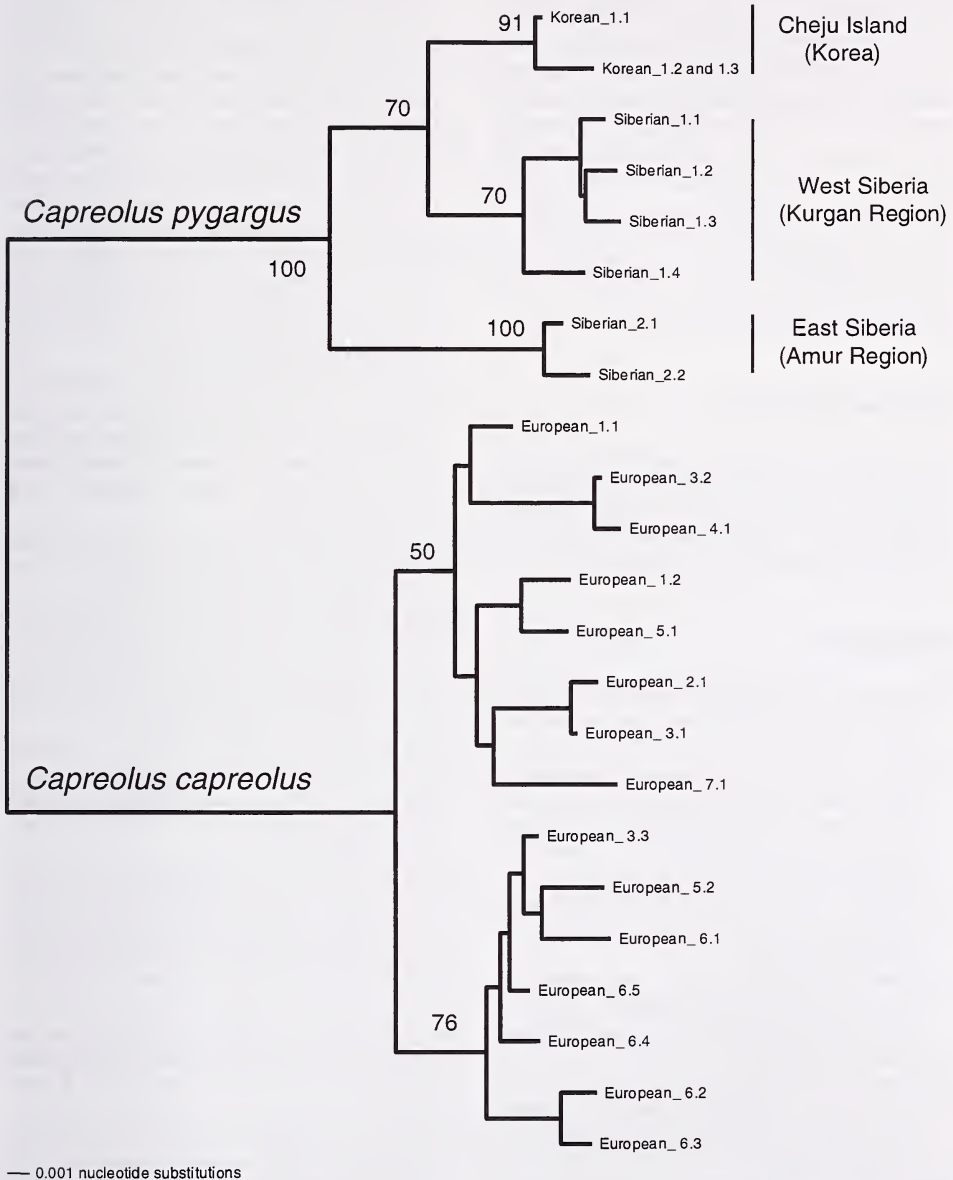


Fig. 1. Neighbor-joining tree (rooted using homologous *C. elaphus* mtDNA CR sequences as outgroups; not shown) clustering mtDNA CR haplotypes from *Capreolus*, computed using TAMURA and NEI (1993) DNA distances. At internodes are reported the bootstrap values $\geq 50\%$. For the identification of *C. pygargus* (Siberian) and *C. capreolus* (European) haplotypes, see: RANDI et al. 1998 b.

tivities at the end of the Tertiary (PARK 1985). Cheju Island was connected to the Korean peninsula during the Pleistocene and separated again at the end of the last glaciation, about 10 000 years ago. Therefore, roe deer in Cheju could have evolved in isolation during the last 10 000 years. KOH et al. (1997) reported that roe deer from the Korean peninsula showed smaller body size and skull length than west and east Siberian roe deer subspecies. Thus, molecular and morphological data lend support to the view that roe deer from Korea do not belong neither to *C. p. tianschanicus* nor *C. p. mantschuricus* (= *bedfordi*), and might belong to a distinct subspecies, *C. p. ochracea*, as described by BARCLAY (1935).

Acknowledgements

This research was in part supported by the Korean Science Foundation (grant 97-0402-05-01-3 to HUNG SUN KOH), and by the Italian Istituto Nazionale per la Fauna Selvatica (I.N.F.S.) and Consiglio Nazionale delle Ricerche (C.N.R.).

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Zeitschrift/Journal: [Mammalian Biology \(früher Zeitschrift für Säugetierkunde\)](#)

Jahr/Year: 2001

Band/Volume: [66](#)

Autor(en)/Author(s): Koh H. S., Randi Ettore

Artikel/Article: [Genetic distinction of roe deer {Capreolus pygargus Pallas} sampled in Korea 371-375](#)