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Authors: Matsuhashi, Tamako, Masuda, Ryuichi, Mano, Tsutomu, Murata, Koichi, and Aiurzaniin, Awirmed

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Phylogenetic Relationships among Worldwide Populations of the Brown Bear *Ursus arctos*

Tamako Matsuhashi[1], Ryuichi Masuda[2*], Tsutomu Mano[3], Koichi Murata[4] and Awirmed Aiurzaniin[5]

[1]*Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*
[2]*Laboratory of Genetic Diversity, Center for Advanced Science and Technology, Hokkaido University, Sapporo 060-0810, Japan*
[3]*Hokkaido Institute of Environmental Sciences, Sapporo 060-0819, Japan*
[4]*College of Bioresource Sciences, Nihon University, Fujisawa 252-8510, Japan*
[5]*Great Gobi National Park, Bayan-Tootoi, Gobi-Altai, Mongolia*

ABSTRACT—Previous phylogenetic studies using mitochondrial DNA sequences of the brown bear *Ursus arctos* have separately defined two major lineages in Europe, three in Alaska, and three in Hokkaido Island of Japan. To reconstruct phylogenetic relationships among worldwide populations of the species, nucleotide sequences of the mitochondrial DNA control region and cytochrome *b* were determined for some additional subpopulations of Asia (Gobi and Tibetan), and then all the data including previously reported sequences were compared. The resultant phylogenetic trees showed that the worldwide populations could be grouped into at least five lineages. One of the five lineages had a wide distributional range covering Eurasia, Alaska, and central Hokkaido. Moreover, it is likely that populations from eastern Hokkaido and eastern Alaska are the direct derivatives of a single lineage. These results suggest that brown bears may have widely colonized Eurasia and North America from their original areas somewhere in Eurasia more than once.

Key words: brown bear, *Ursus arctos*, phylogeny, mitochondrial DNA, migration history

INTRODUCTION

The brown bear *Ursus arctos* is widespread in Eurasia and North America, and its breeding range is the largest of the seven bear species. In phylogeographic studies of brown bears using mitochondrial DNA (mtDNA), Taberlet and Bouvet (1994) and Kohn *et al*. (1995) reported a large genetic differentiation among populations of Europe, and found a clear difference between eastern and western European populations. The western European population split into two lineages: Iberian and Balkan groups (Taberlet and Bouvet, 1994), although Kohn *et al*. (1995) did not recognize any clear difference between those two lineages. In western Europe, brown bear habitats are now highly fragmented through human activity such as hunting and deforestation (Servheen, 1990). In North America, Talbot and Shields (1996b) and Waits *et al*. (1998) showed that there were at least three major mtDNA lineages in Alaska, which are mutually allopatric. In addition, Talbot and Shields (1996b) suggested that the three lineages of Alaska could have diverged in Eurasia prior to their immigration into North America. Meanwhile, the brown bear population of Hokkaido Island of Japan was divided into three lineages, based on phylogenetic analysis of mtDNA control region and cytochrome *b* (Cyt. *b*) (Matsuhashi *et al*., 1999). The distributional boundaries between the three lineages of Hokkaido were quite obvious in spite of the small area of the island. The estimated divergence time suggested that the three lineages of Hokkaido have diverged prior to immigration from the Eurasian continent.

To reveal phylogenetic relationships among those lineages locally recognized, in the present study, we present reconstructed phylogenetic trees of worldwide brown bear populations based on sequence differences of the mtDNA control region and Cyt. b, combining new data and previously reported data together. Migration history of brown bears in Eurasia and North America is then discussed.

* Corresponding author: Tel. +81-11-706-3541;
FAX. +81-11-736-6304.
E-mail: masudary@ees.hokudai.ac.jp

Table 1. Positions of the nucleotide substitution in the control region partial sequence (284 bp).

Haplotype	Position																																									
	3	5	9	10	11	14	15	17	18	20	21	22	23	24	27	40	42	44	45	48	51	56	62	71	74	75	79	80	81	82	83	84	85	86	87	88	89	90	92	93	94	95	96
Gobi-D	T	C	C	C	T	T	C	A	T	T	C	C	C	A	C	C	A	C	T	T	A	G	G	C	C	G	—	—	—	—	—	—	—	—	—	—	—	—	C	T	—	—	—
Pyr	.	.	T	T	.	C	.	G	C	.	.	.	T	.	T	.	.	.	.	C	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
Gre	.	.	T	T	.	.	.	G	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ro1	.	.	T	T	.	.	.	.	.	.	.	T	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T
Rus1	.	.	T	T	.	C	.	.	.	.	.	T	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	T
KD1	.	.	T	T	.	C	.	.	.	.	.	T	T	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HK1-D	.	.	T	T	.	C	.	.	.	.	.	T	T	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	T	T
HK2-D	.	.	T	T	.	C	.	.	.	.	.	T	T	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HK3-D	.	.	T	T	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HK4-D	.	.	T	T	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
HK5-D	.	.	T	T	.	.	.	.	.	.	.	T	T	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	T
HK6-D	.	.	T	T	C	.	.	.	.	.	.	T	T	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Tibet-D	.	.	T	T	.	.	.	.	.	.	.	T	T	.	.	.	.	.	C	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
S. thibetanus	C	T	T	T	.	.	T	.	.	C	T	.	T	G	T	T	.	T	C	.	.	A	.	T	T	.	T	C	C	C	T	C	T	C	C	T	T	T	T	.	.	.	.

A total of 266 nucleotides exclusive of 18 indel sites were used to reconstruct the phylogenetic relationships. Dots indicate identical nucleotides with those of Gobi-D.

MATERIALS AND METHODS

The hypervariable 5' end (approximately 270 base pairs, bp) of the mtDNA control region and a partial portion (700 bp) of Cyt. *b* were used for sequence analysis. Hairs of two Tibetan brown bears (Tibet-1 and 2: from Kobe Municipal Oji Zoo, Japan), blood of one Kodiak brown bear (KD1: from Sapporo Maruyama Zoo, Japan), and dried skin tissues of one Gobi brown bear (Gobi-1: from Great Gobi National Park, Mongolia) were used to extract DNA.

The control region sequence of the Kodiak (included in western Alaska) brown bear (KD1) was determined in the present study, while the other brown bear data were quoted from previous reports: four brown bears of Europe (Accession Nos. are as follows: Pyr, X75878; Gre, X75870; Ro1, X75872; Rus1, X75875) (Taberlet and Bouvet, 1994), six brown bears of Hokkaido (Accession Nos.: HK1-D (central Hokkaido), AB013043; HK2-D (central Hokkaido), AB013055; HK3-D (eastern Hokkaido), AB013057; HK4-D (eastern Hokkaido), AB013058; HK5-D (southern Hokkaido), AB013067; HK6-D (southern Hokkaido), AB013062) (Matsuhashi *et al*., 1999), the Tibetan brown bear (Tibet-D: Accession No. AB010727)(Masuda *et al*., 1998), the Gobi brown bear (Gobi-D: Accession No. AB010728)(Masuda *et al*., 1998) and the Asiatic black bear *Selenarctos thibetanus* as outgroup (Accession No. AB013071)(Matsuhashi *et al*., 1999).

In the present study, 700 bp of the Cyt. *b* sequences were determined from brown bears of Tibet (Tibet-1 and 2) and Gobi (Gobi-1). Sequence data of six brown bears of Hokkaido (Accession Nos.: HK1 and 2-C (central Hokkaido), AB020905; HK3 and 4-C (eastern Hokkaido), AB020907; HK5 and 6-C (southern Hokkaido), AB020909) and *S. thibetanus* (Accession No. AB020910) were quoted from Matsuhashi *et al*. (1999). The sequences of Alaska (Accession Nos.: GB01 (ABC Islands), UAU18870; GB04 (ABC Islands), UAU18873; GB09 (eastern Alaska), UAU18878; GB10 (eastern Alaska), UAU18879; GB19 (Kodiak), UAU18888; GB27 (eastern Siberia), UAU18896) (Talbot and Shields, 1996b) as well as the polar bear *Ursus maritimus* (Accession No. TMU18898)(Talbot and Shields, 1996b) were quoted from the GenBank database.

Sample tissues were subjected to DNA extraction, polymerase chain reaction (PCR), and sequencing due to methods previously reported by Masuda *et al*. (1998) and Matsuhashi *et al*. (1999). DNA analysis of the Tibetan and Gobi brown bears was performed with a permission of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Sequences were aligned by using the computer program GeneWorks Ver. 2.5.1 (IntelliGenetics). Deletions or insertions (indels) found in the mtDNA control region were determined by eye, and excluded from estimation of genetic distance.

Genetic distance was estimated by Kimura's (1980) two-parameter method and the proportion (p)-distance method in MEGA computer program Version 1.01 (Kumar *et al*., 1993). Phylogenetic trees were reconstructed with the neighbor-joining method (Saitou and Nei, 1987) using MEGA.

RESULTS

The control region phylogeny

There were 42 variable sites out of 266 bp within brown bears (Table 1). All substitutions were transitions except one transversion. Percentage sequence differences are shown in Table 2. Thirteen haplotypes of the mtDNA control region (Table 1) were compared to reconstruct a phylogenetic tree among them (Fig. 1a). The four major clades emerging in the phylogeny were named Groups I-IV in the present study, although Groups I and II were supported with only 67% and 54% bootstrap values, respectively.

European brown bears fell into two different clades: Group I (the western European lineage comprising Pyr and Gre) and Group II (the eastern European lineage comprising Ro1 and Rus1) as reported by Taberlet and Bouvet (1994). We confirmed that the two lineages of Europe arose even when all the haplotypes reported by Taberlet and Bouvet (1994) and by Kohn *et al*. (1995) were applied to the phylogenetic tree reconstruction (data not shown). Therefore, in the present study, the four brown bears (Pyr, Gre, Ro1, and Rus1) were selected as representatives of the two lineages of the European brown bears. Meanwhile, the brown bears of Hokkaido were divided into three lineages: HK1-D and HK2-D with a 72% bootstrap value in Group II, HK3-D and HK4-D with a 94% bootstrap value in Group III , and HK5-D and HK6-D with a 90% bootstrap value in Group IV, which correspond to Clusters A, B, and C reported by Matsuhashi *et al*. (1999), respectively, and these three lineages were distributed in central,

97	98	114	115	117	130	158	159	160	161	162	168	170	173	177	178	183	192	193	197	198	201	203	205	207	209	214	215	220	251	252	278
—	T	C	G	C	T	A	C	T	G	T	G	C	A	G	A	C	G	A	–	T	A	T	C	G	T	A	G	T	A	A	T
T	.	T	A	.	C	.	T	.	A	.	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.	.	G	.
T	.	T	A	.	C	.	T	.	.	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	C	.	.	.	.	G	.
T	.	T	A	.	C	.	T	.	A	C	.	.	A	.	T	.	.	.	.	C	.	.	.	.	C	G	T	.	G	.	C
T	.	T	A	.	C	.	T	.	A	C	.	.	A	.	T	.	.	.	.	C	.	.	.	.	C	G	.	.	G	.	C
T	.	T	A	.	C	.	T	.	A	C	.	.	A	.	T	.	.	.	.	C	.	.	.	.	C	G	.	.	G	.	C
T	.	T	A	.	C	.	T	.	A	C	.	.	A	.	T	.	.	.	.	C	.	.	.	.	C	G	.	.	G	.	C
.	—	T	A	.	C	.	T	.	A	C	.	.	A	G	.	.	.	.	.	C	.	.	.	.	C	G	.	.	G	.	C
.	.	T	A	.	.	.	T	.	A	C	.	.	G	A	.	T	.	.	.	C	.	.	.	.	C	G	.	.	.	.	C
.	.	T	A	.	.	.	T	.	A	C	.	.	G	A	.	.	.	.	.	C	.	.	.	.	C	G	A	.	.	.	C
T	.	T	A	.	C	G	T	.	A	C	.	.	.	A	.	T	.	.	.	C	.	C	.	.	C	G	A	C	.	.	.
T	.	T	A	.	C	G	T	.	A	C	.	.	.	A	.	T	.	.	.	C	.	C	.	.	C	G	.	C	.	.	.
T	.	T	A	T	C	G	T	.	A	C	.	.	.	A	.	T	.	.	.	C	G	.	T	A	.	G	.	C	.	.	.
T	.	T	A	.	C	.	T	C	T	.	A	T	.	.	.	.	A	G	G	.	.	.	.	.	C	.	.	.	G	.	C

Table 2. Percentage of pairwise differences of mtDNA control region (266 bp)

	OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	Gobi-D		6.77	5.64	7.52	7.52	7.89	7.89	7.89	6.39	6.39	7.89	7.89	8.65	11.28
2.	PYR			3.38	7.52	6.77	7.14	7.14	7.14	7.14	7.14	7.89	7.89	7.89	10.15
3.	GRE				5.64	5.64	6.02	6.02	6.77	5.26	6.02	6.02	6.02	6.77	10.15
4.	Ro1					0.75	1.13	1.13	1.88	2.63	3.01	3.01	3.38	4.89	10.15
5.	RUS1						0.38	0.38	1.13	1.88	2.63	3.38	3.38	4.89	10.15
6.	KD1							0.75	1.50	2.26	3.01	3.76	3.76	5.26	9.77
7.	HK1-D								0.75	2.26	3.01	3.76	3.76	5.26	10.53
8.	HK2-D									3.01	3.01	4.51	4.51	6.02	10.53
9.	HK3-D										0.75	4.51	4.51	6.02	11.28
10.	HK4-D											4.51	5.26	6.76	11.28
11.	HK5-D												0.75	3.76	12.03
12.	HK6-D													3.76	12.03
13.	Tibet-D														12.78
14.	*S.thibetanus*														

eastern, and southern Hokkaido, respectively. Two individuals were selected as representatives from each of the three lineages of the Hokkaido brown bears. In the Group II, the brown bears of Europe were not intermixed with the Hokkaido brown bear group (Fig. 1a). The Kodiak brown bear (KD1) was included in the Group II, standing near the eastern European brown bear (Rus1, Fig. 1a). Thus, the Group II consisted of the brown bears of eastern Europe, Kodiak, and central Hokkaido, though the bootstrap value is low (54%). The Group III comprised only eastern Hokkaido brown bears. The Tibetan brown bear was clustered with the southern Hokkaido group (Group IV) with a 91% bootstrap value. The Gobi brown bear (Gobi-D) and the western European group (Group I) formed a cluster with a 50% bootstrap value, which was distantly related to the other brown bears (Fig. 1a).

The Cyt. *b* phylogeny

Twelve haplotypes of Cyt. *b* (700 bp) obtained from 15 brown bears (six bears from Alaska, six from Hokkaido, one from Gobi, two from Tibet) and one polar bear *Ursus maritimus* (Table 3) were compared by constructing the neighbor-joining phylogenetic tree (Fig. 1b). A total of 54 variable sites (including two transversions) at all codon positions of Cyt. *b* (700 bp) and 37 variable sites at the third codon positions (235 positions) among 12 haplotypes were identified (Table 3). Percentage sequence differences at the third codon positions are shown in Table 4.

Hokkaido brown bears were divided into three lineages: (1) HK1-C and HK2-C (both from central Hokkaido; their sequences were identical in Group Y); (2) HK3-C and HK4-C (both from eastern Hokkaido; sequences were identical in Group Z); (3) HK5-C and HK6-C (both from southern Hokkaido; sequences were identical in Group W), indicating the same phylogenetic relationships among groups as for the control region. Brown bears from Alaska and eastern Siberia were divided into three groups: (1) GB01 and GB04, both from ABC Islands of Alaska (Group X); (2) GB19 from Kodiak and GB27 from eastern Siberia (Group Y); (3) GB09 and GB10, both from eastern Alaska (Group Z). Haplotypes (GB01 and GB04) in Group X were closer to *U. maritimus* with a 95% bootstrap value. Both Groups Y and Z embraced subpopulations from Alaska and Hokkaido: bears of central Hokkaido (HK1 and 2-C) formed Group Y with Kodiak bears of western Alaska (GB19) and eastern Siberia (GB27) with a 95% bootstrap

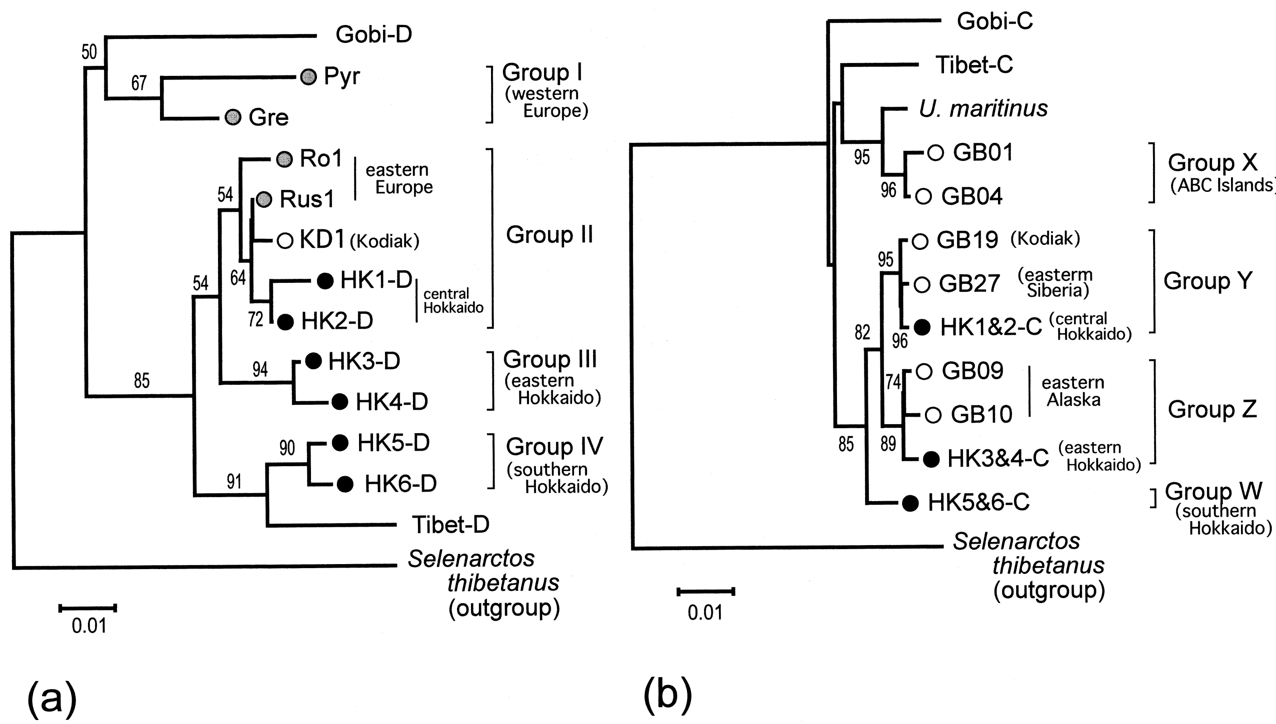

Fig. 1. Molecular phylogenetic trees (a, 266 bp of the control region; b, 700 bp of Cyt. *b*) of Alaskan, European, and Hokkaido brown bears using the neighbor-joining method. Gray, open, and closed circles show the haplotypes from Europe, Alaska or eastern Siberia, and Hokkaido, respectively. Numbers on the branches are bootstrap values (%) derived from 1,000 replications, while values under 50% were not shown.

value; bears of eastern Hokkaido (HK3 and 4-C) were included in Group Z together with those of eastern Alaska (GB09 and GB10) with a 89% bootstrap value. Group W (HK5 and 6-C: bears of southern Hokkaido) split from Groups Y and Z. Two bears of Tibet (Tibet-1 and 2) had identical sequences (Tibet-C). Brown bears of Gobi (Gobi-C) and Tibet (Tibet-C) were remote from both Hokkaido and Alaskan populations.

The sequence data of the control region and Cyt. *b* determined in the present study will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession numbers: the control region sequence of KD1 (AB041258); Cyt. *b* sequences of Tibet-C (AB041259) and Gobi-C (AB041260).

DISCUSSION

Relationships between phylogeny and geographic origins

Each population from Europe, Alaska, and Hokkaido was clearly split into two or three mtDNA lineages (Taberlet and Bouvet, 1994; Kohn *et al.*, 1995; Talbot and Shields, 1996b;

Table 3. Substitution sites of the third codon positions in the partial portion of Cyt. *b* (700 bp).

Haplotype	Position#																																									
	438	444	447	453	459	465	468	474	480	507	516	522	537	549	552	555	558	585	588	594	615	624	627	639	645	657	660	663	681	684	687	723	744	756	762	768	771	780	792	807	813	816	831
Gobi-C	C	T	A	G	T	C	C	G	G	C	A	T	C	T	C	C	G	C	T	A	C	C	C	T	A	A	C	T	G	T	T	C	T	T	T	T	T	T	T	A	G	G	C
Tibet-C	.	.	.	.	.	T	.	.	.	.	.	.	T	C	T	.	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	C	C	.	C	C	.	.	G	.	.	.
U. maritinus	.	.	.	.	.	T	.	.	.	.	G	.	T	.	T	.	.	.	C	G	.	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	C	.	C	.	.	.	.
GB01	.	.	.	.	.	T	.	.	.	.	G	.	T	.	T	.	.	.	C	.	.	.	T	.	.	.	.	C	.	.	.	.	C	C	C	.	C	.	C	.	.	.	.
GB04	.	.	.	.	.	T	.	.	.	.	G	.	T	.	T	.	.	.	C	.	.	.	T	.	.	.	.	C	.	.	.	.	C	C	C	.	C	.	C	.	.	.	.
GB19	.	C	.	.	.	.	.	.	.	.	G	C	T	.	T	.	.	.	.	G	T	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	C	.	.	.	A	.	.
GB27	.	.	.	.	.	.	.	.	.	.	G	C	T	.	T	.	.	.	.	G	T	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	C	.	.	.	A	.	.
HK1&2-C	.	.	.	.	.	.	.	.	.	.	G	C	T	.	T	.	.	.	.	G	T	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	C	.	.	.	A	.	.
GB09	.	.	.	.	.	.	.	.	.	.	G	C	T	.	T	.	.	.	.	G	T	.	T	.	.	C	.	.	A	.	.	.	C	C	.	.	C	.	.	.	A	.	.
GB10	.	.	.	.	.	.	.	.	.	.	G	C	T	.	T	.	.	.	.	G	T	.	T	.	.	C	.	.	A	.	.	.	C	C	.	.	C	.	.	.	A	.	.
HK3&4-C	.	.	.	.	.	.	.	.	.	.	.	C	T	.	T	.	.	.	.	G	T	.	T	.	.	C	.	.	A	.	.	.	C	C	.	.	C	.	.	.	A	.	.
HK5&6-C	.	.	.	.	.	.	.	.	.	.	G	.	T	.	T	.	A	.	.	G	T	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	C	.	.	.	.	.	.
S. thibetanus	T	C	C	A	C	T	T	A	A	T	.	C	T	.	.	T	.	T	.	G	.	T	T	C	G	.	T	C	A	C	C	T	C	C	.	.	C	C	C	.	A	A	T

Positions were counted from the first nucleotide of the Cyt. *b* (1140 bp). There were 235 codons in 3' side of the Cyt. *b* (705 bp). Five unidentified positions were excluded from analysis. Dots indicate identical nucleotides with those of Gobi-C.

Waits *et al*., 1998; Matsuhashi *et al*., 1999). As shown by Taberlet and Bouvet (1994) and by Kohn *et al*. (1995), brown bears of western Europe were genetically distant from those of eastern Europe. Brown bears of Alaska were genetically separated in three regions: ABC Islands, western Alaska including Kodiak Island, and eastern Alaska, while the western Alaskan population is very close to the eastern Siberian population (Talbot and Shields, 1996b). In Japan, there were three lineages distributed in eastern, southern, and central Hokkaido (Matsuhashi *et al*., 1999). The present study suggested that some groups located in distant areas were phylogenetically closer to each other than to geographically neighboring groups. For instance, bears of eastern Europe, Kodiak Island, and central Hokkaido were classified into Group II (Fig. 1a) in the control region phylogenetic tree, though the bootstrap value (54%) is low. On the other hand, bears of western Alaska including Kodiak Island, eastern Siberia, and central Hokkaido were classified into Group Y (Fig. 1b) in the Cyt. *b* tree. The Cyt. *b* data of eastern European brown bears were not included because they were unavailable in the present study. These results suggest that Group II (control region tree, Fig. 1a) and Group Y (Cyt. *b* tree, Fig. 1b) form a common lineage. Brown bears included in this lineage could have been widespread in eastern Europe, Siberia, central Hokkaido, and western Alaska before the final formation of the Bering Strait and the Soya Strait (located between Hokkaido and Sakhalin).

Brown bears of both eastern Alaska and eastern Hokkaido were classified into Group Z (Cyt. *b* tree, Fig. 1b), suggesting that they are also descendent from a common ancestral lineage although they currently inhabit separate and distant areas (Fig. 2).

The control region tree (Fig. 1a) showed that the Tibetan brown bear (Tibet-D) is genetically closer to southern Hokkaido brown bears (Group IV), whereas the Cyt *b* tree (Fig. 1b) did not support it (Tibet-C - Group W). The incongruity between the two phylogenetic trees (Fig. 1a and 1b) might be attributed to the shortage of the length of the control region sequences. Another possibility is that the substitution rates of the control region and Cyt. *b* may be different from one another. For example, at the control region, the sequence difference (excluding indels) between HK1-D and HK6-D was 3.76% and that between HK6-D and *Selenarctos thibetanus* (outgroup) was 12.03% (Table 2). Meanwhile, at the Cyt. *b* gene (transitions at the third codon positions), the pairwise difference between HK1 and 2-C and HK5 and 6-C was 2.56%, but that between HK5 and 6-C and *S. thibetanus* was 24.79%.

In the Cyt. *b* tree (Fig. 1b) in the present study, the position of *Ursus maritimus* was within the brown bear cluster, forming a sister clade to the population of ABC Islands (Group X), as reported by Talbot and Shields (1996a, b) and Shields and Kocher (1991). Fig. 2 shows the worldwide distribution of groups identified from mtDNA lineages.

Divergence time of the brown bear populations

To reconstruct migration history of brown bear populations, the divergence time was calculated from control region sequence differences (Table 3) by using the sequence divergence rate of the homologous human control region sequence (approximately 8.4% difference per million years, Myr) (Vigilant *et al*., 1989). Divergence times were as follows: 0.8 Myr between Gobi + Group I and the others; 0.7 Myr between Gobi and Group I (western Europe); 0.5 Myr between Group IV + Tibetan bear and Groups II + III; 0.3 Myr between Groups II and III; and 1.3 Myr between *Ursus arctos* and *Selenarctos thibetanus*.

The divergence time was also calculated from the Cyt. *b* data. Irwin *et al*. (1991) reported a rate of approximately 10%/Myr for nucleotide substitutions at the third codon positions of Cyt. *b*. Talbot and Shields (1996a) calculated nucleotide substitution rate with fossil records and indicated that transitions at the third codon positions of the ursid Cyt *b* gene occur at an approximately 6% divergence per Myr. Using these two divergence rates and the Cyt *b* data obtained in the

843	844	849	855	858	879	891	894	897	912	915	927	945	947	953	963	981	993	996	1014	1035	1044	1053	1068	1077	1080	1083	1089	1092	1095	1101	1113	1116	1119	1122	1134	1137
A	C	C	T	T	A	T	T	A	T	T	G	G	A	A	C	T	T	A	T	T	T	A	T	T	A	C	A	A	T	C	T	T	A	C	A	A
.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.
.	T	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	G
.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G
.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.
.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	G	G
.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	T	.	C	.	.	G	G
.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	G	G
.	.	T	.	.	.	C	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	G
.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	G
.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	G	G
G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G
.	.	.	C	C	G	.	C	G	C	C	A	A	G	.	T	C	C	.	C	.	C	G	C	C	.	T	G	G	C	.	C	.	G	T	.	.

Table 4. Percentage of pairwise nucleotide differences at the third codon positions in Cyt. *b*(235 positions/700 bp). Above the diagonal: Both transitions and transversions were counted for calculation of divergence time using the method of Irwin *et al*. (1991); below diagnal: only transitions were counted for estimation of divergence time using the method of Talbot & Shields (1996).

	OTU	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	Gobi-C		6.84	8.12	7.69	7.27	7.69	7.69	7.27	8.55	8.12	8.12	6.41	24.79
2.	Tibet-C	6.84		4.70	5.13	4.70	5.98	5.98	5.56	6.84	6.41	6.41	4.70	24.79
3.	*U. maritimus*	7.69	4.27		2.14	2.56	5.56	5.56	5.13	6.41	5.98	6.84	4.27	23.93
4.	GB01	7.69	5.13	1.71		0.43	5.98	5.98	5.56	6.84	6.41	7.27	4.70	24.79
5.	GB04	7.27	4.70	2.14	0.43		6.41	6.41	5.98	7.27	6.84	7.69	5.13	24.36
6.	GB19	7.69	5.98	5.13	5.98	6.41		0.85	0.43	2.56	2.14	2.99	2.99	23.93
7.	GB27	7.69	5.98	5.13	5.98	6.41	0.85		0.43	2.56	2.14	2.99	2.99	24.79
8.	HK1&2	7.27	5.56	4.70	5.56	5.98	0.43	0.43		2.14	1.71	2.56	2.56	24.36
9.	GB09	8.12	6.41	5.56	6.41	6.84	2.14	2.14	1.71		0.43	1.28	3.85	23.93
10.	GB10	7.69	5.98	5.13	5.98	6.41	1.71	1.71	1.28	0.43		0.85	3.42	24.36
11.	HK3&4	7.69	5.98	5.98	6.84	7.27	2.56	2.56	2.14	1.28	0.85		4.27	23.50
12.	HK5&6	6.41	4.70	3.85	4.70	5.13	2.99	2.99	2.56	3.42	2.99	3.85		25.21
13.	*S. thibetanus*	24.36	24.36	23.08	24.36	23.93	23.50	24.36	23.93	23.08	23.50	22.65	24.79	

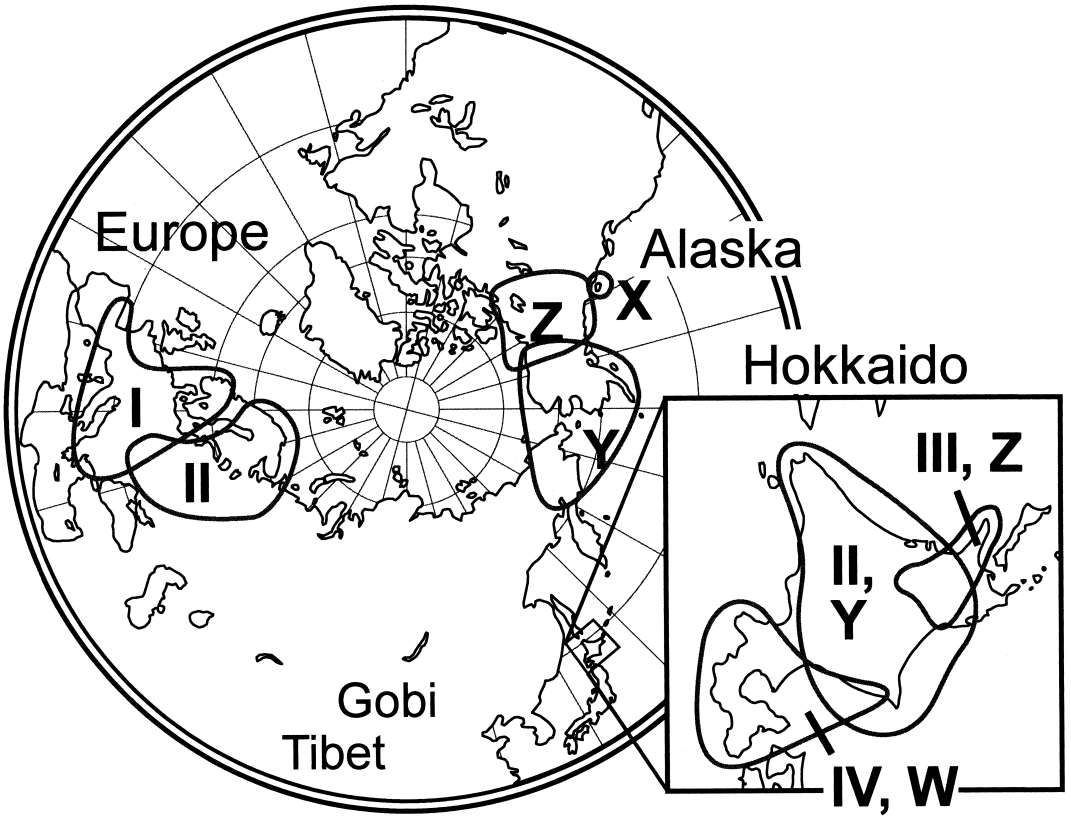

Fig. 2. Worldwide distribution of eight groups (I-IV, X-W) in brown bear populations.

present study (Table 4), divergence times were estimated as follows (when using the rates of 10%/6% per Myr): 0.8/1.2 Myr between Gobi and others; 0.6/0.9 Myr between Group X + Tibet and Hokkaido + the other Alaskan brown bears; 0.3/0.5 Myr between Group W and Groups Y + Z; 0.2/0.3 Myr between Groups Y and Z; 0.2/0.3 Myr between Group X and *U. maritimus*; and 2.4/4.0 Myr between *U. arctos* and *S. thibetanus*.

Mazza and Rustioni (1994) suspected that some brown bears in Asia immigrated into Europe at the end of the Early Pleistocene. Taberlet and Bouvet (1994) suggested that the western and eastern Europe lineages diverged 0.85 Myr ago. Waits *et al*. (1998) estimated 0.11–0.14% divergence (Kimura's two-parameter distance) of the control region per 0.01 Myr, from the data of Talbot and Shields (1996b). Using this divergence rate, Waits *et al*. (1998) estimated the divergence time as follows: 0.146–0.185 Myr between *U. maritimus* and the ABC Island brown bear (the same population as Group X in the present study); 0.245–0.31 Myr between western (Group Y) and eastern Alaskan bears (Group Z); 0.28–0.356

Myr between western Alaskan (Group Y) and Rocky Mountain brown bears; and 0.404–0.515 Myr between eastern Alaskan (Group Z) and Rocky Mountain brown bears. These values were relatively close to our estimates, although we did not examine any sequences of Rocky Mountain brown bears.

From the estimated divergence time, each of the four lineages (Gobi, Group I, Group X , and other groups) classified in the present study are likely to have diverged from the others approximately 0.6–0.9 Myr ago. These ages may relate to the early stage of the evolution of the brown bear. The ancestral lineage of Group II/Group Y could have been distributed in the extensive area ranging from eastern Europe to eastern Siberia, and some populations from this lineage could have crossed the land bridge (Beringia) between Siberia and Alaska and immigrated into North America. Other populations from this lineage could have migrated through Sakhalin to Hokkaido. This presumption does not conflict with Kurten's (1968) report on the basis of the fossil record that the brown bear could have existed in North America at least 0.5 Myr ago. Thus, our results indicate that some minor (probably relict) lineages of mtDNA likely occur in western Europe, ABC Islands of Alaska, Gobi of Mongolia, Tibet, and Hokkaido (Fig. 2), which are considered to be refugia of brown bears during the last glacial age of Pleistocene. Further studies involving more data of the same DNA sequence regions on worldwide brown bear populations will more fully illustrate their origin and migration history.

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