Insights into Korean Red Fox (*Vulpes vulpes*) Based on Mitochondrial Cytochrome *b* Sequence Variation in East Asia

Author(s): Jeong-Nam Yu, Sang-Hoon Han, Bang-Hwan Kim, Alexey P. Kryukov, Soonok Kim, Byoung-Yoon Lee and Myounghai Kwak
Source: Zoological Science, 29(11):753-760. 2012.
Published By: Zoological Society of Japan
DOI: http://dx.doi.org/10.2108/zsj.29.753
URL: http://www.bioone.org/doi/full/10.2108/zsj.29.753

ZOOLOGICAL SCIENCE **29**: 753–760 (2012)

Insights into Korean Red Fox (*Vulpes vulpes*) Based on Mitochondrial Cytochrome *b* Sequence Variation in East Asia

Jeong-Nam Yu[1], Sang-Hoon Han[1], Bang-Hwan Kim[2], Alexey P. Kryukov[3], Soonok Kim[1], Byoung-Yoon Lee[1], and Myounghai Kwak[1]*

[1]*National Institute of Biological Resources, Environmental Research Complex, Incheon 404-708, Korea*
[2]*The Korea Environmental Association, Yu-i do-dong Yeongdeungpo-gu, Seoul 150-870, Korea*
[3]*Institute of Biology and Soil Science, Far Eastern Branch Russian Academy of Sciences, Vladivostok-22, 690022, Russia*

The red fox (*Vulpes vulpes*) is the most widely distributed terrestrial carnivore in the world, occurring throughout most of North America, Europe, Asia, and North Africa. In South Korea, however, this species has been drastically reduced due to habitat loss and poaching. Consequently, it is classified as an endangered species in Korea. As a first step of a planned red fox restoration project, preserved red fox museum specimens were used to determine the genetic status of red foxes that had previously inhabited South Korea against red foxes from neighboring countries. Total eighty three mtDNA cytochrome *b* sequences, including 22 newly obtained East Asian red fox sequences and worldwide red fox sequences from NCBI, were clustered into three clades (i.e., I, II, and III) based on haplotype network and neighbor-joining trees. The mean genetic distance between clades was 2.0%. Clade III contained South Korean and other East Asian samples in addition to Eurasian and North Pacific individuals. In clade III, South Korean individuals were separated into two lineages of Eurasian and North Pacific groups, showing unclear phylogeographic structuring and admixture. This suggests that South Korean red fox populations may have been composed of individuals from these two different genetic lineages.

Key words: Korean red fox, *Vulpes vulpes*, genetic variation, restoration, *Cyt b*

INTRODUCTION

The red fox (*Vulpes vulpes*, Canidae) is a small carnivorous mammal, widely distributed in Eurasia, northern Africa, and America; generally, its status is known to be stable (Garshelis and Steinmetz, 2008). However, illegal killing to protect livestock or for fur, secondary poisoning as a consequence of a nationwide rodenticide program, and habitat loss and fragmentation have caused a drastic decrease in the red fox population in South Korea (Won and Smith, 1999). In the 1980s, the red fox was recognized as extinct in nature in South Korea and it was designated as "endangered species I" (Ministry of the Environment of Korea, 2005). Except for the unexpected discovery of a dead red fox in Yanggu-gun, Gangwon province, wild individuals have not been seen since the 1980s.

In 2011, the Korean government initiated a project to restore the red fox population in Sobaek National Park. Sobaek National Park was chosen because of the species richness of possible prey species, including small to medium-sized mammals, birds, reptiles, amphibians, insects, carrion, and fruit. Because Canidae, including the red fox, is generally highly adaptable to the environment and have good natural fertility, natural breeding after restoration was expected to be achieved. However, propagation using original individuals from South Korea is practically impossible, and the best alternative is to introduce the individuals most closely phylogenetically related to the South Korean population (Kleiman, 1989).

The native Korean red fox has been described as subspecies *Vulpes vulpes peculiosa* based on morphological characteristics (Kishida, 1927), but the existence of subspecies in red fox was doubtful (Cobert, 1978; Wilson and Mittermeier, 2009). Recently, molecular genetic studies of the red fox in Japan, Europe, the USA, and Canada have been performed using the mitochondrial cytochrome *b* (*Cyt b*) gene and control region (Inoue et al., 2007; Perrine et al., 2007; Aubry et al., 2009). The Japanese red fox is separated into two groups: a widely distributed group and a local group on Hokkaido. The widely distributed group comprises haplotypes from Hokkaido, Kyushu, eastern Russia, and Europe (Inoue et al., 2007). In Europe and North America, red fox populations are largely separated into Holarctic and Nearctic clades (Aubry et al., 2009). The Holarctic clade comprises haplotypes from Eurasia, Alaska, and Western Canada, and the Nearctic clade is only found in North America, and predominates in the USA and Eastern Canada. However, the genetic status of Korean red fox populations

* Corresponding author. Tel. : +82-32-590-7127;
Fax : +82-32-590-7230;
E-mail: mhkwak1@korea.kr
doi:10.2108/zsj.29.753

has not been investigated to date.

The small circular mitochondrial genomes are extracted easily, even from degraded specimens such as old bones or dry specimens (Higuchi et al., 1988). Their high mutation rate and low recombination via maternal inheritance has led to the popular use of mitochondrial molecular markers for evolutionary and population genetics studies (Brown et al., 1979; Clayton, 1982; Lansman et al., 1983; Hayashi et al., 1985). The *Cyt b* gene is an especially good marker for intra- and interspecific phylogenetic studies, as it has a moderate mutation rate (Irwin et al., 1991; Esposti et al., 1993; Avise, 1994; Hillis et al., 1994). Many species of fish (Rocha-Olivares et al., 1999; Park et al., 2000; Apostolidis et al., 2001; Moller and Gravlund, 2003), birds (Edwards and Wilson, 1990; Randi et al., 2001), and mammals (Ma et al., 1993; Lee et al., 2008) have been studied using *Cyt b* gene sequences.

For red fox restoration, the genetic status of the red fox breed that was native to South Korea should be determined. For comparison with other red fox populations, we used *Cyt b* gene sequences that have been used in previous studies (Inoue et al., 2007; Aubry et al., 2009). In this study, we analyzed *Cyt b* gene sequences from 22 individuals, including four specimens that are definitely from South Korea and 18 individuals from China, Russia, Mongolia and North Korea, and compared them with *Cyt b* gene sequences from 61 haplotypes worldwide from the GenBank database.

MATERIALS AND METHODS

Sampling and DNA extraction

Samples from red foxes native to South Korea were obtained from three stuffed specimens in university museums and one alcohol-preserved muscle sample from a dead fox found in Yanggu-Gun, Gangwon province, in 2004 (Table 1). The remaining 18 individuals included two from the Kaema Highlands, North Korea; three from Primorsky, Russia; six from northeastern China; two from the Mongolia and five from Seoul Grand Park Zoo (from North Korea and China), Korea. Total DNA was isolated from blood, muscle tissue, or hair of these animals using a DNeasy Blood & Tissue Kit (QIAGEN Co., Hilden, Germany).

DNA sequencing

The *Cyt b* gene was amplified by polymerase chain reaction (PCR) using two primer sets, designed by Inoue et al. (2007). PCR mixtures were prepared in 20 μl reaction volumes containing 100 ng template DNA, 0.5 μl 25 mM dNTPs, 20 pmole each primer, 1.5 mM $MgCl_2$, 1 unit of Takara Ex-*Taq*™ (Takara, Japan), and 10 × PCR buffer. The amplification process consisted of 95°C for 5 min; 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s; and a final 10 min at 72°C. The PCR products were checked on 1.2% agarose gels and purified with the QIAGEN purification kit (QIAGEN Co. Hilden, Germany). All samples were sequenced on an Applied Biosystems 3730 XL DNA sequencer at Biomedic (Bucheon-Si, South Korea). The chromatograms and alignments were checked visually and verified. Nucleotide sequences of the 17 novel haplotypes were deposited in the DDBJ/EMBL/GenBank database under accession numbers JN652603–JN652617 and JQ003577–JQ003578.

Population genetic analysis

We compared the 22 East Asian *Cyt b* gene sequences from

Table 1. Specimens used in this study.

Sample abbreviation	Sampling locations	Year sampled
KW2	South Korea: Mt. Odae (Natural History Museum Hannam University)	1990
KW3	South Korea: Demilitarized zone (Ewha Womans University Natural Museum)	1980
KW4	South Korea: Mt. Jiri (Kyunghee University Natural History Museum in Korea)	1978
KW5	South Korea: Gangwondo Yanggu	2004
KS2	South Korea: Seoul Grand Park Zoo (from North Korea and China)	
KS3		
KS4		
KS8		
KS10		
CD5	North China	2007–2008
CD6		2008
CD7		2007
CD8		2007
CD9		2006
CD10		2004
NK02	North Korea, Kaema Highlands	
NK03		
RW1328	Russia, Primorsky	2008
RW1601		2009
RW1651		2010
MO06	Mongolia	2006
MO09		2009

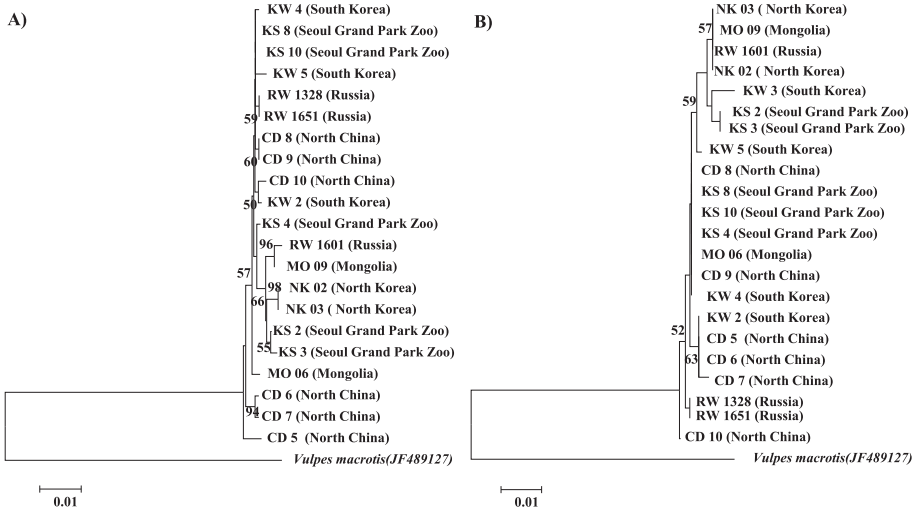

Fig. 1. Neighbor-joining tree based on the *Cyt b* gene sequences (A, 1,103 bp; B, 338 bp) of 21 or 22 specimens in this study. The numbers at the nodes are bootstrap values computed using 1,000 replications and Kimura's 2-parameter distance model. See table 1 for the sample abbreviations.

Table 2. Haplotype definition and distribution of the red fox, *Vulpes vulpes*. A and B represent partial *Cyt b* gene sequences of 1,033 and 338 bp, respectively. See Table 1 for the sample abbreviations. **Vulpes vulpes* (AM181037) from GenBank was used as the reference.

A)

Individuals	Variable site																																	Accession number
	4	6	8	1	1	2	3	4	4	4	4	4	4	5	5	5	5	6	6	7	7	7	8	8	8	9	9	9	9	1 0	1 0	1 0	1 1	1 1	
	2	7	4	0	1	9	2	3	5	6	7	8	4	6	7	8	2	3	3	0	0	4	0	2	6	5	7	9	9	1	5	5	0	3	
				2	4	4	1	9	9	8	7	6	6	8	4	2	1	3	3	5	4	4	2	3	4	0	0	3	2	0	3	6	4	
**Vulpes vulpes* (AM181037)	A	G	T	G	T	G	T	A	T	C	T	A	C	G	T	G	T	C	C	C	T	T	T	T	A	C	A	C	A	T	C	T	A	
NK02, NK03	C	.	.	.	C	A	.	.	C	.	.	G	.	.	.	.	C	.	.	T	.	C	C	.	G	.	.	.	G	.	.	.	.	JN652603
KW5	C	A	.	.	.	.	.	.	C	.	.	.	.	A	.	.	C	T	.	.	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652604
KS8, KS10	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652605
CD10	C	.	.	A	.	.	.	.	C	T	.	.	.	.	.	A	C	.	.	.	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652606
RW1328, RW1651	C	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652607
RW1601	C	.	.	.	C	A	.	.	C	.	C	G	.	.	.	.	C	.	A	.	.	C	C	.	.	.	T	.	.	C	.	JN652608
KS2	C	A	.	.	C	A	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	C	C	.	.	T	.	.	.	.	.	.	.	JN652609
KW2	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652610
KS3	C	A	.	.	C	A	.	.	C	.	.	G	.	.	.	.	C	.	.	T	.	C	C	.	.	T	.	.	.	.	.	.	.	JN652611
KS4	C	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	C	.	.	T	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652612
KW4	C	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	T	.	.	JN652613
CD5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	A	.	.	.	.	C	.	.	.	JN652614
CD6	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	C	.	.	.	.	.	C	.	.	.	G	.	.	.	.	G	JN652615
CD7	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	G	.	.	.	.	G	JN652616
CD8	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	C	C	C	.	.	.	.	.	.	.	T	.	.	JN652617
MO06	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	C	C	.	T	T	.	.	.	.	.	.	.	JQ003577
MO09	C	.	.	.	C	A	.	.	C	.	C	G	.	.	.	.	C	.	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	JQ003578

B)

Haplotypes	Variable site									Samples	Corresponding to haplotypes	
				1	1	2	3	3	3		Aubry et al. (2009)	Inoue et al. (2007)
	4	6	8	0	1	9	2	3	5			
	2	7	4	2	4	4	1	1	9			
**Vulpes vulpes* (AM181037)	A	G	T	G	T	G	T	G	T			
Hap_1	C	.	.	.	C	A	.	.	.	NK02,NK03, RW1601, MO09	W	C10-D15, C10-D16, C8-D11
Hap_2	C	A	.	.	C	A	.	.	.	KS2,KS3		
Hap_3	C	.	.	.	.	.	.	.	.	KS4, KS8, KS10, KW4, CD8,CD9, MO06	U	C6-D10, C6-D5, C6-D6, C6-D7
Hap_4	.	.	.	.	.	.	.	.	.	KW2, CD5, CD6	U4	C1-D1, C1-D3
Hap_5	C	A	.	.	.	A	.	A	G	KW3		
Hap_6	C	A	.	.	.	.	.	.	.	KW5		
Hap_7	.	.	A	.	.	.	.	.	.	CD7		
Hap_8	C	.	.	A	.	.	.	.	.	CD10		
Hap_9	C	.	.	.	.	.	C	.	.	RW1328, RW1651		

this study with 61 *Cyt b* gene sequences obtained from the GenBank database. To analyze the *Cyt b* gene sequences, multiple alignments of the nucleotide sequences were performed with GENETYX-WIN version 4.0.1 (Genetyx Co., Tokyo, Japan) to identify nucleotide variation, from which the haplotypes were defined. A parsimonious haplotype network of the *Cyt b* gene haplotypes was drawn using TCS ver. 1.21 and the Templeton-Crandall-Sing parsimony algorithm with 95% probability (Clement et al., 2000). The divergence time was estimated using DnaSP ver 4.5 (Rozas and Rozas, 1955) with the *Cyt b* divergence rate of 2% per million years for large mammals (Brown et al., 1980; Avise et al., 1998). The phylogenetic relationships among maternal lineages of red foxes were constructed with the program MEGA. The stability of internal nodes was assessed by bootstrap analysis (1,000 replicates were used for neighbor-joining). The hierarchical nesting of genetic diversity was estimated using the analysis of molecular variance (AMOVA) approach with the ARLEQUIN software (Schneider et al., 2000).

RESULTS

Partial *Cyt b* gene sequences (1,033-bp) were obtained from 21 individuals. We obtained only 400-bp sequences in the 5′ region of KW3 (Table 2). Among the 21 *Cyt b* sequences, except for KW3, 28 transitions and six transversions at 33 variable sites were observed (Table 2). The neighbor-joining (NJ) tree was constructed using obtained 1,033-bp sequences with the kit fox (*Vulpes macrotis,* JF489127) sequence. None of the individuals from the different regions were separated (Fig. 1).

To compare previously studied red foxes, 338-bp *Cyt b* sequences from the 22 individuals were extracted from 1,033-bp *Cyt b* sequences. Nine haplotypes were observed from the 22 East Asian individuals (Table 2). The four wild

individuals from South Korea were Hap_3, Hap_4, Hap_5, and Hap_6 with no shared haplotype. While Hap_5 and Hap_6 were found only in South Korean individuals, Hap_3 and Hap_4 were observed in individuals from South Korea, China and the Seoul Grand Park Zoo (Table 2). Hap_3 was the most frequent haplotype, and was found in six individuals (30%). Two North Korean foxes and one Russian fox had Hap_1. The other two Russian red foxes had Hap_9, Hap_7 and Hap_8 were found only in Chinese foxes. All of the haplotypes, including missing haplotypes, had one to nine substitutions, and Hap_3, Hap_4, Hap_5, and Hap_6 differed by one to five substitutions (Table 2). The NJ tree with the small extracted fragments of 338-bp *Cyt b* gene sequences with the kit fox as the out-group was similar to that of the original 1,103-bp *Cyt b* sequences (Fig. 1). Thus, we performed further analysis with 388-bp sequences to compare previously reported red fox sequences. AMOVA was performed using the 338-bp *Cyt b* sequences assuming that the individuals were separated according to their countries of origin. However, the AMOVA result showed no significant genetic differentiation among the geographical groups and the majority of the total *Cyt b* variation (84%, $P < 0.05$) was distributed within populations, suggesting no further subgroups (Table 3).

Finally, we compared the 22 East Asian red foxes with 61 haplotypes of red foxes distributed worldwide from the GenBank database. For the 338-bp *Cyt b* sequences, 51 haplotypes were obtained with 49 variable sites (data not shown). The haplotype network formed three distinct clades (Fig. 2) and the mean genetic distances were 2.0% between clade I and clade II, 3.1% between clade I and clade III, and 2.1% between clade II and clade III. Clade I was composed of only Hokkaido individuals and clade II was composed of individuals from North American group. Clade III was separated into Eurasian and North Pacific groups. Interestingly, some East Asian red foxes, including South Korean red foxes, belonged to the Eurasia group and other haplotypes were assigned to the North Pacific group. Taking the *Cyt b* divergence rate of 2% per million years for large mammals (Brown et al., 1980; Avise et al., 1998), the divergence time of the three clades was estimated to have occurred around one million years ago (MYA) between clades I and II, 1.55 MYA between clades I and III, and 1.05 MYA between clades II and III during the Late Pleistocene era. In Korea, four red fox individuals were separated into two lineages (Figs. 2 and 3) and the divergence between the two lineages was roughly estimated to be 0.5 MYA. Nine haplotypes from 22 East Asian individuals, including the South Korean indi-

Table 3. Analyses of molecular variance based on *Cyt b* gene sequences from geographic groups of red fox.

Source of variation	Variance components	Percentage of variation	*P* value
Among groups	–0.21934	–21.48	0.85533 (> 0.05)
Among populations within groups	0.38015	37.22	0.05767 (> 0.05)
Within populations	0.86042	84.25	0.03519 (< 0.05)

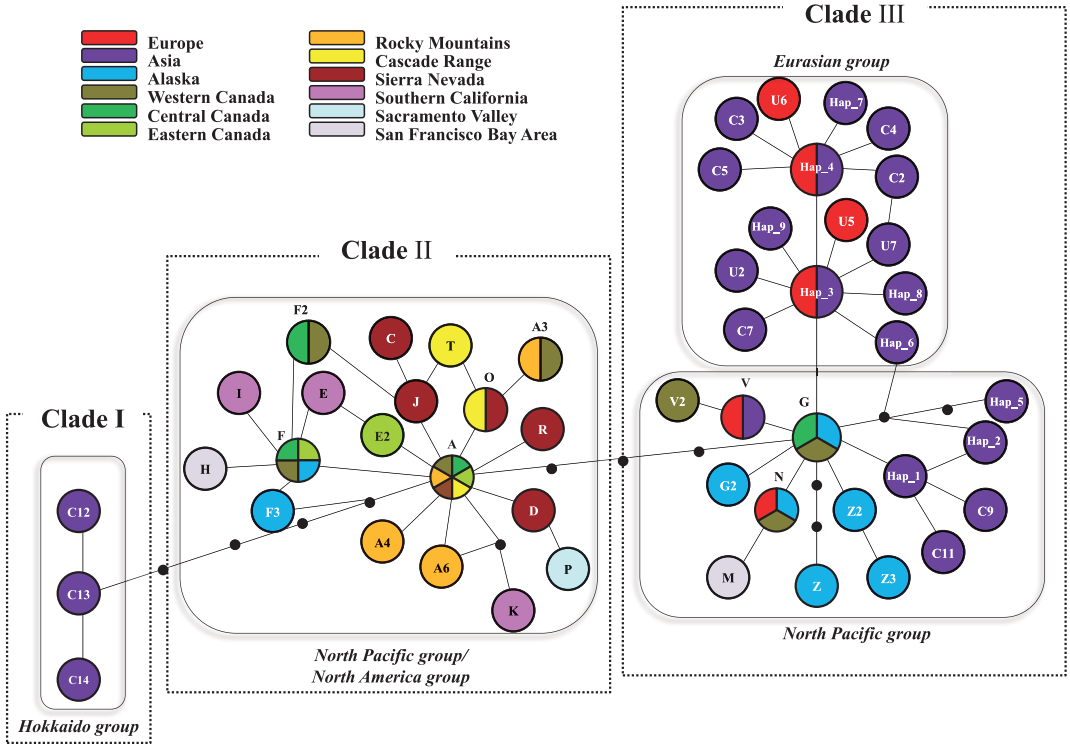

Fig. 2. Haplotype network of *Cyt b* gene based on 338-bp sequences for 22 red fox specimens in this study and 61 sequences from GenBank. One branch is proportional to the number of substitutions, and black circles show missing haplotypes. The boxes with the solid line are subclades, and boxes with the dashed line are major clades. The haplotype abbreviations refer to Aubry et al. (2009), Inoue et al. (2007), Perrine et al. (2007), and haplotypes of table 2 B.

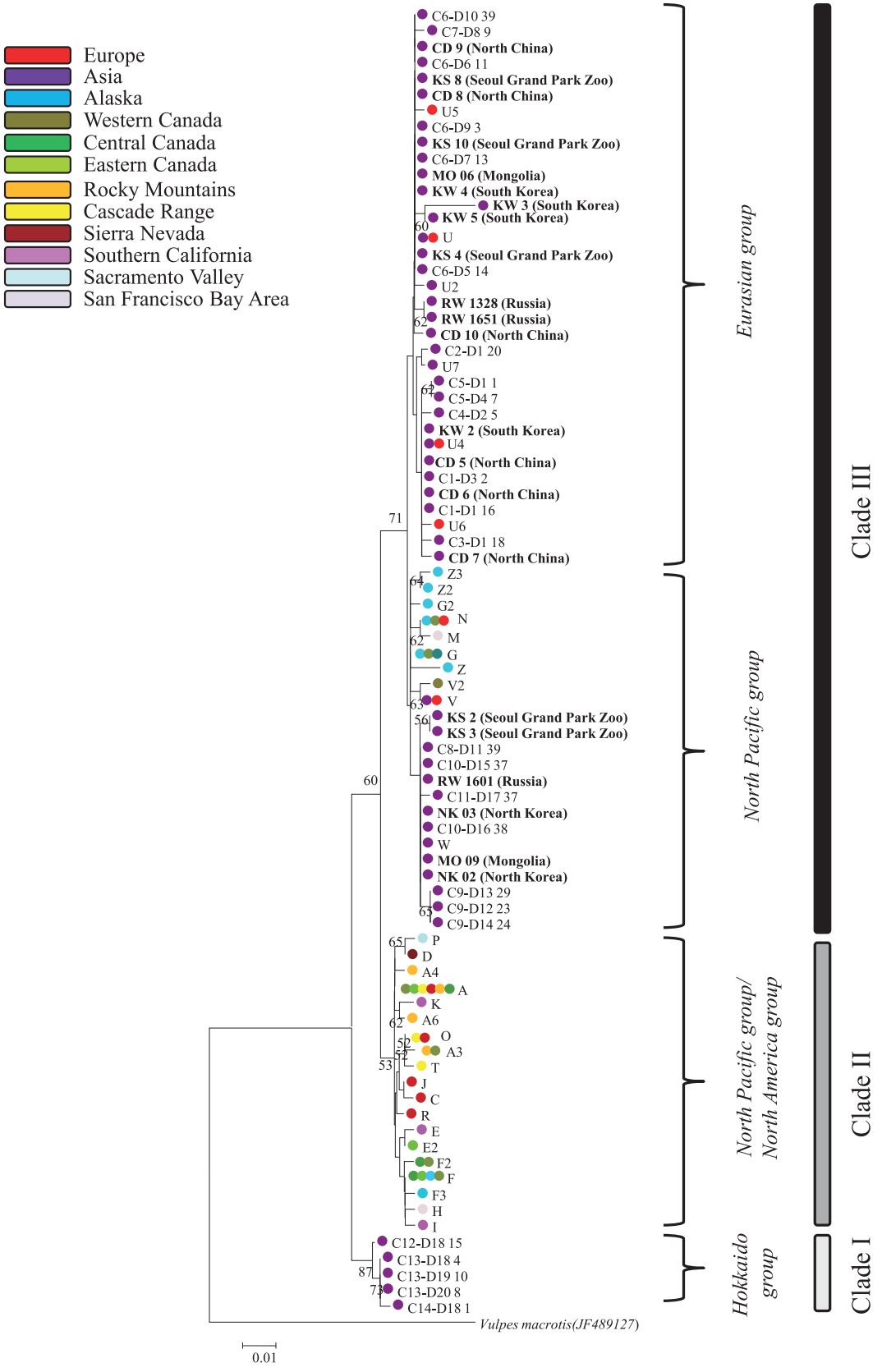

Fig. 3. Neighbor-joining tree based on the 338-bp *Cyt b* sequences of 22 specimens in this study and GenBank data. The numbers at the nodes are bootstrap values computed from 1,000 replications using Kimura's 2-parameter distance. The color bar indicates the clade divisions that correspond to those shown in Fig. 2. Bold indicates 22 individuals in this study. The 22 individuals and haplotype abbreviations refer to Aubry et al. (2009), Inoue et al. (2007), Perrine et al. (2007) and Table 1.

viduals, were assigned into Eurasian region and North Parcific region in the clade III (Fig 2). The NJ tree constructed using the 338-bp *Cyt b* sequences with the kit fox as the outgroup was consistent with the haplotype network (Fig. 3).

DISCUSSION

This study showed that the Korean red fox is basically clustered into clade III with Eurasian and North Pacific individuals (Fig. 2). In clade III, however, South Korean individuals assigned into the two groups with haplotypes from the Eurasian and from the North Pacific lineages, implying at least two recent migrations. Recent molecular phylogenetic studies showed that some parts of the Eurasian population were very close to those of the Alaskan population, and that ancestral populations moved from Eurasia to North America or North America to Eurasia via Beringia of the Late Pleistocene (Matsuhashi et al., 1999; Leonard et al., 2000; Matsuhashi et al., 2001; Mahmut et al., 2002; Skong et al., 2009). The divergence time between clades was estimated to be 0.5–1.5 MYA, suggesting that divergence predated the last glaciations (LGM; 10,000 to 50,000 years ago). Our results were in accordance with the divergence time estimation between the Hokkaido and the Honshu/Kyusyu/Russian red fox populations using *Cyt b* and *D-loop* (Inoue et al., 2007). The divergence time between lineages of the brown bear (*Ursus arctos*) and red deer (*Cervus elaphus*) estimated based on *Cyt b* and *D-loop* variations were also relatively close to our estimates (Matsuhashi et al., 1999; Leonard et al., 2000; Matsuhashi et al., 2001; Mahmut et al., 2002; Skong et al., 2009). Thus, the large Asian mammals mentioned above and the red fox share a similar history of intercontinental migration and evolution in the Pleistocene.

Of the nine haplotypes from the 22 individuals using the 338-bp *Cyt b* sequence, Hap_3 and Hap_4 were common in East Asia and South Korea (Table 2). This is consistent with a worldwide study; Hap_3 corresponds to the U haplotype and Hap_4 to the U4 haplotype, which are common in Europe and Asia (Aubry et al., 2009). In contrast, Hap_1, Hap_2, Hap_5, and Hap_6 may be local haplotypes distributed in East Asia, including South and North Korea, Japan (Honshu and Kyushu), and Russia (Table 2; Inoue et al., 2007; Aubry et al., 2009). The haplotype network showed that the 22 East Asian red fox in this study belongs to the Eurasian region and few haplotypes were assigned to the North Pacific region (Fig. 2; Aubry et al., 2009). Before the Quaternary, there was gene flow in plants and animals between eastern Siberia and Alaska via the Bering land bridge (estimated before 20,000–10,000 years ago) (Kawamura, 2007; Elias and Crocker, 2008; Dixon, 2011). Consequently, the Alaskan red fox genotypes in East Asia are a signature of past gene flow via this land bridge. During the Pleistocene, red fox populations underwent migration or dispersal during the glacial and inter-glacial periods, which might have caused the present genetic admixture of widely distributed haplotypes and local haplotypes (Taberlet et al., 1998; Hewitt, 1999; Inoue et al., 2007; Aubry et al., 2009). Thus, these results suggest that the East Asian red fox, including the South Korean, may have been divided into two or more genetic lineages. Consequently, multiple genetic lineages might attribute high genetic diversity in local wild population and the maintaining the certain level of genetic diversity in the restored population should be monitored.

The NJ tree of the East Asian individuals did not show a distinct geographic grouping (Fig. 1). In addition, AMOVA showed low genetic differentiation among the geographic groups, and the majority of total variation was within populations (Table 3). This unclear geographic structuring of at least two lineages in red fox is consistent with previous studies with the Hokkaido red fox (Inoue et al., 2007; Oishi et al., 2011). A study of red fox in Japan using mtDNA *Cyt b* and *D-loop* revealed that two distinct lineages in Hokkaido (Inoue et al., 2007). However, these distinct two haplotypes did not show any geographical structuring in Hokkaido. This admixture in the Hokkaido red fox was also supported with the study using microsatellites (Oishi et al., 2011). A previous study on the gray wolf (*Canis lupus*: Canidae) reported similar results of admixture (Vila et al., 1999). Although the gray wolf is distributed widely in Eurasia and most of the haplotypes were restricted to local groups, there was no clear phylogeographic structure (Vila et al., 1999). These unclear geographic patterns might reflect the absence of significant population structure and the presence of active gene flow among red fox populations and among gray wolf populations (Inoue et al., 2007; Aubry et al., 2009; Oishi et al., 2011).

Canidae, such as the fox and wolf, are very mobile and highly adaptable to various habitats. Some behavioral studies have suggested that the red fox can disperse over long distances (Strom et al., 1976). For example, some red foxes on Hokkaido in Japan were reported to have dispersed more than 30 km (Uraguchi, 2008). Red foxes are also reported to move up to 100 km in Europe and over 300 km in North America, and the average movement distance is 2.8–43.5 km for males and 1.8–38.6 km for females (Trewhella et al., 1988; Saunders et al., 1995). Movement exceeding 1000 km has been observed in the wolf (Fritts, 1983; Mech, 1987). Such behavioral characteristics of the red fox and gray wolf may cause the unclear phylogeographic genotype distribution (Vila et al., 1999; Inoue et al., 2007; Oishi et al., 2011). Compared to this unclear phylogeographic genetic structuring, distinct genetic differentiation and a well-differentiated genetic structure have been found in the grizzly bear (*Ursus arctos*) and Asiatic black bear (*Ursus thibetanus*), although they also had gene flow between Eurasian and North American populations via the Bering land bridge (Kohn et al., 1995; Weiss and Ferrand, 2007). A smaller-scale analysis of the Siberian chipmunk (*Tamias sibiricus*), an endangered species in Korea, separated it into northern and southern populations within South Korea individuals (Lee et al., 2008). Bears and the Siberian chipmunk have small mobility, and thus a low rate of admixture between different populations, and consequently, clear genetic structuring patterns (Leonard et al., 2000; Weiss and Ferrand, 2007; Lee et al., 2008; Ohnish et al., 2009). Therefore, the widespread admixture in red foxes and gray wolves is caused by high gene flow, which might be explained by the behavioral and ecological characteristics of Canidae, including their high mobility (Frati et al., 1998; Vila et al., 1999; Aubry et al., 2009).

In an Asiatic black bear restoration project in Korea, individuals from closely related populations were introduced

to prevent inbreeding and extinction, although a few isolated wild individuals remain (Han, 2006). In contrast, for the red fox in Korea, there is no pure breed for propagation. Consequently, the introduction of individuals from genetically and geographically close northeastern populations such as North Korea, China and Russia are the best alternative (Kleiman, 1989; Sawyer, 2008). However, although two genetically identical North Korean individuals in this study are insufficient to elucidate general genetic characteristics of North Korean red fox populations, three South Korean individuals (KW2, KW3, and KW5 with Hap_4, Hap_5 and Hap_6, respectively) were collected from near the border from North Korea so that the high genetic diversity in current North Korean population were also expected. Thus, the further studies with more intensive sampling from diverse geographic regions of North Korea, China, and Russia will be needed to select individuals for introduction.

Additionally, genetic markers of highly variable and biparental inheritance are needed, such as microsatellites for characterizing each individuals and family histories. Also, introduction should be preceded by ecological studies, including studies of the potential of introduced individuals to spread disease, the distribution of rodents as a food source for red fox, the potential effect of red fox on competitors, such as the leopard cat, raccoon dog, and raptors, and the identification of suitable habitats (Snyder et al., 1996; Beissinger and Westphal, 1998; Sawyer, 2008). Recent habitat fragmentation in South Korea will limit animal mobility and consequently decrease gene flow between populations. Inbreeding in the local population will pose a serious risk to the survival of the population because of low genetic diversity. For example, the Florida panther population declined sharply in the 1990s, leading to increased inbreeding and the risk of extinction from disease caused by the decreased genetic diversity and offspring sex ratio imbalance (Sawyer, 2008). Consequently, long-term provision for gene flow is needed and artificial outbreeding may be required in some cases, especially for endangered animals with high mobility (Hedrick and Fredrickson, 2008). Lastly, sustainable management and monitoring should be carried out continuously to ensure the persistent and stable settlement, breeding of introduced individuals and the level of genetic diversity.

ACKNOWLEDGMENTS

The authors thank Mi-Sook Min of Seoul National University, Ui-wook Hwang of Kyungbook National University, and Ho Young Suk of Yeungnam University for their valuable comments on this research. We also thank the Species Restoration Center of Korea National Park and Seoul Grand Park Zoo for providing red fox individuals for this study. This research was supported by "The Genetic Evaluation of Important Biological Resources (2011)" from the National Institute of Biological Resources of Korean government.

REFERENCES

Apostolidis AP, Mamuris Z, Triantaphyllidis C (2001) Phylogenetic relationships among four species of Mullidae (Perciformes) inferred from DNA sequences of mitochondrial cytochrome *b* and 16S rRNA genes. Biochem Syst Ecol 29: 901–909

Aubry KB, Statham MJ, Sacks BN, Perrine JD, Wisely SM (2009) Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. Mol Ecol 18: 2668–2686

Avise JC (1994) Molecular markers, natural history and evolution. Chapman and Hall, New York, pp 1–511

Avise JC, Walker D, Johns GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc Biol Sci 265: 1707–1712

Beissinger SR, Westphal MI (1998) On the use of demographic models of population viability in endangered species management. J Wildlife Manage 62: 821–841

Brown WM (1980) Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. Proc Natl Acad Sci USA 77: 3605–3609

Brown WM, George MJ, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76: 1967–1971

Clayton DA (1982) Replication of animal mitochondrial DNA. Cell 28: 693–705

Clement M, Posaka D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1657–1659

Corbet GB (1978) The mammals of the Palaearctic Region: A Taxonomic Review. British Museum (Natural History), London, p 314

Dixon EJ (2011) Late Pleistocene colonization of North America from Northeast Asia: New insights from large-scale paleogeographic reconstructions. Quaternary International: 1–11

Edwards SV, Wilson AC (1990) Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostomus*). Genetics 126: 695–711

Elias SA, Crocker B (2008) The Bering Land Bridge: a moisture barrier to the dispersal of steppe-tundra biota? Quaternary Sci Rev 27: 2473–2483

Esposti MD, De Vries S, Crimi M, Ghelli A, Patarnello T, Meyer A (1993) Mitochondrial cytochrome *b*: evolution and structure of the protein. Biochem Biophys Acta 1143: 243–271

Frati F, Hartl GB, Lovari S, Delibes M, Mrkov G (1998) Quaternary radiation and genetic structure of the red fox *Vulpes vulpes* in the Mediterranean Basin, as revealed by allozymes and mitochondrial DNA. J Zool Lond 245: 43–51

Fritts SH (1983) Record dispersal by a wolf from Minnesota. J Mammal 64: 166–167

Garshelis DL, Steinmetz R (2008) *Ursus thibetanus*. In IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. <www.iucnredlist.org>

Han S-H (2006) The Status of Bears and Restoration Projects on the Korean Peninsula. Understanding Asian Bears to Secure Their Future. Japan Bear Network, Ibaraki, Japan, pp 102–106

Hayashi J-I, Tagashira Y, Yoshida MC (1985) Absence of extensive recombination between inter- and intraspecies mitochondrial DNA in mammalian cells. Exp Cell Res 160: 387–396

Hedrick PW, Fredrickson RJ (2008) Captive breeding and the reintroduction of Mexican and red wolves. Mol Ecol 17: 344–350

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biol J Linnean Soc 68(1–2): 87–112

Higuchi C, von Beroldingen H, Sensabaugh GF, Erlich HA (1988) DNA typing from single hairs. Nature 332: 543–546

Hillis DM, Huelsenbeck JP, Cunningham CW (1994) Application and accuracy of molecular phylogenies. Science 264: 671–677

Inoue T, Nonak N, Mizuno A, Morishima Y, Sato H, Katakura K, Oku Y (2007) Mitochondrial DNA phylogeography of the red fox (*Vulpes vulpes*) in Northern Japan. Zool Sci 24: 1178–1186

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of cytochrome *b* gene of mammals. J Mol Evol 32(2): 128–144

Kawamura Y (2007) Last glacial and Holocene land mammals of the Japanese islands: their fauna, extinction and immigration. Quaternary Res 46(3): 171–177

Kleiman DG (1989) Reintroduction of captive mammals for conservation: guidelines for reintroducing endangered species into the wild. BioSciences 39: 152–161

Kohn M, Knauer F, Stoffella A, Schroeder W, Paabo S (1995) Conservation genetics of the European brown bear—A study using

excremental PCR of nuclear and mitochondrial sequences. Mol Ecol 4: 95–103

Lansman RA, Avise JC, Huettel MD (1983) Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. Proc Natl Acad Sci USA 80: 1969–1971

Lariviery S, Pasitschniak-Arts M (1996) *Vulpes vulpes*. Mammalian Species 537: 1–11

Lee M-Y, Lissovsky AA, Park S-K, Obolenskaya EV, Dokuchaev NE, Zhang Y-p, et al. (2008) Mitochondrial cytochrome b sequence variations and population structure of Siberian chipmunk (*Tamias sibiricus*) in Northeastern Asia and population substructure in South Korea. Mol Cells 26: 566–575

Leonard JA, Wayne RK, Cooper A (2000) Population genetics of Ice Age brown bears. PNAS 97: 1651–1654

Ma DP, Zoharkikh A, Graur D, Vandeberg JL, Li WH (1993) Structure and evolution of opossum, guinea pig and porcupine cytochrome b genes. J Mol Evol 36: 327–334

Mahmut H, Masuda R, Onuma M, Takahashi M, Nagata J, Suzuki M, Ohtaishi N (2002) Molecular phylogeography of the red deer (*Cervus elaphus*) populations in Xinjiang of China: Comparison with other Asian, European, and North American populations. Zool Sci 19: 485–495

Matsuhashi T, Masuda R, Mano T, Yoshida MC (1999) Microevolution of the mitochondrial DNA control region in the Japanese brown bear (*Ursus arctos*) population. Mol Biol Evol 16: 676–648

Mech LD (1987) Age, season, distance, direction, and social aspects of wolf dispersal from a Minnesota pack. In "Mammalian Dispersal Patterns: The Effects of Social Structure on Population Genetics" Ed by BD Chepko-Sade, ZT Halpin, University of Chicago Press, Chicago, pp 55–74

Ministry of the Environment of Korea (2005) Government Notification 2005–20. Gwancheon-shi, Gyeongggi-do, Korea

Moller PR, Gravlund P (2003) Phylogeny of the eelpout genus *Lycodes* (Pisces, Zoarcidae) as inferred from mitochondrial cytochrome b and 12S rDNA. Mol Phylogenet Evol 26: 369–388

Ohnish N, Uno R, Ichibashi Y, Tanate HB, Oi T (2009) The influence of climatic oscillations during the Quaternary Era on the genetic structure of Asian black bears in Japan. Heredity 102: 579–589

Oishi T, Uraguchi K, Takahashi K, Masuda R (2011) Population structures of the red fox (*Vulpes vulpes*) on the Hokkaido Island, Japan, revealed by microsatellite analysis. J Heredity 102: 38–46

Park JY, Lee HJ, Kim WJ, Lee JH, Min KS (2000) Mitochondrial cytochrome b sequence variation in Korean salmonids. J Fish Biol 56: 1145–1154

Randi E, Luccini V, Hennache A, Kimball RT, Braun EL, Ligon JD (2001) The mitochondrial DNA control region and cytochrome b genes and the inference of phylogenetic relationships in the avian genus *Lophura* (Galliformes). Mol Phylogenet Evol 19: 187–201

Perrine, JD, Pollinger JP, Sacks BN, Barrett RH, Wayne RK (2007) Genetic evidence for the persistence of the critically endangered Sierra Nevada red fox in California. Conservation Genetics 8: 1083–1095

Rocha-Olivares A, Kimbrell CA, Eitner BJ, Vetter RD (1999) Evolution of a mitochondrial cytochrome b gene sequence in the species-rich genus *Sebastes* (Teleostei, Scorpaenidae) and its utility in testing the monophyly of the subgenus *Sebastomus*. Mol Phylogenet Evol 11: 426–440

Rozas J, Rozas R (1995) DnaSP, DNA sequence polymorphism: an interactive program for estimating Population Genetics parameters from DNA sequence data. Comput Applic Biosci 11: 621–625

Saunders G, Coman B, Kinnear J, Braysher M (1995) Managing Vertebrate Pests: Foxes. Australian Government Publishing Service, Canberra

Sawyer R (2008) The endangered Florida panther (*Puma* [=*Felis*] *concolor coryi*) conservation status: *in situ* breeding and shrinking habitat. http://jhir.library.jhu.edu/handle/1774.2/34161

Schneider S, Roessli D, Excoffier L (2000). ARLEQUIN: A Software for Population Genetics Data Analysis. User Manual ver. 2.000. (Geneva, Switzerland: University of Geneva)

Skog A, Zachos FE, Rueness EK, Feulner PGD, Mysterud A, Langvatn R, et al. (2009) Phylogeography of red deer (*Cervus elaphus*) in Europe. J Biogeogr 36: 66–77

Snyder NFR, Derricksons SR, Beissinger SR, Wiley JW, Smith HTB, Toone WD, Miller B (1996) Limitations of captive breeding in endangered species recovery. Conserv Biol 10: 338–348

Taberlet P, Funagalli L, Wust-Saucy A-G, Cosson J-F (1998) Comparative phylogeography and postglacial colonization routes in Europe. Mol Ecol 7: 453–464

Storm GL, Andrews RD, Phillips RL, Bishop RL, Siniff DB, Tester JR (1976) Morphology, reproduction, dispersal, and mortality of Midwestern red fox populations. Wildl Monogr 49: 1–82

Trewhella WJ, Harris S, McAllister FE (1988) Dispersal distance, home-range size and population density in the red fox (*Vulpes vulpes*): a quantitative analysis. J Applied Ecol 25: 423–434

Uraguchi K (2008) Epidemiology and ecology of the red fox. In "Mammalogy in Japan 2" Ed by N Takatsuki, J Yamagiwa, University of Tokyo Press, Tokyo, pp 149–171 (in Japanese)

Vila C, Amorim IR, Leonard JA, Posada D, Castrociejo J, Petrucci-Fonseca F, et al. (1999) Mitochondrial DNA phylogeography and population history of the gray wolf *Canis lupus*. Mol Ecol 8: 2089–2103

Weiss SJ, Ferrand N, eds. (2007) Phylogeography in Southern European Refugia: Evolutionary Perspectives on the Origins and Conservation of European Biodiversity. Kluwer Academic Publishers, Dordrecht, Netherlands

Wilson DE, Mittermeier RA (2009) Handbook of the Mammals of the World. Vol. 1. Carnivores. Lynx Editions, Barcelona

Won C, Smith KG (1999) History and current status of mammals of the Korean peninsula. Mammal Rev 29: 3–33

(Received September 18, 2011 / Accepted June 2, 2012)