

Genetic Diversity of Mitochondrial DNA D-Loop Sequence of Siberian Roe Deer (*Capreolus Pygargus*) Inhabiting Mongolia

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Abstract: Roe deer, the only species among the twelve wild artiodactyls in Mongolia that is considered to have large population density and vast area of habitat, live in a herd throughout the year. Roe deer play important role for forest biodiversity. Balanced population density of roe deer affects sufficient regeneration and diversity of flora and fauna in forest. Living area of roe deer is getting disrupted by uncontrolled mining and overgrazing. In this case some small population of roe deer might be endangered. Therefore, if phylogenetic analysis among roe deer populations inhabiting Mongolia is carried out, propagation of the small and endangered roe deer population can be promoted. We have amplified and sequenced 1220 nucleotides of the mitochondrial DNA D-loop region in 12 Siberian (*Capreolus pygargus*) roe deer from 5 localities in Mongolia. The sequences were aligned with 51 published sequences of roe deer mtDNA D-loop region from GenBank by using the CLUSTAL W version 2.1. software. Phylogenetic reconstructions were performed by Maximum likelihood. Calculations and Maximum likelihood and Neighbor-Joining trees construction were conducted using the MEGA 5.1 software based on Kimura – 2 – parameter model considering transitions, transversions, and irrespective of the nucleotide position. Stability of the clustering order was assessed using bootstrap (100 permutations). The alignment showed 20 polymorphic sites including 13 transitions and 7 transversions, defined 12 haplotypes within our samples. The mean nucleotide divergence of roe deer haplogroups was 2.8% between Mongolian and Russian, 3.5% between Mongolian and Korean, 3.5% between Mongolian and Kyrgystani and 2.4% between Mongolian and Chinese roe deer. Phylogenetic tree showed every cluster has haplotypes representing roe deer

in Mongolia, which could mean that Mongolia might be the center of migration for Siberian roe deer.

Keywords: Siberian Roe Deer, mtDNA, D-loop, cluster, phylogenetics

Introduction

Roe deer, the only species among the twelve wild artiodactyls in Mongolia that is considered to have large population density and vast area of habitat, live in a herd throughout the year. A herd of roe deer consists of different aged male and female deer. Each herd is headed by a leader, the best of the herd, which is usually an adult female roe deer (“Zur” in Mongolian). Gravid roe deer run away from the herd from April and give birth in a calm and withdrawn area in June (Munkhbat, 2011).

Roe deer play important role for forest biodiversity. Balanced population density of roe deer affects sufficient regeneration and diversity of flora and fauna in forest (Lorenzini, 2002; 2003; 2006). If population density of roe deer is around 15 individuals, it is ideal for forest regeneration but too low or high population density has negative effect on forest ecosystem (Robin, 2000).

In Mongolia, hunting is permitted between September 1st and December 1st. But illegal hunting is growing in the spring season due to some folk rumors where it is believed that eating fresh meat and drinking fresh blood of wild animal brings strength to the body. In addition, living area of roe deer is getting disrupted by uncontrolled mining and overgrazing. In this case some small population of roe deer might be endangered (Munkhbat, 2011).

Therefore, if phylogenetic analysis among roe deer populations inhabiting Mongolia is carried out, propagation



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of the small and endangered roe deer population can be promoted.

I. MATERIALS AND METHODS

Materials: We used 12 sequences of D-loop regions obtained from tissue samples of Siberian roe deer *C. pygargus* collected in Mongolia (Fig. 1)

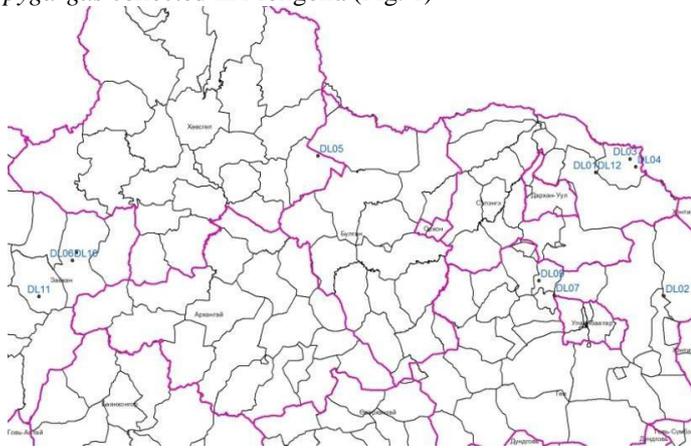


Figure 1. Sampling locations. DL01, DL03, DL04, DL12 from Selenge province, DL02, DL07, DL09 from Tuv province, DL05, DL06, DL08, DL10, DL11 from Zavkhan province.

Data analysis: The sequences were aligned using the CLUSTAL W version 2.1 software (Larkin, 2007.). Haplotype, nucleotide diversity and the percentage of inter- and between populational variability was determined by Kimura – 2 – parameter model. Sequences of homologous fragment of mtDNA control region of Siberian roe deer from Genbank/NCBI database with numbers AY860055-57, GU811820-23, AY785540-55, GU811835-38, AY958266-72, GU811839-46, AJ311188-89, AY854040-45, Z70317 were used for the analysis of our data. While constructing phylogenetic trees, the sequences of homologous fragment

of mtDNA control region of *Cervus elaphus* (accession number in GenBank AF005199 was used as an outgroup (Vorobieva, 2011; Xiao, 2007; Lorenzini, 2003; 2006).

Phylogenetic reconstructions were performed by Maximum likelihood. Calculations and Maximum likelihood and Neighbor-Joining trees construction were conducted using the MEGA 5.1 software based on Kimura – 2 – parameter model considering transitions, transversions, and irrespective of the nucleotide position. Stability of the clustering order was assessed using bootstrap (100 permutations).

II. RESULTS AND DISCUSSION

We obtained 12 mtDNA control region sequences (1220 bp), all of which have been sent to GenBank (accession nos. JQ958970-81). The alignment showed 20 polymorphic sites including 13 transitions and 7 transversions, defined 12 haplotypes (DL01, DL03, DL04, DL12 from Selenge province, DL02, DL07, DL09 from Tuv province, DL05, DL06, DL08, DL10, DL11 form Zavkhan province, the submitted sequences include all 12 haplotypes) (Table 1). Nucleotide diversity was 0.027. To determine the relationship between Siberian roe deer’s population , we used 51 published sequences of roe deer mtDNA control region from GenBank (accession nos. AY625875-AY625892, AY625732-AY625751, Z70318, Z70317, AJ311188, and AJ311189), corresponding to 51 haplotypes, namely, cn, XP, WD,SP, Ja, KS, Alt, Ns, Ts and T312 (cn, XP, WD, SP, China; Ja, Jakuta Russia; KS, Korea South; Alt, Altai Russia; Ns, Novosibirsk Russia; Ts, Tien Shan Kyrgystan; T312, Amur region Russia) and 12 haplotypes of our samples which was located in Mongolia and named as DL. Average interhaplotype sequence divergence within northeastern Chinese, Russian, Korean, Kyrgyzstani and Mongolian roe deer was 1.78%, 3.06%, 2.17%, 1.95% and 2.8%, respectively (Table 2). The mean nucleotide

Table 1. Variable positions of mtDNA control region (1220 bp) in Siberian roe deer of Mongolia. *n*,

Haplotypes	Position of Variable site																	
	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4
	5	4	5	6	6	7	7	9	0	0	3	3	4	6	6	6	7	7
	9	2	7	2	6	0	2	6	6	8	3	5	0	1	4	9	0	2
DL01	C	T	G	C	G	A	T	G	C	A	T	T	C	G	A	A	G	T
DL02	-	.	A	.	.	.	C	A	T	C	.	.	.
DL03	-	C	A	A
DL04
DL05	.	C	.	T	.	.	C	A	T	G	.	.	.	A	.	.	A	.
DL06	T	.	.	T	.	.	C	A	T	G	.	A	.	.	C	G	A	C
DL07	A	.	C	A	T	.	C	.	.	.	T	.	.	.
DL08	T	.	.	T	.	.	C	A	T	G	.	A	.	.	C	G	A	C
DL09	T	A
DL10	G	C	A	A
DL11	G	C	A	A
DL12	.	.	.	T	.	.	C	A	T	A	.	.	A	.

divergence of roe deer haplogroups was 2.8% between Mongolian and Russian, 3.5% between Mongolian and Korean, 3.5% between Mongolian and Kyrgyzstani and 2.4% between Mongolian and Chinese roe deer (Table 3). Maximum likelihood (Fig. 2) and Neighbor-Joining (Fig. 3) trees grouped the 63 roe deer haplotypes into eight distinct clusters. Cluster “A” belongs to Novosibirsk of Russia because of including all Novosibirsk’s roe deer population’s haplotypes (Ns). It is also includes DL01, DL04, DL09 haplotypes of Mongolia. Cluster “B” contains Altai’s roe deer haplotypes of Russia, Mongolian roe deer haplotypes and several other haplotypes (Ts3, Ts5, cn1, 0001, Z70317). Jakuta’s haplotypes construct cluster “B” which included some of Mongolian haplotypes (DL03, DL10, DL11) and some haplotypes of China (cn4, cn12, cn14, cn16, cn8, XP1, WD1, 0002). DL12, DL05 haplotypes of Mongolian roe deer belong to cluster “F”, including Chinese roe deer mtDNA control region haplotypes (Fig. 1). Rest of other haplotypes separated into other four clusters (D, E, G, H). And Korean roe deer haplotypes dominate the clusters “E”, “G”, “H”. These results are almost same for studies of Randi et al., (1998), Vorobieva et al., (2011). It is observed from the phylogenetic trees that D-loop of mtDNA of roe deer inhabiting Mongolia is polymorphic. This polymorphism could be caused by mobility of roe deer that can travel 100-200 km per day while they are migrating (Coulon, 2008). On the other hand, every cluster has haplotypes representing roe deer in Mongolia, which could mean that Mongolia might be the center of migration for Siberian roe deer.

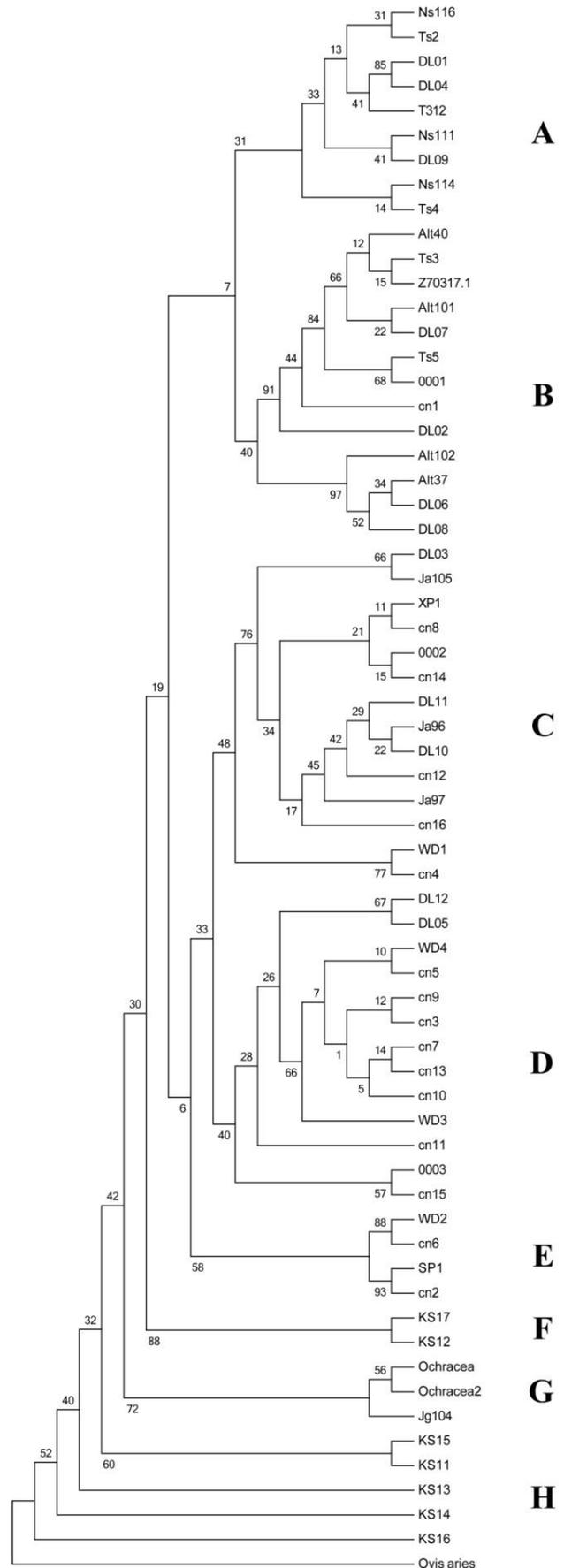
Table 2. Genetic Diversity within populations

Korea	0,0217
Russia	0,0306
Kyrgyzstan	0,0195
Mongolia	0,028
China	0,0178

Table 3. Estimates of Evolutionary Divergence over Sequence Pairs between populations

Korea	Russia	Kyrgyzstan	Mongolia	China
Korea				
Russia	0,035			
Kyrgyzstan	0,032	0,025		
Mongolia	0,035	0,028	0,026	
China	0,032	0,028	0,027	0,024

Figure 2. Maximum likelihood tree of Siberian roe deer mtDNA control region haplotypes. We compared 12 haplotypes of Mongolian roe deer with 51 haplotypes from Genbank. The major clusters and bootstrap values are indicated. The sequence of the Red deer (AF005199) is taken as an outgroup. Abbreviations: “DL” indicate Mongolian samples, “Alt” – samples from Altai; “Ns” – Novosibirsk region samples; “Ts” – Tian Shan samples; “Ja” – Yakutian samples (Vorobieva, 2011); Z70317 - Russian Far East samples (Randi, 1998); “WD”, “XP”, “SP” – Chinese samples (Xiao, 2007); “ochracea”, “KS” – Korean samples (Hung, 2004). Clusters (A–H) are marked with different colors.



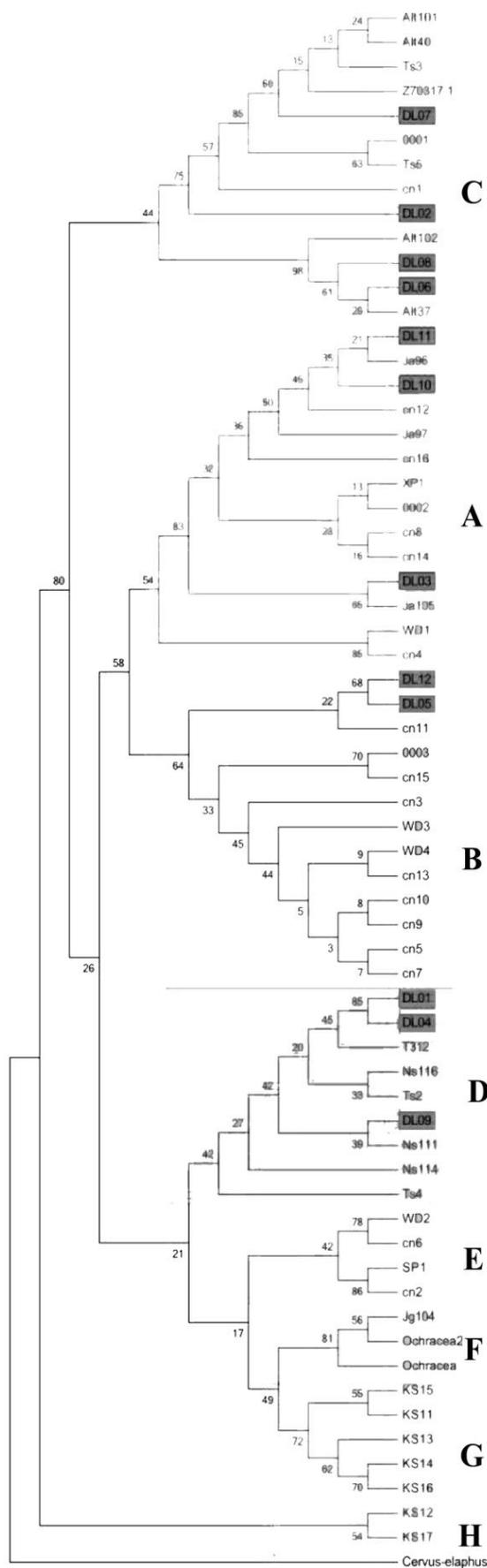


Figure 3. Neighbor-Joining tree of Siberian roe deer mtDNA control region haplotypes. We compared 12 haplotypes of Mongolian roe deer with 51 haplotypes from Genbank. The major clusters and bootstrap values are indicated. The sequence of the Red deer (AF005199) is taken as an outgroup. Abbreviations: “DL” indicate Mongolian samples, “Alt” – samples from Altai; “Ns” – Novosibirsk region samples; “Ts” – Tian Shan samples; “Ja” – Yakutian samples (Vorobieva, 2011); Z70317 - Russian Far East samples (Randi, 1998); “WD”, “XP”, “SP” – Chinese samples (Xiao, 2007); “ochracea”, “KS” – Korean samples (Hung, 2004). Clusters (A–K) are marked with different colors.

ACCESSION NUMBERS

GenBank accession numbers for the sequences presented in our study are: DL JQ958970-81, cn AY785540-55, Ja/g GU811835-38, KS AY958266-72, Ns GU811839-41, Ochracea AJ311188-89, Ts GU811842-46, WD AY854041-44, XP AY854045, SP AY854040, Alt GU811820-23, 000 AY860055-57

ACKNOWLEDGMENT

This work was funded by Mongolian Foundation for Science and Technology grant.

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