Mol. Cells *26*, 566-575, December 31, 2008

Molecules and Cells

Mitochondrial Cytochrome *b* Sequence Variations and Population Structure of Siberian Chipmunk (*Tamias sibiricus*) in Northeastern Asia and Population Substructure in South Korea

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Twenty-five chipmunk species occur in the world, of which only the Siberian chipmunk, *Tamias sibiricus*, inhabits Asia. To investigate mitochondrial cytochrome *b* sequence variations and population structure of the Siberian chipmunk in northeastern Asia, we examined mitochondrial cytochrome *b* sequences (1140 bp) from 3 countries. Analyses of 41 individuals from South Korea and 33 individuals from Russia and northeast China resulted in 37 haplotypes and 27 haplotypes, respectively. There were no shared haplotypes between South Korea and Russia - northeast China. Phylogenetic trees and network analysis showed 2 major maternal lineages for haplotypes, referred to as the S and R lineages. Haplotype grouping in each cluster was nearly coincident with its geographic affinity. In particular, 3 distinct groups were found that mostly clustered in the northern, central and southern parts of South Korea. Nucleotide diversity of the S lineage was twice that of lineage R. The divergence between S and R lineages was estimated to be 2.98-0.98 Myr. During the ice age, there may have been at least 2 refuges in South Korea and Russia - northeast China. The sequence variation between the S and R lineages was 11.3% (K2P), which is indicative of specific recognition in rodents. These results suggest that *T. sibiricus* from South Korea could be considered a separate species. However, additional information, such as details of distribution, nuclear genes data or morphology, is required to strengthen this hypothesis.

INTRODUCTION

During the Pleistocene Era, the climate of the Northern hemisphere fluctuated frequently and dramatically (Andersen and Borns, 1997; Hewitt, 2000; Markov et al., 1965). The effects of these extreme climate changes have been found through the study of isolation in glacial refugia, local extinctions, biological diversity and extreme demographic oscillations from widely distributed species. Such research can be used to determine the evolutionary history of a species, as well as for identification of hidden population structures (Gündüz et al., 2007). These revelations can potentially define species or subspecies, an Evolutionary Significant Unit (ESU) and / or Management Unit (MU) (Allendorf and Luikart, 2007). The conservation and management of wildlife would not be where they are today without this evidence.

The 25 known chipmunk species have a complex geographical distribution and are differentiated by minute morphological differences. The Siberian chipmunk, *T. sibiricus*, inhabits the northern part of the Eurasian continent, while the eastern chipmunk, *T. striatus,* occurs throughout the eastern United States. The remaining 23 species are distributed throughout the western United States and Mexico (Banbury and Spicer, 2007; Piaggio and Spicer, 2001). To date, most phylogeographic and population genetics studies have been conducted for chipmunks in North America (Demboski and Sullivan, 2003; Good et al., 2003; Good and Sullivan, 2001; Schulte-Hostedde et al., 2001). There have been few molecular genetic studies of Siberian chipmunks. However, acoustic studies revealed an unidentified variation pattern in Siberian chipmunks (Lissovsky et al., 2006), demonstrating the need for population genetics study of this species.

The Siberian chipmunk (*Tamias sibiricus*) is distributed from northwest Russia eastward through Siberia and Far East, Mongolia, northeastern and central China, northern Japan and Ko-

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Received June 18, 2008; revised October 7, 2008; accepted October 9, 2008; published online October 13, 2008

Keywords: cytochrome *b* sequence variation, population structure, Siberian chipmunk

rea (Ognev, 1940). Historically, in Korea, most chipmunks were distributed in mountain and forest habitats. However, human activities have fragmented these populations. For example, the construction of express highways and local roads in South Korea resulted in potential physical barriers. Moreover, trapping for commercial trade occurred (Won and Smith, 1999) and population fluctuations in some local regions of South Korea might have occurred due to predators, such as wild cats (2004 Report of national survey on natural environment. DVD. National Institute of Environmental Research, Ministry of Environment). Siberian chipmunks are good companion animals caught in the wild of South Korea, so they may have been randomly spread out by humans. Populations are now found in urban settings as the result of captive bred individuals having been artificially released (Won and Smith, 1999). Until now, the study of genetic diversity of *T. sibiricus* in South Korea has been limited, in spite of the need for conservation and management strategies. Basic knowledge of the genetic diversity, phylogenetic relationships and population genetics of chipmunk would provide valuable information for the development of a protection strategy, and would provide important insights on chipmunk evolutionary history.

Several studies have focused on chipmunk systematics and phylogenetic relationships among Sciruidae species, including Siberian chipmunks, using nuclear and mitochondrial markers to determine the patterns of their evolution, dispersal and relatedness (Banbury and Spicer, 2007; Oshida et al., 1996; Steppan et al., 2004). Jones and Johnson (1965) reported that Siberian chipmunks on the Korean peninsula are classified into two subspecies: *T. s. barberi* in the central and southern parts of the peninsula, *and T. s. orientalis* in the extreme north-eastern part of Korea. Cheng (1991) found no differences between chipmunks from northeast China and Korean using morphological and cytogenetic analyses. At present, no study elucidates the genetic diversity and population structure of the Siberian chipmunks from diverse regions such as Russia, China and South Korea.

In the present study, *Tamias sibiricus* specimens from different localities in South Korea, Russia and China were compared by analyzing molecular data from Mt cytochrome *b* gene. The goals of the present study are: 1) to investigate genetic variations and evolutionary history of Siberian chipmunks from 3 regions (South Korea, northeast China and some regions of Russia) and 2) to reveal population substructure of *T. sibiricus* in South Korean populations. Based on molecular phylogenetic and population genetics data, we discuss population distinctiveness and population structure of this species.

MATERIALS AND METHODS

Materials

A total of 72 specimens of Siberian chipmunk, *Tamias sibiricus*, from 25 localities distributed across the species' range were analyzed (Fig. 1; Table 1). The samples collected in South Korea were provided by local rescue centers from road kills, while samples in Russia and China were collected by trapping and from carcasses. Samples were preserved at -70°C or in absolute ethanol following the storage standard of CGRB (Conservation Genome Resource Bank for Korean Wildlife). Two sequences were obtained from GenBank (AF147666, AF147667; Piaggio and Spicer, 2001) for phylogenetic analysis. We used sequences of 4 species from the subgenus *Tamias* (*T. senex, T. dorsalis, T. siskiyou, T. townsendii*) as outgroups in the phylogenetic analyses.

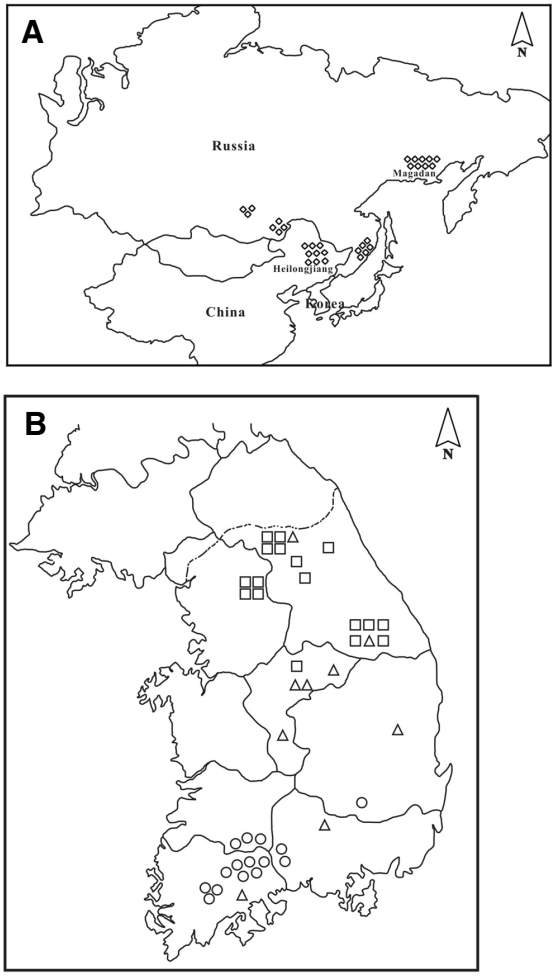

Fig. 1. Map of Eurasia (A) and South Korea (B) showing collection sites for *Tamias sibiricus*.

DNA extraction, PCR amplification and sequencing

Whole genomic DNA was extracted from muscle or liver using the DNeasy tissue kit (Qiagen, USA) following the protocol for animal tissue as recommended by the manufacturer. Two primers were used to amplify the entire cytochrome *b* gene sequence (1140 bp) with a polymerase chain reaction (PCR); L14724 and H15915 (Kocher et al., 1989). Three primers, L14724, L15162 (Irwin et al., 1991) and H15915, were used for sequencing. The amplification process was conducted as follows: 94°C for 5 min; 35 cycles of 94°C for 45 s, 45°C for 60 s, and 72°C for 90 s; and final 72°C for 7 min. PCR mixtures were prepared in 20 ml reaction volume containing 10-50 ng template DNA, 100 μM each dNTPs, 10 pmole each primer, 1.5 mM $MgCl_2$, 1 unit Takara Taq™ (Takara, Japan), and 10× PCR buffer. PCR products were checked on 2% agarose gels and PCR products were purified with the Zymoclean Gel DNA Recovery Kit (Zymo research, USA). All samples were sequenced on an Applied Biosystems 3730 XL DNA sequencer following manufacturer's instructions. Chromatograms and alignments were visually checked and verified. The sequences generated in this study have been deposited in GenBank (EU754751- EU754822).

mtDNA sequence analysis and phylogeny

Multiple sequence alignments were made using CLUSTAL_X

Table 1. List of samples examined in this study

NO.	CGRB ID	Collecting Localities	Type of sample	Country	No. of haplotype
1	cgrb288	Chuncheon, Gangwon-do	Muscle	South Korea	Hap1
2	cgrb289	Hwacheon, Gangwon-do	Muscle	South Korea	Hap2
3	cgrb290	Yanggu, Gangwon-do	Muscle	South Korea	Hap3
4	cgrb2389	Cheolwon, Gangwon-do	Muscle	South Korea	Hap4
5	cgrb2605	Cheolwon, Gangwon-do	Muscle	South Korea	Hap5
6	cgrb3103	Chungju, Chungcheongbuk-do	Muscle	South Korea	Hap6
7	cgrb3478	Gapyeong, Gyeonggi-do	Muscle	South Korea	Hap7
8	cgrb3479	Gapyeong, Gyeonggi-do	Muscle	South Korea	Hap7
9	cgrb3480	Gapyeong, Gyeonggi-do	Muscle	South Korea	Hap8
10	cgrb3481	Gapyeong, Gyeonggi-do	Muscle	South Korea	Hap9
11	cgrb3494	Cheolwon, Gangwon-do	Muscle	South Korea	Hap10
12	cgrb3894	Cheolwon, Gangwon-do	Muscle	South Korea	Hap11
13	cgrb4098	Teaback, Gangwon-do	Muscle	South Korea	Hap12
14	cgrb4100	Teaback, Gangwon-do	Muscle	South Korea	Hap12
15	cgrb4099	Teaback, Gangwon-do	Muscle	South Korea	Hap13
16	cgrb4102	Teaback, Gangwon-do	Muscle	South Korea	Hap14
17	cgrb1276	Okcheon, Chungcheongbuk-do	Muscle	South Korea	Hap15
18	cgrb1567	Cheolwon, Gangwon-do	Muscle	South Korea	Hap16
19	cgrb1975	Cheongsong, Gyeongsangbuk-do	Muscle	South Korea	Hap17
20	cgrb2096	Hapcheon, Gyeongsangnam-do	Muscle	South Korea	Hap18
21	cgrb2125	Goesan, Chungcheongbuk-do	Muscle	South Korea	Hap19
22	cgrb3407	Suncheon, Jeollanam-do	Muscle	South Korea	Hap20
23	cgrb3510	Goesan, Chungcheongbuk-do	Muscle	South Korea	Hap21
24	cgrb3750	Danyang, Chungcheongbuk-do	Muscle	South Korea	Hap22
25	cgrb4103	Teaback, Gangwon-do	Muscle	South Korea	Hap23
26	cgrb1129	Gurye, Jeollanam-do	Muscle	South Korea	Hap24
27	cgrb1144	Gurye, Jeollanam-do	Muscle	South Korea	Hap25
28	cgrb1145	Namwon. Jeollabuk-do	Muscle	South Korea	Hap26
29	cgrb1151	Namwon. Jeollabuk-do	Muscle	South Korea	Hap27
30	cgrb1213	Gurye, Jeollanam-do	Muscle	South Korea	Hap29
31	cgrb1267	Gurye, Jellanam-do	Muscle	South Korea	Hap28
32	cgrb3659	Daegu, Kyeongsangnam-do	Muscle	South Korea	Hap29
33	cgrb3679	Gwangju, Jellanam-do	Muscle	South Korea	Hap29
34	cgrb2134	Gurye, Jeollanam-do	Muscle	South Korea	Hap30
35	cgrb3345	Gurye, Jeollanam-do	Muscle	South Korea	Hap31
36	cgrb3346	Gurye, Jeollanam-do	Muscle	South Korea	Hap32
37	cgrb3653	Gwangju, Jellanam-do	Muscle	South Korea	Hap33
38	cgrb3672	Gwangju, Jellanam-do	Muscle	South Korea	Hap34
39	cgrb3714	Namwon, Jellanam-do	Muscle	South Korea	Hap35
40	cgrb3814	Sangcheong, Gyeongsangnam-do	Muscle	South Korea	Hap36
41	cgrb3815	Sangcheong, Gyeongsangnam-do	Muscle	South Korea	Hap37
42	157	Magadan vicinity	Muscle	Russia	Hap38
43	165	Magadan vicinity	Muscle	Russia	Hap38

(Continued)

NO.	CGRB ID	Collecting Localities	Type of sample	Country	No. of haplotype
44	166	Magadan vicinity	Muscle	Russia	Hap38
45	AF147666	Badzhal_Range (Khabarovsk Territory)	-	Russia	Hap38
46	158	Magadan vicinity	Muscle	Russia	Hap39
47	162	Magadan vicinity	Muscle	Russia	Hap39
48	159	Magadan vicinity	Liver	Russia	Hap40
49	160	Magadan vicinity	Muscle	Russia	Hap41
50	161	Magadan vicinity	Muscle	Russia	Hap42
51	164	Magadan vicinity	Muscle	Russia	Hap43
52	AF147667	Ola_River (Magadan Region)	-	Russia	Hap44
53	cgrb4111	Heilongjiang Province	Muscle	China	Hap45
54	cgrb4113	Heilongjiang Province	Muscle	China	Hap46
55	cgrb4115	Heilongjiang Province	Muscle	China	Hap47
56	cgrb4116	Heilongjiang Province	Muscle	China	Hap48
57	cgrb4118	Heilongjiang Province	Muscle	China	Hap49
58	cgrb4120	Heilongjiang Province	Muscle	China	Hap50
59	cgrb3506	Lazo Reserve, Primorye Territory	Muscle	Russia	Hap51
60	cgrb4112	Heilongjiang Province	Muscle	China	Hap52
61	cgrb4114	Heilongjiang Province	Muscle	China	Hap53
62	cgrb4119	Heilongjiang Province	Muscle	China	Hap54
63	cgrb2709	Lazo Reserve, Primorye Territory	Muscle	Russia	Hap55
64	cgrb2710	Lazo Reserve, Primorye Territory	Muscle	Russia	Hap56
65	cgrb2711	Lazo Reserve, Primorye Territory	Muscle	Russia	Hap56
66	cgrb3504	Lazo Reserve, Primorye Territory	Muscle	Russia	Hap57
67	cgrb3505	Lazo Reserve, Primorye Territory	Muscle	Russia	Hap58
68	83	Chita Region, Southeast Transbaikalia	Liver	Russia	Hap59
69	84	Chita Region, Southeast Transbaikalia	Muscle	Russia	Hap60
70	132	Chita Region, Southeast Transbaikalia	Liver	Russia	Hap61
71	136	Chita Region, Southeast Transbaikalia	Liver	Russia	Hap62
72	cgrb3698	Oshurkovo, Buriatia Republic, west Transbaikalia	Muscle	Russia	Hap63
73	cgrb3699	Oshurkovo, Buriatia Republic, west Transbaikalia	Muscle	Russia	Hap64
74	cgrb3700	Oshurkovo, Buriatia Republic, west Transbaikalia	Muscle	Russia	Hap64

(Thompson et al., 1997), with further modification by eye using Bioedit (Hall, 1999). Haplotype (*h*) and nucleotide (π) diversities among geographic locations were estimated as implemented in DnaSP version 4.10 (Rozas et al., 2003). The pairwise sequence difference between populations was calculated by the Kimura 2-parameter using MEGA version 4 (Tamura et al., 2007). The phylogenetic relationships among maternal lineages of Siberian chipmunks were constructed with the program PAUP 4b10 (Swofford, 2001). The appropriate model of sequence evolution was chosen on the basis of hierarchical likelihood-ratio tests as implemented in MODELTEST 3.06 (Posada and Crandall, 1998), and had the following parameters: base frequencies of 0.3124, 0.2796, 0.0999, and 0.3082 for A, C, G, and T nucleotides, respectively; Substitution rate of 1, 14.9535, 1, 1, 18.8950, and 1 for A-C, A-G, A-T, C-G, C-T, and G-T nucleotide rate; a gamma shaper parameter=0.1535. The NJ, ML and MP topologies were constructed using a heuristic search with a tree-bisection-reconnection (TBR) algorithm. The stability of internal nodes was assessed by bootstrap analysis (1000 replicates were used for NJ, and 100 for ML and MP). Median-joining network (Bandelt et al., 1999) was drawn using the program Network 4.0 to investigate possible relationships among haplotypes of Siberian chipmunks. This approach has been verified to generate the best genealogies among other rooting and network procedures (Cassens et al., 2003).

Population genetics and historical demographic analyses

To assess whether there was significant geographical differentiation, the partitioning of total genetic variation was hierarchically examined by an analysis of molecular variance (AMOVA) in ARLEQUIN ver. 3 (Excoffier et al., 2005). Pairwise differences between haplotypes were used as a molecular distance measure. The statistical significance of variance components in AMOVA was tested with 10000 permutations. We also examined the demographic history of populations and geographical units by constructing a distribution of pairwise differences, the

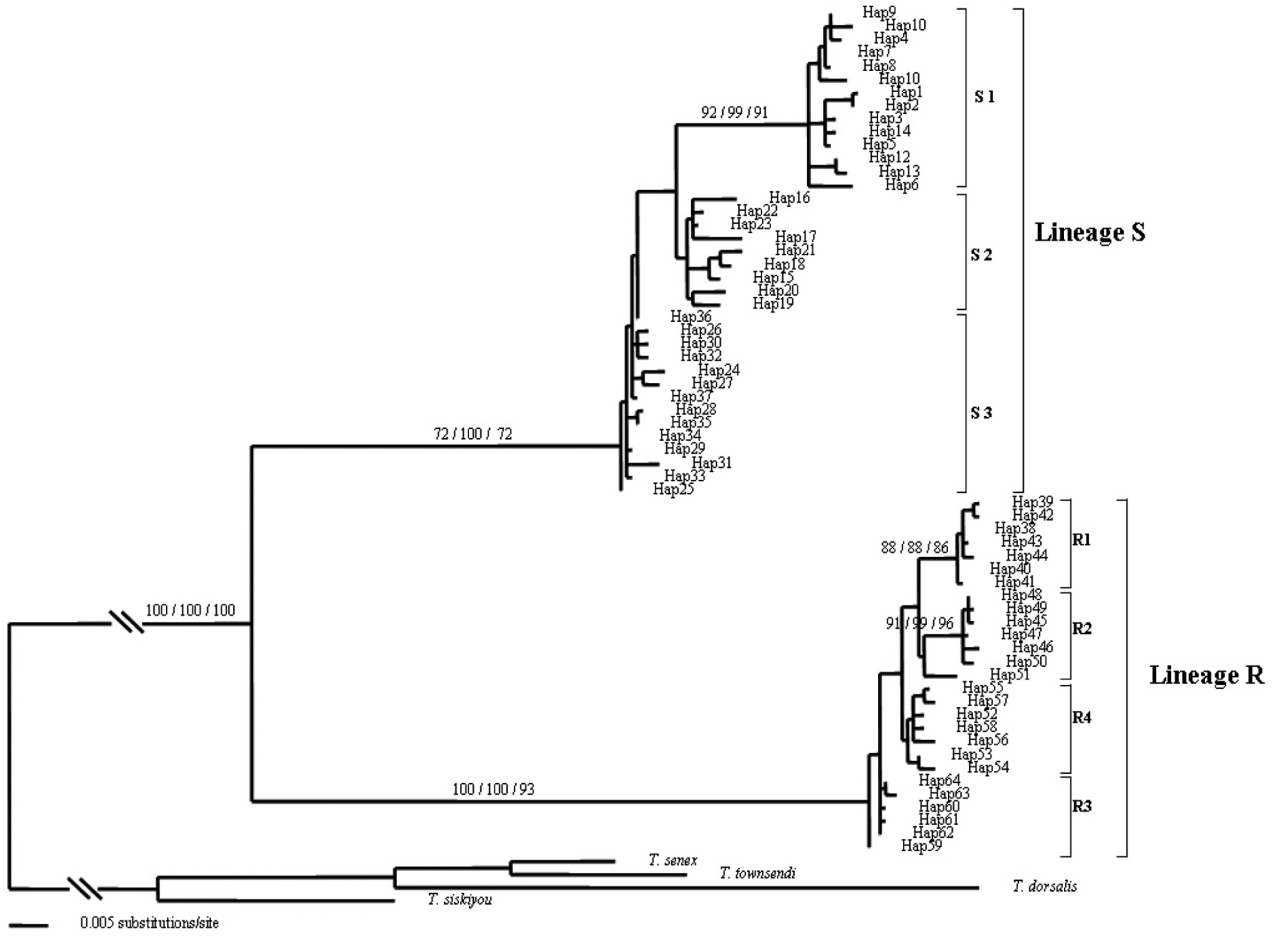

Fig. 2. Maximum likelihood tree of 64 haplotypes of Siberian chipmunk sequences, which were separated into two major clades: S and R. Clade S has three groups (S1, S2, S3). Numbers above or below the branches correspond to bootstrap support obtained from the NJ, MP and ML analyses, respectively.

mismatch distribution, which aids in distinguishing between populations that have been stable over time with those that have experienced recent expansion or reduction. This method is based on an assumed stepwise growth model only and does not consider alternatives. Fu's *Fs* statistic (Fu, 1997) was computed to test for neutrality and demographic expansions with ARLEQUIN ver. 3. Relative ratio test was estimated by two cluster test in Phyltest (Kumar, 1996) using Kimura 2-parameter.

RESULTS

Mitochondrial DNA sequence variation

A complete Mt cytochrome *b* sequence (1140 bp) defined 64 haplotypes in a total of 74 individuals of *T. sibiricus*, of which 37 haplotypes were from 41 individuals from South Korea, 18 haplotypes from 24 individuals from Russia and 9 haplotypes from 9 individuals from China (Table 1). Nucleotide substitutions were detected at 213 (19.4%) positions, and 170 (14.9%) of the sites were parsimony informative. Among 213 variable sites, 193 transitions, 26 transversions and no indels (insertions and deletions) were found. The largest haplotype group (Hap 38) consisted of 4 individuals from Russia, while 4 haplotypes from South Korea (Hap7 and Hap12) and Russia (Hap 42 and Hap 56) included 2 individuals and 2 haplotypes from South Korea (Hap29) and Russia (Hap 38) included 3 individuals. No individuals among populations from Korea and Russia - northeast China shared haplotypes.

As summarized in Table 2, overall haplotype diversity (*h*) and nucleotide diversity (π) were 0.995 and 5.915%, respectively. Nucleotide diversity was higher for entire regions than within regions (π of entire regions = 5.915 and π of each region = 0.809-1.886), whereas haplotype diversity was similar among regions (Overall h = 0.995 and h of each region = 0.967-1). The level of nucleotide diversity within South Korea (π = 1.886 $\pm$ 0.08%) was twice that of other regions (China, π = 0.809 $\pm$ 0.14%; Russia, 0.865 $\pm$ 0.05%). The mean genetic distances among all localities from South Korea, Russia and northeast China were, respectively, 1.9% (0-3.5%), 0.9% (0-1.5%) and 0.8% (0.1-1.5%). The genetic distance between the clade containing the only haplotypes from South Korea and the clade containing the haplotypes from northeast China and Russia was 0.113 substitutions per site for cytochrome *b* (Kimura 2 parameter).

Phylogenetic relationships and population structure

The ML construction for phylogenetic relationships among haplotypes is shown in Fig. 2. The MP and NJ trees had identical topologies with 2 major clades and few differences in the details.

Table 2. Summary of sample sizes, number of haplotypes (*n*), nucleotide diversity (π), haplotype diversity (*h*), polymorphic site, singleton, parsimony informative sites for the specimens used in this study.

	n	No. of haplotypes	π (% ± SE)	*h* (SE)	No. of Polymorphic sites	Singleton	Parsimony informative sites
South Korea	41	37	1.886 (0.08)	0.994 (0.007)	113	51	62
China	9	9	0.809 (0.14)	1.000 (0.052)	26	12	14
Russia	24	18	0.865 (0.05)	0.967 (0.024)	44	20	24
Overall	74	64	5.915 (0.11)	0.995 (0.004)	213	43	170

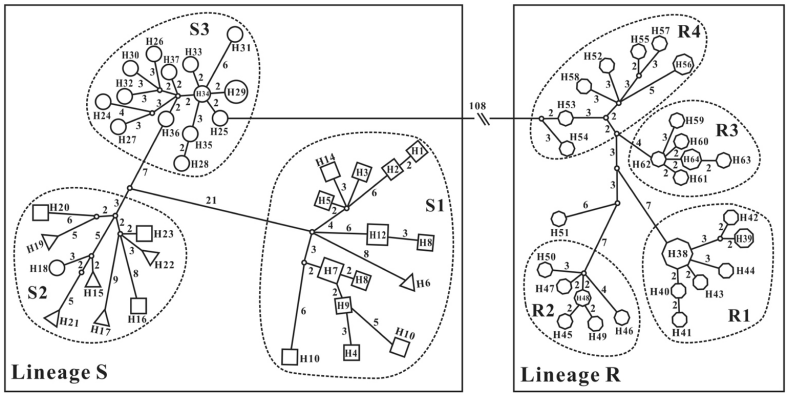

Fig. 3. Median-joining network showing the distribution of Siberian chipmunk cytochrome *b* gene in phylogeographic groups. Circles, triangles, tetragons, and hexagons represent haplotypes and their sizes are proportional to the frequencies observed. The letter "H" indicates the haplotypes and numbers on the nodes indicate the number of mutation steps between haplotypes.

The 3 trees constructed from 64 haplotypes clearly showed that Siberian chipmunks were divided into 2 distinct mtDNA lineages: S and R (Fig. 1). Clade S comprised 37 haplotypes from 41 individuals from South Korea, while clade R comprised 27 haplotypes from 33 individuals from Russia and northeast China.. These lineages are well supported with a high bootstrap value (BP) of 100% and separated by a high degree of the mean pairwise distance between haplotypes for lineages S and R (11.3% of K2P genetic distance). This was much higher than distances within either lineage S (1.9%) or lineage R (1.0%). The lineage R haplotypes partially clustered geographically (China and Magadan region). Lineage S was divided into 3 groups (S1, S2, and S3) (Fig. 1 and Table 5) with no haplotype shared between groups S1 and S3. Haplotypes in group S1 only occurred in the northern part of South Korea, except for one haplotype (Hap6), and haplotypes in group S3 only occurred in the southern part of South Korea.

The pattern from the median-joining network tree (Fig 3.) for 74 individuals of *T. sibiricus* from South Korea, Russia and northeast China showed a large differentiation (108 mutation steps) between South Korea and Russia - northeast China. As with the results from phylogenetic trees, the median-joining network tree gave a similar genealogical structure in South Korea: 3 phylogeographical groups. Group S1, comprised of individuals from the northern part of South Korea, was well differentiated and was separated from groups S2 and S3 by 24 to 28 fixed mutations. Group S2 was from individuals likely distributed in the central part of South Korea, but also included the haplotypes from the southern and northern regions. For lineage R, there were 4 phylogeographic groups based on geographical information. Groups R1 and R2 were for individuals from the Magadan region and northeast China, respectively.

Population structure analyses reinforced the genetic patterns found with the different phylogenetic constructions. Analysis of molecular variance (AMOVA) showed that the majority of the total mtDNA variation (74.70%) was distributed among geographical groups (Korea and Russia - northeast China), whereas a low percentage (14.64%) was observed among populations within the groups (Table 3). Moreover, the ϕ statistic between lineages S and R indicated a very high degree of genetic differentiation, suggesting low levels of gene flow between populations (ϕ_{st} = 0.893, P < 0.000).

Historical demography of populations has had a large effect on the patterns of genetic variation of mtDNA and genetic diversity (Donnelly et al., 1996; Reich and Glodstin, 1998). *Fs* values were -10.41 (P < 0.00) and -13.85 (P < 0.00) for lineages R and S, respectively, which indicates recent population growth. A signature of population growth is distinctly evident in the distribution of pairwise distributions (bell-shaped distribution) in both lineage S and lineage R (Fig. 4). This result coincided with Fu's *Fs* statistic. The relative ratio test result derived by Phyltest (Kumar, 1995) indicated no significant rate heterogeneity for cytochrome *b* between lineages S and R (0.01 ± 0.008).

DISCUSSION

Genetic diversity and population structure

Nucleotide diversity (π = 5.915%) of the assayed fragment of the cytochrome *b* gene in Siberian chipmunks is higher than has been reported for the same locus for other rodents (0.95% for Russian flying squirrel, Oshida et al., 2005; 0.79-0.93% for ground squirrels, Gündüz et al., 2007; 2.82% for root vole, Brunhoff et al., 2003; 4.53% for *Hylaeamys megacephalus*, Miranda et al., 2007). The high levels of mtDNA nucleotide diversity in each Siberian chipmunk lineage (0.806-1.886%; Table 2) suggest relatively large population sizes during the last glacial period and subdivisions within lineages. Moreover, the estimates for diversity are as high, or higher, than those observed in the Russian flying squirrel (Oshida et al., 2005) and the Eurasian red squirrel (Lee et al., being prepared for publication), which inhabit similar regions. In particular, a high sequence variation in lineage S was found (π =1.886%; Table 2).

One of the most notable results of our study was the extremist

Table 3. Analyses of molecular variance based on MtDNA data from geographical groups of Siberian chipmunk (*Tamias sibiricus*). The geographic groups were assigned to seven groups identified by the phylogenetic trees and haplotype network.

Source of variation	variance components	Percentage of variation	P value
Among groups	38.542	74.41	< 0.00
Among populations/groups	7.554	14.64	< 0.00
Within populations	5.494	10.65	< 0.00

genetic structure within Siberian chipmunks. No cytochrome *b* haplotypes were shared between the two lineages representing South Korea and Russia - northeast China. Results from the phylogenetic analysis (Fig. 2), median-joining network (Fig. 3) and AMOVA clearly confirm the existence of 2 large allopatric phylogeographic groups (lineages S and R) in Siberian chipmunks from Russia, northeast China and South Korea. Moreover, 3 subgroups in lineage S, consistent with their geographical affinities, were observed throughout South Korea, although this is a relatively small region in terms of the migratory and dispersal capabilities of mammals.

Divergence time and phylogeographical patterns

Cytochrome *b* sequence divergence was used to set an approximate time frame for the ages of the maternal lineages. In general, the standard divergence rate for the mammalian cytochrome *b* gene is 2% per million years (Myr) (Avise et al., 1998). However, some studies reported that there was substantial heterogeneity in the cytochrome *b* substitution rates among rodents (Bradley and Baker, 2001; Spardling et al., 2000), and different divergence rates have been used. Fedorov and Stenseth (2001) used 5% per Myr for the divergence rate in their phylogeographical study of Norwegian lemmings (*Lemmus lemmus*). Eddingsaas et al. (2004) and Arbogast et al. (2001) applied 3.04% divergence per Myr for North American tree squirrels and 10-12% divergence per Myr for the arctic ground squirrel (*Spermophilus parryii*). In this study, we applied a divergence of 2-10%, which resulted in 2.93-0.58 Myr between lineage S and lineage R. Divergence between the Korean and Russian - Chinese populations may have been initiated during the Pleistocene and the late Pliocene. During the glacial events, Siberian chipmunks might have been isolated in South Korea and Russia, leading to limited gene flow and genetic differentiation.

The median joining network showed that 7 regional clades displayed star-like patterns. Such patterns are suggestive of local differentiation from a single haplotype (Fig. 3). There are 3 exceptions in lineage R (H51 from eastern Russia and H53, 54 from northeast China). The groups R1, R2 and R3 comprised individuals from the Magadan region, northeast China and Russian Transbaikalia, respectively. However, group R4 is from one individual (H52) from northeast China and the remainder are from eastern Russia (Primorye Territory), which shows that divergence between the regions occurred, but that there was still gene flow. Because the H51 haplotype from eastern Russia was closer to group R2, even if it didn't cluster exactly, and the geographical distance is not much farther than other regions support this result.

Based on a large genetic divergence and the population structures detected here, we hypothesize that there may have been 2 refuges, one in South Korea and the other somewhere in Russia and China, during the Quaternary glaciation period,

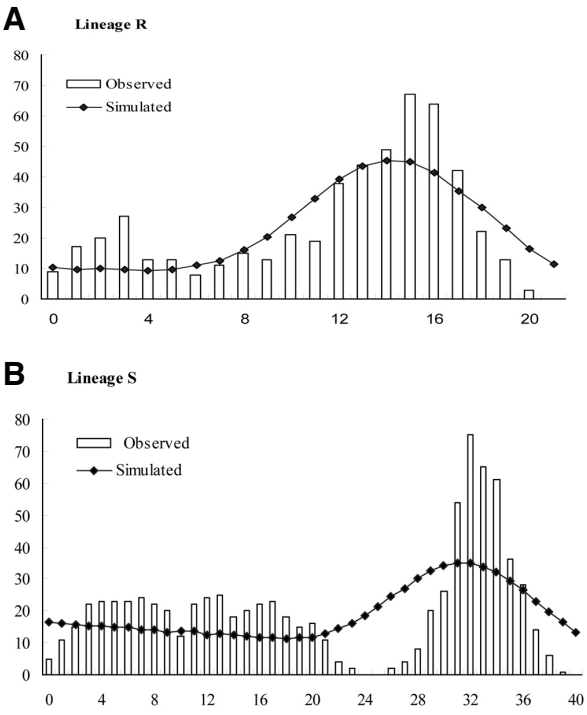

Fig. 4. Mismatch distribution of MtDNA lineages of Siberian chipmunks.

although the specific sites cannot be defined from this study. During the ice age, there may have been a physical barrier, such as a glacial mass, between Korea and Russia - China, which may have prevented gene flow between the 2 refuges. After the ice age, the individuals in the Korean refuge might have adapted to the Korean natural environment, and might not have moved to other regions (Russia and China), although we did not collect samples from North Korea. On the other hand, the individuals in the Russian and Chinese refuge might have spread out from Russia and China. The hypothesis is supported by the fact that the time (2.98-0.58 Myr) to diverge lineages S and R is roughly coincident with the ice age.

Another unusual feature of the phylogeographic patterns is that there are 3 groups in South Korea. There have been a few reports regarding phylogeographic patterns or genetic differentiation in South Korea (Cho et al., 2007; Kang et al., 2005; Park et al., 2004a; 2004b; Suzuki et al., 2004). However, these were studies of species having low dispersal ability, such as fish and insects. Until now, the southern part of the Korean peninsula was generally considered as a single management unit for mammal species, as South Korea is a relatively small region and species with good dispersal abilities, like mammals, could readily migrate across the country. However, our results showed 3 distinct groups in South Korea, and that group S1 was quite different from groups S2 and S3. Some haplotypes in group S2 occur in the southern and northern parts of South Korea, which suggests the possibility of gene flow or migration (Fig. 3). This phylogeographic pattern is quite different from that of the Asiatic black bear (*Ursus thibetanus*), striped field mice (*Apodemus agrarius*) and the Korean spider shrew (*Sorex caecutiens*). A study of the Asiatic black bear, which was used for a re-introduction project, revealed that the populations from the far eastern part of Russia and Korea are considered a single evolutionary significance unit (ESU) based on genetic data

Table 4. Mitochondrial cytochrome *b* haplotype distribution of *T. sibiricus* in South Korea

Province	Gangwon-do					Gyeonggi-do	Chungcheong-do			Gyeongsang-do					Jellanam-do			
Locality	Cheorwon	Chuncheon	Hwacheon	Yanggu	Taebaek	Gapyeong	Okcheon	Goesan	Chungju	Danyang	Cheongsong	Hapcheon	Sancheong	Deagu	Namwon	Gurye	Gwangju	Suncheon
N (41)	5	1	1	1	5	4	1	2	1	1	1	1	2	1	2	7	4	1
Hap1		S1																
Hap2			S1															
Hap3				S1														
Hap4	S1																	
Hap5	S1																	
Hap6									S1									
Hap7						S1/S1												
Hap8						S1												
Hap9						S1												
Hap10	S1																	
Hap11	S1																	
Hap12					S1													
Hap13					S1/S1													
Hap14					S1													
Hap15							S2											
Hap16	S2																	
Hap17											S2							
Hap18												S2						
Hap19								S2										
Hap20																		S2
Hap21								S2										
Hap22										S2								
Hap23					S2													
Hap24																S3		
Hap25																S3		
Hap26															S3			
Hap27															S3			
Hap28																S3		
Hap29														S3		S3	S3	
Hap30																S3		
Hap31																S3		
Hap32																S3		
Hap33																	S3	
Hap34																	S3	
Hap35																	S3	
Hap36													S3					
Hap37													S3					

(H. Lee et al., 2005, Analysis of genetic information on the Asiatic black bear in Korea. A research report submitted to Jirisan Nambu Office of Korea National Park Service). In addition, a phylogenetic study of insectivores showed that individuals from the middle and eastern parts of Russia, Finland and Japan, except for Hokkaido, and the Korean spider shrew in Korea clustered together (Ohdachi et al., 2003), and there is no phylogeographic pattern for striped field mice on the Korean peninsula (Koh et al., 2000). The discrepancy in results observed between the Siberian chipmunk and others shows that some mammal populations on the Korean peninsula might be structured into a few separate management units. In terms of management, intensive research will be needed with more mammal species in South Korea for effective conservation and man-

Table 5. Sequence divergence of Siberian chipmunk from three parts of South Korea, Russia, and China (Below diagonal) and population pairwise ϕst of *T. sibiricus* (Above diagonal)

	Northern part	Central part	Southern part	Russia	China
Northern part		0.37216*	0.63382*	0.89708*	0.88850*
Central part	0.39919		0.47789*	0.90012*	0.89014*
Southern part	0.63581	0.39605		0.92653*	0.93629*
Russia	0.90668	0.90450	0.91621		0.33935*
China	0.89856	0.89312	0.92270	0.33906	

*P < 0.0001

agement strategies.

Taxonomic considerations

Until now, there have been insufficient data, especially from phylogenetic studies, for defining the taxonomic status of the Siberian chipmunk. The present study collected samples from South Korea, northeast China and some parts of Russia. The results from phylogenetic tree analysis, haplotype network and genetic variations based on the cytochrome *b* gene showed that the current taxonomic classification may not be an accurate reflection of the evolutionary relationships within *Tamias sibiricus*. As mentioned above, the Siberian chipmunk populations in South Korea and Russia - northeast China are significantly differentiated and distinctive. Compared to Bradley and Baker's study (2001) showing levels of sequence variations in the cytochrome *b* gene using rodents and bats, the 11.3% (K2P) sequence variation observed in this study can be considered indicative of specific recognition in rodents. It highlights that we need further examination of this species' taxonomy with appropriate collections of samples and additional genetic markers. In addition, a large divergence and different structures among populations were found in South Korea (Figs. 2 and 3; Table 3). These results are quite discordant with the current subspecies status of *Tamias sibiricus*. Based on morphological characteristics and skull data, John and Johnson (1965) reported that *Tamias sibiicus barberi* from central and southern parts of Korea is different from *T. s. orientalis* that inhabits the extreme northern parts of Korea. *T. s. senescens*, from 15 miles west of Beijing, China differs from *T. s. barberi* and *T. s. orientalis* as well. Some researchers in Russia have shown that the subspecies *T. s. orientalis* inhabits extreme southeastern Siberia and border parts of Manchuria and Korea (Ognev, 1940). Our results clearly show that the chipmunks from South Korea and the chipmunks from Russia and China might be different species. However, because the Mt cytochrome *b* gene only was used in this study as a genetic marker, this is not powerful enough for defining a species, even though the cytochrome *b* gene has shown significant genetic differentiation among chipmunks very well. In addition to more comprehensive sampling from diverse regions of China and Russia, highly variable genetic markers are needed, such as microsatellites, as well as nuclear markers and morphological data to establish more concrete data for the taxonomic status of Siberian chipmunks.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent assistance with data analysis and review from Warren E. Johnson and Stephen J. O'Brien at the National Cancer Institute, USA and Kyung Seok, Kim at the USDA-Agricultural Research Service, USA. Also, we would like to thank many contributors who donated valuable samples to the CGRB for use in this study (Sung Yong Han, Mu Shik Kang, Yang Hoon Kim, Woo Jin Jung, Taek Woo Nam, Sang Yong Jung, Sang Wook Jung, Jin Jae Kim, Chul Un Jung, Jung Suk Song, Hang Kyu Lim, Seung Gu Kang, Chan Ryul Park, Seok wan Cheong, Young-Dae Kim, Dong-Ki Choi, Chun-Gweon Choi, Chang-Yong Choi, and Su-Ho Kim). This study was supported, in part, by a Korea Research Foundation Grant funded by the Korean Government (Ministry of Education and Human Resources Development; KRF-2007-C00193-I00755), Brain Korea 21 Program for Veterinary Science and Research Institute of Veterinary Science, Seoul National University.

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