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Authors: Mahmut, Halik, Masuda, Ryuichi, Onuma, Manabu, Takahashi, Manami, Nagata, Junko, et al.

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Molecular Phylogeography of the Red Deer (*Cervus elaphus*) Populations in Xinjiang of China: Comparison with other Asian, European, and North American Populations

Halik Mahmut^{1,2}, Ryuichi Masuda^{3*}, Manabu Onuma², Manami Takahashi², Junko Nagata⁴, Masatsugu Suzuki² and Noriyuki Ohtaishi²

¹Department of Biology, Xinjiang University, Urumqi, Xinjiang 830046, China

²Laboratory of Wildlife Biology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

³Laboratory of Genetic Diversity, Center for Advanced Science and Technology, Hokkaido University, Sapporo 060-0810, Japan

⁴Wildlife Management Laboratory, Forestry and Forest Products Research Institute, Ibaraki 305-8687, Japan

ABSTRACT—To illustrate phylogeography of red deer (*Cervus elaphus*) populations of Xinjiang, we determined their mitochondrial DNA (mtDNA) control region sequences, and then investigated geographic variations and phylogenetic relationships between Xinjiang populations and other populations from Asia, Europe, and North America. The *C. elaphus* mtDNA control region shared different copy numbers of tandem repeats of 38 to 43-bp motifs which clearly distinguished the Western lineage from the Eastern lineage of this species in Eurasia. The western lineage comprised the Tarim populations from southern Xinjiang and the European populations, all of which had four copies of the motifs. By contrast, the Eastern lineage consisted of populations from northern Xinjiang (Tianshan and Altai Mountains), other Asian areas (Alashan, Gansu, Tibet, Mongolia, and northeastern China), and North America, all of which shared six copies of the motifs. MtDNA phylogenetic trees showed that there are two major clusters of haplotypes which referred to the Western and Eastern lineages, and that subgroupings of haplotypes in each cluster were congruent with their geographic distributions. The present study revealed that a boundary separating the Western lineage from the Eastern lineage occurs between Tarim Basin and Tianshan Mountains in Xinjiang. Meanwhile, North American populations were genetically closer to those of northern Xinjiang, northeastern China, and Mongolia, supporting that *C. elaphus* immigrated from northeastern Eurasia to North America through the glacier-induced land-bridge (Beringia) which had formed between the two continents after Late Pleistocene.

Key words: red deer, *Cervus elaphus*, phylogeography, mitochondrial DNA, Xinjiang

INTRODUCTION

The red deer (*Cervus elaphus*) is widely distributed in Palaearctic and Nearctic regions (Fig. 1), and their characteristics have well diverged. Based on coat colors and morphological characters of skulls and antlers, this species was classified into 22 subspecies (Ellerman and Morrison-Scott, 1966; Flerov, 1952; Ueckermann, 1987; Heptner *et al.*, 1989; Ohtaishi and Gao, 1990; Dulamtseren *et al.*, 1990; Ohtaishi, 1992; Ohtaishi, 1995). In addition, this species of

the world was roughly divided into three groups: the European group (*elaphoid* type) from Europe and northwestern Africa; the Central Asiatic group (Hangul type or wallich type) consisting of seven subspecies, four of which were from China (*C. e. yarkandensis*, *C. e. wallichii*, *C. e. kansuensis*, and *C. e. macneilli*); the East Asia-North America group or Maral-Canadensis type consisting of eight subspecies, four of which were from China (*C. e. sibiricus*, *C. e. songaricus*, *C. e. alashanicus*, and *C. e. xanthopygus*) (Ohtaishi, 1992, 1995).

Cervus elaphus is considered to have evolved from the sika deer-like ancestor in the Himalayan foothill land, and expanded from the Chinese continent, through Middle Asia,

* Corresponding author: Tel. +81-11-706-2798;

FAX. +81-11-737-0536.

E-mail: masudary@ees.hokudai.ac.jp

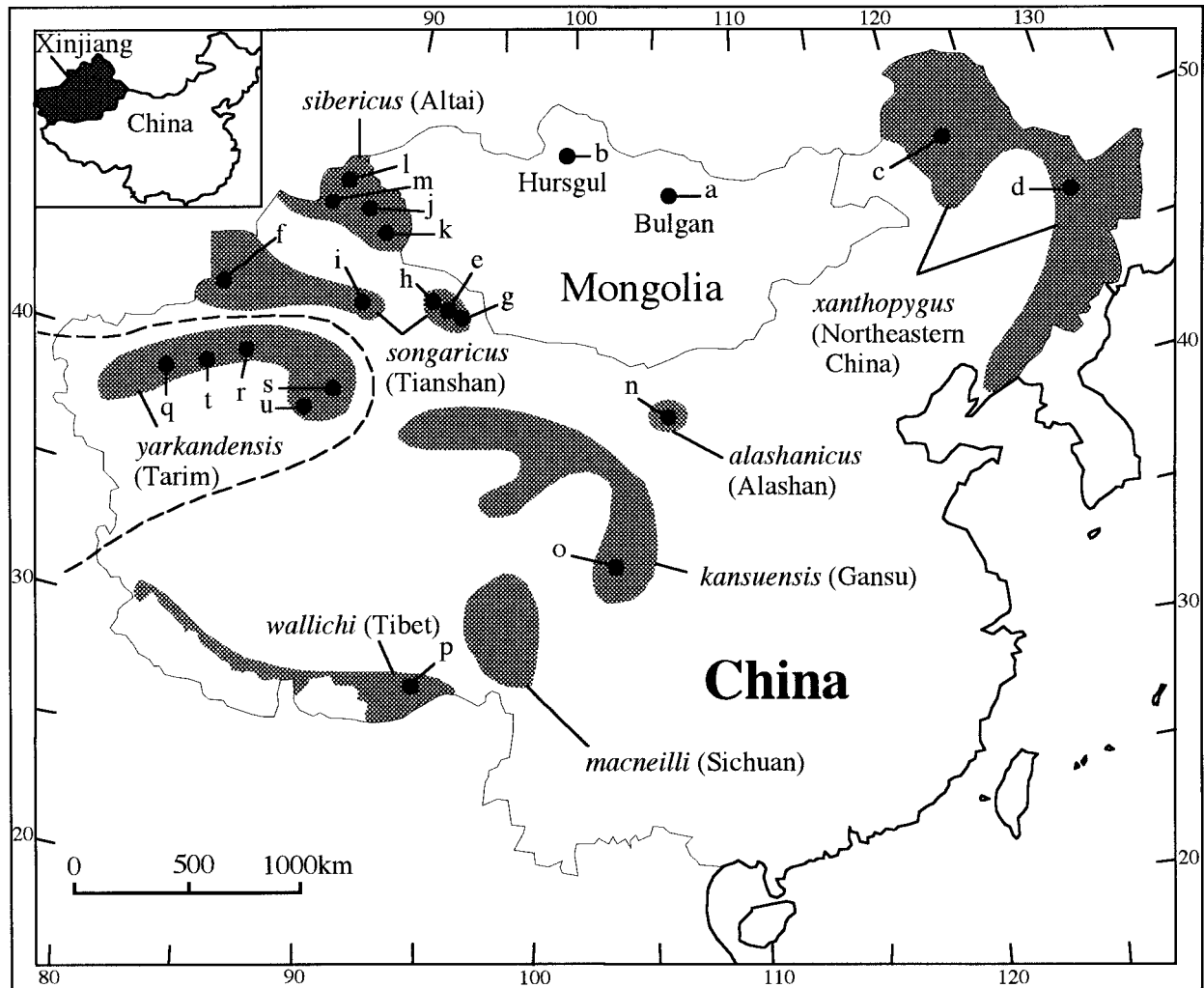


Fig. 1. Distribution (shaded areas and localities in parentheses) of red deer (*Cervus elaphus*) subspecies in China and sampling points (a-u, referring to Table 1) in the present study. The broken line located in Tarim Basin of Xinjiang shows a boundary between the Western and Eastern lineages of mtDNA. The small map at the upper left indicates the location of Xinjiang in China

to Europe (Geist, 1971, 1983; Groves and Grubb, 1987; Ohtaishi, 1992, 1995). Geist (1971, 1983) and Groves and Grubb (1987) also suggested populations of Middle Asia and North America were derived from European populations which were originated from the Himalayan land. Thus, Middle Asia has been colonized by *C. elaphus* at least twice and populations of this area must have been involved deeply in the genetic communication between eastern and western populations of Eurasia. Therefore, characteristics of Middle Asian populations will provide invaluable information to further understand the evolution and migration history of *C. elaphus*. Polziehn and Strobeck (1998) investigated the mtDNA control region phylogeny of *C. elaphus*, and reported occurrence of two lineages: Europe vs. Siberia and North America, although they did not include populations from Middle Asia and East Asia. In China, studies of distributions, habitats, population sizes, reproduction, farming, and conservation of *C. elaphus* have been carried out since so far (Gao and Gu, 1985; Gao and Hu 1993; Chen *et al.*,

1993; Qiao and Gao, 1997; Li *et al.*, 1998), however, information on phylogenetic relationships between Asian populations including Chinese one is still missing.

In the present study, we investigate mitochondrial DNA (mtDNA) control region sequences to illustrate phylogeographic status of *C. elaphus* populations of Xinjiang in Middle Asia, compared with those from other areas of Asia, Europe, and North America. Based on molecular phylogenetic data, we discuss evolution and migration history of this species in Eurasia.

MATERIALS AND METHODS

Sample collection

A total of 47 red deer samples (dried skins, hairs, or bloods) from eight populations were collected (Fig. 1 and Table 1). No specimens from the subspecies *macneilli* (see Fig. 1) were available. Dried skins of two Mongolian red deer were offered from Dr. Sumiya Ganzorig of Hokkaido University. Hairs of one Hungarian and one

Table 1. Sample profiles of *Cervus elaphus* in the present study

Subspecies	Locality ¹⁾	Sample No.	Deletion		mtDNA haplotype	Cluster ⁴⁾	Accession no. ⁵⁾	
			D1 ²⁾	D2 ³⁾				
<i>manitobensis</i>	Manitoban		–	–	CE-01	E1	AF016957#	
<i>nelsoni</i>	Rocky Mts.		–	–	CE-02	E1	AF016966#	
<i>nannodes</i>	Sierra Nevada Mts.		–	–	CE-03	E1	AF016976#	
<i>roosevelti</i>	Coast Mts.		–	–	CE-04	E1	AF016970#	
<i>xanthopygus</i>	a: Bulgan, Mongolia	a 1	–	–	CE-05	E2	AB074538	
	b: Hursgul, Mongolia	b 1	–	–	CE-06	E2	AB074539	
	c: Da Hinggan Ling	c 1	–	–	CE-07	E3	AB074540	
		c 2	–	–	CE-08	E3	AB074541	
	d: Xiao Hinggan Ling	d 1	–	–	CE-09	E3	AB074542	
		d 2	–	–	CE-09	E3		
	<i>songaricus</i>	e: Junmachang	e 1	–	–	CE-10	E4	AB074543
			e 2	–	–	CE-10	E4	
		f: Aktax	f 1	–	–	CE-11	E4	AB074544
f 2			–	–	CE-11	E4		
g: Hongshan		g 1	–	–	CE-12	E4	AB074545	
		g 2	–	–	CE-12	E4		
		g 3	–	–	CE-12	E4		
		g 4	–	–	CE-12	E4		
h: Gulja		h 1	–	–	CE-13	E4	AB074546	
		h 2	–	–	CE-13	E4		
		h 3	–	–	CE-13	E4		
<i>sibericus</i>		i: Qitai	l 1	–	–	CE-14	E4	AB074547
	j: Altai	j 1	–	–	CE-15	E5	AB074548	
		j 2	–	–	CE-15	E5		
	k: Koktokay	k 1	–	–	CE-15	E5		
		k 2	–	–	CE-15	E5		
	l: Kanas	l 1	–	–	CE-16	E5	AB074549	
		l 2	–	–	CE-16	E5		
	m: Kaba	m 1	–	–	CE-16	E5		
m 2		–	–	CE-16	E5			
<i>alashanicus</i>	n: Helanshan	n 1	–	–	CE-17	E6	AB074550	
		n 2	–	–	CE-18	E6	AB074551	
<i>kansuensis</i>	o: Gansu	o 1	–	–	CE-19	E7	AB074552	
		o 2	–	–	CE-19	E7		
<i>wallichii</i>	p: Tibet	p 1	–	–	CE-20	E8	AB074553	
		p 2	–	–	CE-20	E8		
<i>yarkandensis</i>	q: Awat	q 1	–	+	CE-21	W1	AB074554	
		q 2	–	+	CE-22	W1	AB074555	
	r: Shayar	r 1	–	+	CE-23	W1	AB074556	
		r 2	–	+	CE-23	W1		
		r 3	–	+	CE-23	W1		
	s: Qarkilik	s 1	–	+	CE-26	W1	AB074559	
		s 2	–	+	CE-24	W1		
		s 3	+	+	CE-25	W1		
	t: Alar	t 1	+	+	CE-25	W1		
		t 2	+	+	CE-25	W1		
	u: Qarqan	u 1	+	+	CE-26	W1		
		u 2	+	+	CE-26	W1		
		u 3	+	+	CE-26	W1		
	<i>hippelaphus</i>	v: Poland	v 1	–	+	CE-27	W2	AB074560
		w: Hungary	w 1	–	+	CE-27	W2	
<i>elaphus</i>	Europe		–	+	CE-28	W2	AF016972#	
	Europe		–	+	CE-29	W2	AF016973#	

¹⁾ Alphabets of localities refer to those in Fig. 1.

²⁾ D1: 19-bp deletion.

³⁾ D2: +, four copies of motifs; –, six copies of motifs.

⁴⁾ Clusters refer to those of Fig. 3.

⁵⁾ Sequence data will appear in DDBJ nucleotide sequence databases with those accession numbers.

Cited from Polziehn and Strobeck (1998).

Polish red deer were supplied from Mr. Geza Voros of Director of Oreglak Game Processing Plant of Hungary. Total DNAs from those tissues were extracted using the phenol/proteinase K/sodium dodecyl sulphate method of Sambrook *et al.* (1989) with slight modifications (Masuda and Yoshida, 1994) or using Chelex 100 (Bio-Rad) (Walsh *et al.*, 1991). The following sequences were obtained from GenBank (Table 1): European and North American *C. elaphus*, accession Nos. AF016976, AF016970, AF016957, AF016966, AF016972, AF016973; *Cervus nippon*, AF016974 (Polziehn and Strobeck, 1998).

PCR and direct sequencing of mtDNA control region fragments

Primers (LD5 and HD8) for polymerase chain reaction (PCR) amplification and sequencing (Nagata *et al.*, 1998) were used as shown in Fig. 2. An aliquot (10 μ l) of the DNA extract was subjected to PCR amplification in a reaction mixture of 50 μ l, which included 10 mM Tris (pH 8.3), 50 mM KCl, and 1.5 mM MgCl₂, each dNTP at 0.2 mM, 1.25U of *Taq* DNA polymerase (Takara), and each primer at 0.5 μ M. Step programs for PCR were as follows: 94°C for 1 min; 50°C for 1 min and 72°C for 2 min. The cycle was repeated 35 times followed by a completion reaction at 72°C for 10 min. PCR products were purified with the QIAquick PCR purification kit

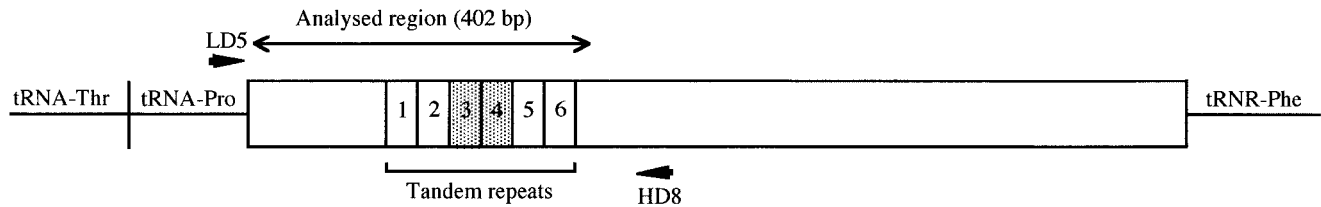


Fig. 2. Schematic diagram of the *Cervus elaphus* mtDNA control region. Numbers 1–6 indicate tandem repeats of 38 to 43-bp motifs. Arrows show positions of primers used for amplification and sequencing. Shaded motifs 3 and 4 were absent in both Tarim and European populations.

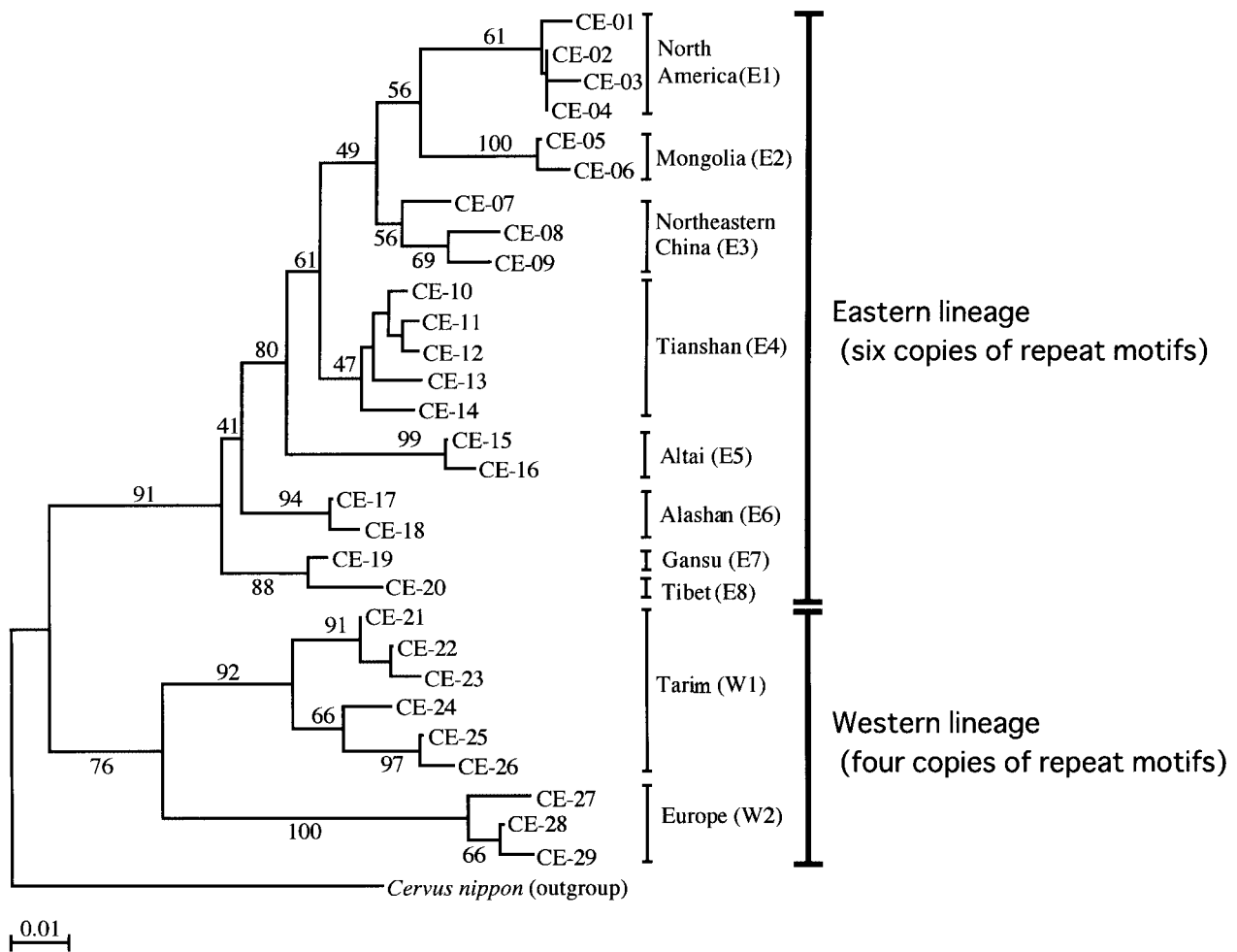


Fig. 3. Phylogenetic relationships among haplotypes of the *Cervus elaphus* mtDNA control region, constructed by the neighbor-joining method. Haplotype names refer to those of Appendix. Scale bars represent genetic distances calculated by the Kimura's two-parameter method, exclusive of indel sites. E1-8 and W1-2 were clusters with sampling locality names. Numbers near internal branches are bootstrap values (%) derived from 1,000 replications.

(Qiagen). Then, 5 μ l of purified PCR products were sequenced using the ABI Prism™ 377 DNA sequencing system (Perkin-Elmer).

Sequence analysis

Sequence alignment was performed using a computer software GeneWorks (Intelligenetics). A phylogenetic tree was reconstructed using the neighbor-joining method (Saitou and Nei, 1987) in a computer software MEGA (Kumar *et al.*, 1993). Numbers of nucleotide substitutions per site were estimated for multiple substitutions using the Kimura's (1980) two-parameter method in MEGA. Insertion or deletion (indel) sites were eliminated for sequence analysis. Bootstrap (Felsenstein, 1985) values were derived from 1,000 replications. Parsimonious relationships between haplotypes were presented by hand-written networks. The *C. nippon* sequence was used as outgroup to root the neighbor-joining phylogenetic tree.

RESULTS

Tandem repeats and polymorphisms in mtDNA control region sequences

Nucleotide sequencing in the present study yielded 402-bp fragments of the *C. elaphus* mtDNA control region. From the 47 individuals, we identified 23 haplotypes (CE-05 to -27) (Table 1). Haplotypes CE-01 to -04 and CE-28 and -29 were cited from previous reports of Polziehn and

Strobeck (1998) as mentioned in Materials and Methods. A sequence alignment showed that 57 of 304 sites (19%: 57/304 sites) excluding indels were polymorphic (see Appendix).

This fragment included tandem repeats (four or six copies) of 38 to 43-bp motifs: four copies in populations of Tarim (CE-21 to -26) and Europe (CE-27 to -29), while six copies in populations of Alashan (CE-17 and -18), Gansu (CE-19), Tibetan (CE-20), northeastern China (CE-07 to -09), Tianshan (CE-10 to -14), Altai (CE-15 and -16), Mongolia (CE-05 and -06), and North America (CE-01 to -06) (Table 1 and Appendix). Consequently, two copies (third and fourth copies) of the motifs were absent in populations of both Tarim and Europe, compared with those of the other areas (Fig. 2 and Appendix). In addition, CE-25 and CE-26 from the Tarim population shared a 19-bp deletion within the first copy of the motif (Table 1 and Appendix).

Phylogenetic relationships among populations

The neighbor-joining tree showed that mtDNA haplotypes from Asia, Europe, and North America were classified into two major clusters, named the Eastern (consisting of

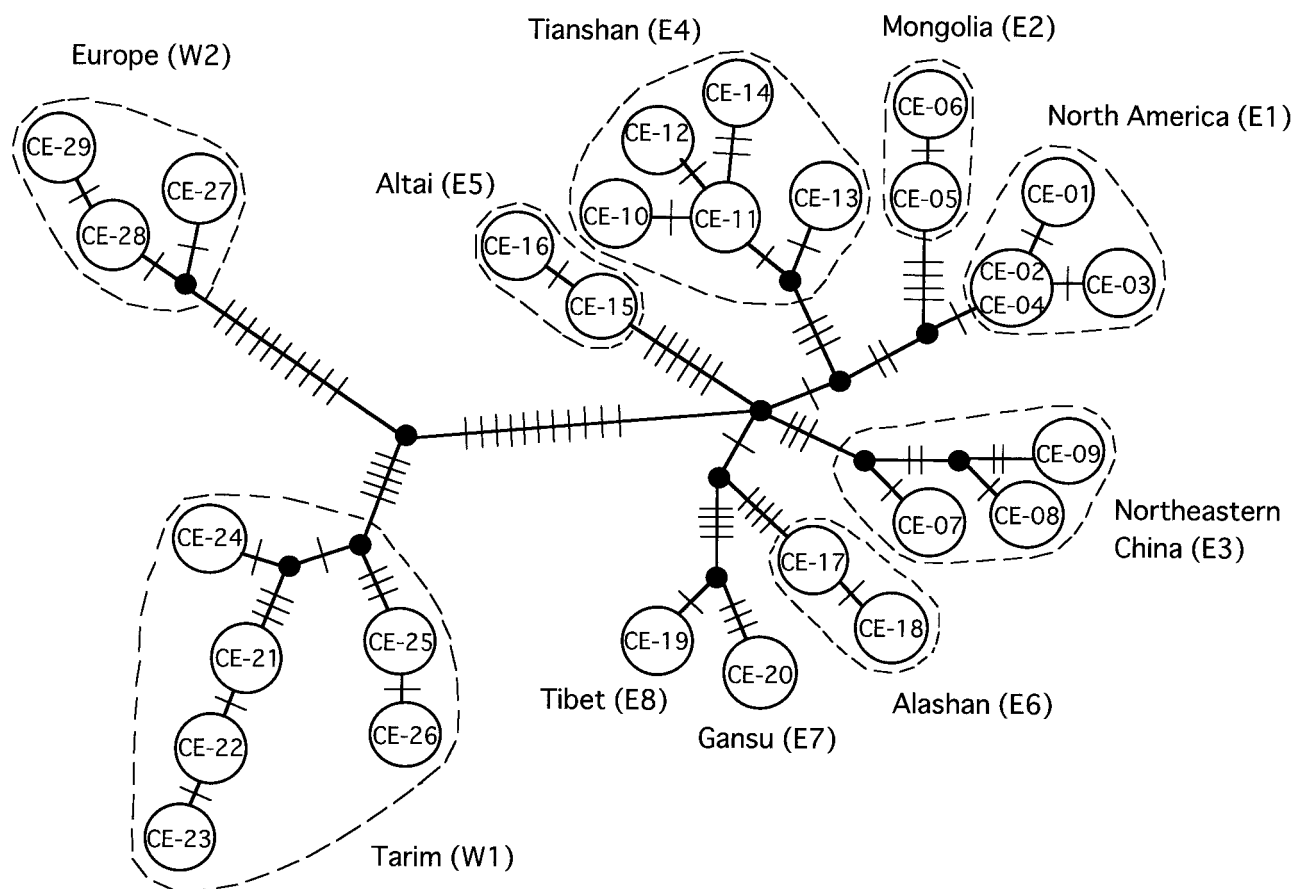


Fig. 4. Parsimonious networks between haplotypes of the *Cervus elaphus* mtDNA control region. Haplotype names in open circles correspond to those of Fig. 3. Closed circles indicate predicted haplotypes. One slash means a nucleotide substitution, excluding indel sites. The networks basically support the separation between the Western and Eastern lineages, the ten clusters (E1-8 and W1-2), and the haplotype relationships which were formed in the neighbor-joining tree (Fig. 3).

Table 2. Genetic distances (%) between clusters, calculated using Kimura's two-parameter method. Clusters E1–8 are from the Eastern lineage, while W1 and W2 are from the Western lineage (see Fig. 3).

	E1	E2	E3	E4	E5	E6	E7	E8	W1	W2
E1		2.0	1.8	2.4	3.8	3.4	3.6	4.4	8.4	9.8
E2			3.6	3.5	4.9	4.5	4.7	5.4	8.8	9.9
E3				2.8	4.0	3.6	3.8	4.5	8.1	9.2
E4					3.3	3.5	3.6	4.4	7.7	9.7
E5						3.3	3.4	4.1	8.8	9.8
E6							3.1	2.9	6.9	7.5
E7								1.0	7.8	8.8
E8									8.2	8.9
W1										6.3
W2										

CE-01 to -20) and Western (consisting of CE-21 to -29) lineages with 91% and 76% bootstrap values, respectively (Fig. 3). The separation of the two lineages was in congruence with haplotype classification by copy numbers of tandem repeats: six copies for the Eastern lineage and four copies for the Western lineage (Table 1).

Since haplotypes from geographically closer localities (see Fig. 1) were genetically closer to each other and formed their own clusters with 47–100% bootstrap values, we named clusters E1–8 and W1–2 (Fig. 3). Parsimonious networks supported the existence of the Western and Eastern lineages and the haplotype clusters found in the neighbor-joining tree (Fig. 4).

In the Western lineage, haplotypes (CE-21 to -26) from the Tarim populations of southern Xinjiang formed their own cluster (W1) with a 92% bootstrap value, while haplotypes (CE-27 to -29) from Europe (W2) were clustered with a 100% bootstrap value (Fig. 3). CE-25 and CE-26, both which shared the 19-bp deletion (Table 1), formed a sub-cluster within the Tarim cluster (W1) with a 97% bootstrap value.

Meanwhile, in the Eastern lineage, CE-19 from Gansu (E7) and CE-20 from Tibet (E8) were closer to each other with a 88% bootstrap value (Fig. 3). They were closely related to the Alanshan population (Cluster E6; CE-17 and CE-18). Haplotypes CE-15 and CE-16 from Altai Mountains of northern Xinjiang (Cluster E5; 99% bootstrap value) and haplotypes (CE-10 to -14) from Tianshan Mountains of northern Xinjiang (E4; 47% bootstrap value) formed one larger cluster with CE-07 to -09 from northeastern China (E3; 56% bootstrap value), CE-05 and CE-06 from Mongolia (E2; 100% bootstrap value), and CE-01 to -04 from North America (E1; 61% bootstrap value), showing a 80% bootstrap value (Fig. 3). Fig. 3 showed that North American populations were relatively closer to the Mongolian population (Cluster E2). In addition, Tianshan (E4) and Altai (E5) populations from northern Xinjiang were included in the Eastern lineage, whereas the Tarim population (W1) from southern Xinjiang was grouped into the Western lineage (Fig. 3). Thus, mtDNA lineages of southern and northern Xinjiang were different from each other. The boundary of the two lin-

eages could have occurred between Tianshan Mountains and Tarim River in Tarim Basin. Parsimonious networks (Fig. 4) also showed that the relationships among haplotypes were the same as those of Fig. 3.

Genetic distances were 6.3% within the Western lineage (W1 and W2), 1.8–5.4% within the Eastern lineage (E1–8), and 6.9–9.9% between the Western and Eastern lineages (Table 2).

DISCUSSION

Phylogeography of *Cervus elaphus* in the Holarctic region

The present study demonstrated that there are two major mtDNA lineages in *C. elaphus* of Eurasia: the Western and Eastern lineages (Table 1; Figs. 3 and 4). It was supported by geographic distribution of four (Western lineage) or six (Eastern lineage) copies of motifs in tandem repeats identified in the present study, in addition to the molecular phylogenetic relationships between haplotypes. The similar tandem repeats of motifs in the mtDNA control region were found in the Japanese sika deer *Cervus nippon*, whose repeat numbers were more variable (four to seven copies) and population-specific within Japanese islands (Nagata *et al.*, 1998, 1999). Compared with the data of *C. nippon*, it is noticed that *C. elaphus* from much wider ranges (Eurasia and North America) shared only two kinds (four or six) of copy numbers.

Each cluster of the trees was almost congruent with their geographical distribution as well as subspecies classification (Figs. 3 and 4). This means that genetic characterization in the present study was consistent with morphological differentiation in local populations of *C. elaphus*.

The genetic distances (6.9%–9.9%, Table 2) between the Western and Eastern lineages corresponded to 0.3–0.4 million years ago for the divergence time, based on the mutation rate (0.118×10^{-6} substitutions/site/year) of the human mtDNA control region reported by Stoneking *et al.* (1992). After the divergence between the two lineages, some populations with the Western lineage could have immigrated into Europe. Fossil data showed that *C. elaphus*

appeared in Europe in Middle Pleistocene (approximately 0.7 to 0.55 million years ago) (Kurtén, 1968; Nilsson, 1983; Lister, 1984, 1990, 1993). Divergence times estimated by us supported that *C. elaphus* occurred in Europe in Middle Pleistocene, although the estimated divergence times were somewhat younger than the fossil data.

The Eastern lineage included North American populations which were closer to Mongolian and Northeastern Chinese populations (Figs. 3 and 4). This fact indicates that the ancestral populations of *C. elaphus* immigrated from northeastern Eurasia to North America via Beringia which was formed during the last glacial period, as considered from fossil data (Geist, 1998). From the genetic distances (2.0%, Table 2) between North American and the Mongolian populations, the divergence time was estimated to be 80,000 years ago. Polziehn and Strobeck (1998) estimated that the divergence time between Siberian and North American populations of *C. elaphus* was 52,000 years ago based on the mtDNA control region data. Geist (1971, 1983, 1998) described from fossil data that at the Riss (Illinoian) age the *C. elaphus* remained in Alaska, moved into Alberta approximately 40,000 years ago, and entered southern parts of North America. Our results were not in discordance with the divergence time estimated by previous DNA analysis (Polziehn and Strobeck, 1998) and fossil data (Geist, 1971, 1983, 1998). Recent molecular phylogenetic studies on intercontinental populations of brown bears *Ursus arctos* showed that some parts of Eurasian populations are very close to those of Alaska and that ancestral populations immigrated from northeastern Eurasia to North America, via Beringia of Late Pleistocene (Leonard *et al.*, 2000; Matsushita *et al.*, 2001). Thus, large mammals such red deer and brown bears, which have adapted themselves to relatively cold climates, likely have a similar history on intercontinental migration in Pleistocene.

Migration history within Eurasia

Cervus elaphus is considered to have evolved from the sika deer-like ancestor when they immigrated from the Himalayan foothills to the Chinese continent. In addition, *C. elaphus* expanded their distribution to Europe through Middle Asia, then, in connection with glacial retreats and glacial cup disappearance in Middle Pleistocene, went back to northern parts of the Chinese continent through Xinjiang, and finally immigrated to Siberia and North America (Geist, 1971, 1983; Groves and Grubb, 1987; Ohtaishi, 1992, 1995). The present study, however, demonstrated that the European populations did not belong to the Eastern lineage but to the Western lineage with populations of Tarim Basin (southern Xinjiang). This result indicates that the European populations were not a direct ancestor of current populations in Northeastern Asia and North America. Figure 5 summarizes a conceivable migration history of *C. elaphus* using clusters defined in the present study. Combining our data with the previous reports on *C. elaphus* evolution (Geist, 1971, 1983; Groves and Grubb, 1987; Ohtaishi, 1992, 1995), it is likely that the Eastern lineage (Altai and Tianshan Mountains in northern Xinjiang, northeastern China, Mongolia, and North America) evolved from ancestral populations around the Tibetan plateau.

Occurrence of the mtDNA lineage boundary within Xinjiang

Our results showed that the boundary between the Western and the Eastern lineages occurred between Tianshan Mountains and Tarim River within Tarim Basin of Xinjiang (Table 1; Figs. 1, 3, and 4). Green belts along Tarim River in Tarim Basin are current habitats of *C. elaphus* (Fig. 1). Desert areas lying north of Tarim River could have been geographic barriers between the Tianshan and Tarim populations, and genetically differentiated them. Genetic characteristics in Xinjiang populations could reflect their migration history in Middle Asia. When ancestral populations of *C. ela-*

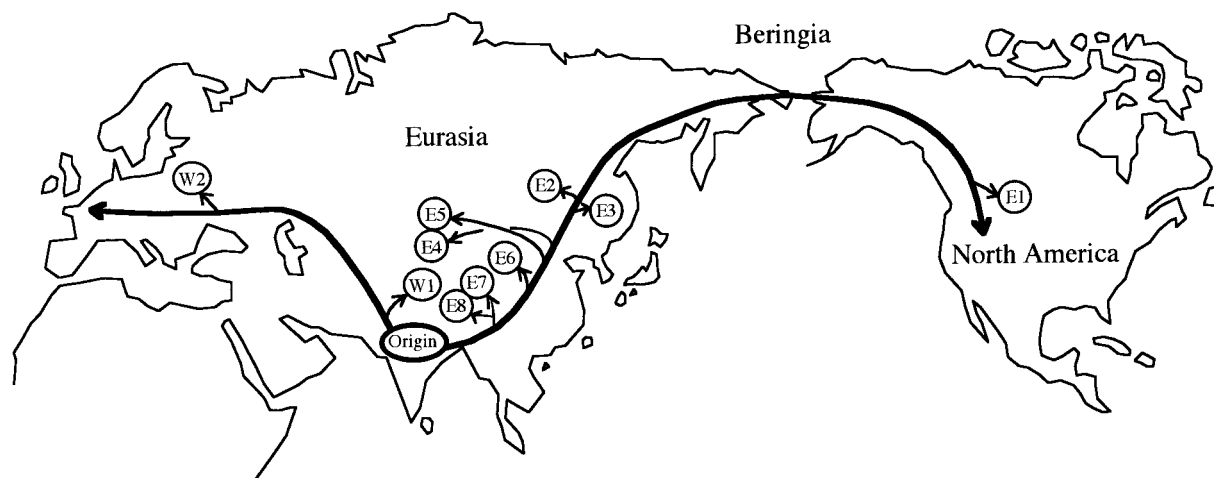


Fig. 5. Migration history of *Cervus elaphus* in Eurasia and North America. Clusters E1-8 and W1-2 were the same as in Fig. 3. 'Origin' means an assumed ancestor of *C. elaphus*.

phus immigrated into Tarim Basin from the south in Middle Pleistocene and adapted themselves to the habitat, Tianshan Mountains had been already covered with glacial sheets (Wen, 1994). Therefore, they might not be able to reach northern Xinjiang across glacial sheets, and might have stayed in Tarim Basin. Meanwhile, formation of deserts had started from eastern Tarim since the end of Early Pleistocene (Wen, 1994). Even after the glacial retreat in Holocene (Wen, 1994), Tarim populations could not cross Tianshan Mountains by geographic barriers of deserts formed between the Tarim River and Tianshan Mountains, and then they have been isolated in green belts developing along the river. As shown in Fig. 5, only populations having the Eastern lineage of mtDNA could have colonized northern Xinjiang (Altai and Tianshan Mountains) from East Asia.

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REFERENCES

- Chen H, Ma J, Li F, Wang Y, Wang H, Li F (1993) Regional variation in winter diets of red deer in Heilongjiang, Northeastern China. In "Deer of China: Biology and Management" Ed by N Ohtaishi, H Sheng, Elsevier Sci Publ, Amsterdam, pp 181–186
- Dulamtsuren S, Danilkin AA, Lushechina AA, Neronov VM, Sokolov VE (1990) Wild ungulates. In "The National Atlas of the Mongolian People's Republic" Ed by SCH Tsegmed, VV Vorobiev, Academy of Sci Mongolia (in Mongolian)
- Ellerman JR, Morrison-Scott TCS (1966) Checklist of Palaearctic and Indian Mammals 1758 to 1946. Trustees of the British Museum (Nat Hist), London
- Felsenstein DG (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791
- Flerov KK (1952) Fauna of USSR, Mammals Vol. 1, No. 2: Musk Deer and Deer. The Academy of Sciences of the USSR (The Israel Program for Scientific Translation, 1960)
- Gao X, Gu J (1985) Red Deer in Xinjiang. *Chinese Wildlife* 2: 24–26 (in Chinese)
- Gao X, Hu D (1993) Status of wild and farmed red deer in Xinjiang. In "Deer of China: Biology and Management" Ed by N Ohtaishi, H Sheng, Elsevier Sci Publ, Amsterdam, pp 159–164
- Geist V (1971) The relation of social evolution and dispersal in ungulates during the Pleistocene, with emphasis on the Old World deer and genus *Bison*. *Quat Res* 1: 283–315
- Geist V (1998) *Deer of the World*. Stackpole Books, Mechanicsburg
- Geist V (1983) On the evolution of ice age mammals and its significance to an understanding of speciations. *ASB Bulletin* 30: 109–133
- Groves CP, Grubb P (1987) Relationships of living deer. In "Biology and Management of the Cervidae" Ed by CM Wemmer, Smithsonian Inst Press, pp 21–59
- Heptner VG, Nasimovich AA, Bannikov AG (1989) Mammals of the Soviet Union. Vol. I Ungulates. EJ Brill Publ, Leiden
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111–120
- Kumar S, Tamura K, Nei M (1993) MEGA: Molecular Evolutionary Genetics Analysis Version 1.01. Pennsylvania State Univ, Pennsylvania
- Kurten B (1968) Pleistocene Mammals of Europe. Weidenfeld and Nicolson, London
- Leonard JA, Wayne RK, Cooper A (2000) Population genetics of ice age brown bears. *Proc Natl Acad Sci USA* 97: 1651–1654
- Li M, Wang X, Sheng H, Masuda R, Nagata J, Ohtaishi N (1998) Origin and genetic diversity of four subspecies of red deer (*Cervus elaphus*). *Zool Res* 19: 177–183 (in Chinese with English abstract)
- Lister A M (1984) Evolutionary and ecological origins of British deer. *Proc Royal Soc Edinburgh* 82B: 205–229
- Lister A M (1990) Critical appraisal of the middle Pleistocene deer species "*Cervus*" *elaphoides* Kahlke. *Quaternary* 3–4: 75–192
- Lister A M (1993) The stratigraphical significance of deer species in the Cromer Forest bed formation. *J Quat Sci* 8: 95–108
- Masuda R, Yoshida MC (1994) Nucleotide sequence variation of cytochrome b genes in three species of weasels, *Mustela itatsi*, *Mustela sibirica*, and *Mustela nivalis*, detected by improved PCR product-direct sequencing technique. *J Mamm Soc Japan* 19: 33–43
- Matsuhashi T, Masuda R, Mano T, Murata K, Aiurzaniin A (2001) Phylogenetic relationships among worldwide populations of the brown bear *Ursus arctos*. *Zool Sci* 18: 1137–1143
- Nagata J, Masuda R, Kaji K, Kaneko M, Yoshida MC (1998) Genetic variation and population structure of the Japanese sika deer (*Cervus nippon*) in Hokkaido Island, based on mitochondrial D-loop sequences. *Mol Ecol* 7: 871–877
- Nagata J, Masuda R, Tamate HB, Hamazaki S, Ochiai K, Asada M, Tatsuzawa S, Suda K, Tado H, Yoshida MC (1999) Two genetically distinct lineages of the sika deer, *Cervus nippon*, in Japanese islands: Comparison of mitochondrial D-loop region sequences. *Mol Phylogenet Evol* 13: 511–519
- Nilssen T (1983) The Pleistocene. Reidel Publishing, Dordrecht
- Ohtaishi N, Gao Y (1990) A review of the distribution of all species of deer (Tragulidae, Moschidae and Cervidae) in China. *Mammal Rev* 20: 125–144
- Ohtaishi N (1992) The origins and evolution of the deer in China. In "The Deer in China" Ed by H Sheng, East China Normal Univ Press, Shanghai, pp 8–18 (in Chinese with English abstract)
- Ohtaishi N (1995) Red deer—the most prosperous deer. *Chikusan Kenkyu* (Livestock Research), 49: 132–139 (in Japanese)
- Polziehn RO, Strobeck C (1998) Phylogeny of wapiti, red deer, sika deer, and other North American cervids as determined from mitochondrial DNA. *Mol Phylogenet Evol* 10: 249–258
- Qiao J, Gao X (1997) Study on food habit of Yarkand red deer (*C. e. yarkandensis*) *Endemic Diseases Bulletin, Supplement (Zoology)* 12: 12–17 (in Chinese with English abstract)
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, New York
- Stoneking M, Sherry ST, Redd AJ, Vigilant L (1992) New approaches to dating suggest a recent age for the human mtDNA ancestor. *Phil T Roy B* 337: 34–37
- Ueckermann E (1987) Managing German red deer (*Cervus elaphus* L.) populations. In "Biology and Management of the Cervidae" Ed by CM Wemmer, Smithsonian Inst Press, Washington D.C.,

pp 505–516

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Bio Techniques* 10: 506–513

Wen Q (1994) Quaternary geology and environment of Xinjiang region, China. Agriculture Publishing Company of China, Beijing (in Chinese with English abstract)

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Appendix

Sequence alignment of the *Cervus elaphus* mtDNA control region. Dashes indicate indel sites. Dots denote identity with nucleotides of haplotype CE-01. Arrows under sequences show tandem repeats (four or six copies) of 38 to 43-bp motifs.

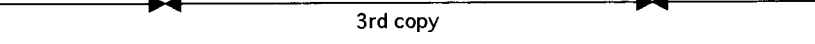
	10	20	30	40	50	60	70
CE-01	CCCAAAGCTG	AAGTTCTATT	TAAACTATTC	CCTGACGCTT	ATTAATATAG	TCCATAAAA	ATCAAGAACT
CE-02
CE-03
CE-04
CE-05
CE-06
CE-07
CE-08
CE-09
CE-10
CE-11
CE-12
CE-13
CE-14
CE-15
CE-16
CE-17
CE-18
CE-19
CE-20
CE-21G..
CE-22G..
CE-23G..
CE-24G..
CE-25G..T..
CE-26G..T..
CE-27
CE-28T..
CE-29T..
CN

	80	90	100	110	120	130	140
CE-01	TTATCAGTAT	TAAATTCCA	AAAAAATTTA	ATATTTAAT	ACAGCTTTCT	ACTCAACATC	CAATTTACAT
CE-02T..
CE-03T..
CE-04T..
CE-05T..
CE-06T..G..
CE-07T..T
CE-08T..C
CE-09
CE-10T..
CE-11T..
CE-12T..
CE-13T..
CE-14T..
CE-15TC..
CE-16TC..
CE-17T..
CE-18T..
CE-19T..
CE-20T..C..
CE-21T..C..T..
CE-22TT..C..T..
CE-23TT..C..T..
CE-24T..C..T..
CE-25T..C..T..
CE-26G..T..C..T..
CE-27GT..C..CC..T..
CE-28GT..C..CC..T..
CE-29GT..C..CC..T..
CNT..C..C..

	150	160	170	180	190	200	210
CE-01	TTTATGTCCT	ACTAATTACA	CAGCAAAACA	CGTGATATAA	CCTTATGCGC	TCG-TAGTAC	ATAAAATCAA
CE-02
CE-03C.....
CE-04	...A.....
CE-05	...CA....A.....
CE-06	...CA....A.....
CE-07	...CA....T.....
CE-08	...CA....C.....	..T.....
CE-09	...CA....C.....	..T.....
CE-10	...C.....A.....
CE-11A.....
CE-12A.....
CE-13
CE-14A.....
CE-15	...CC....T.....
CE-16	...C.....T.....
CE-17	...C.....C.....	..T.....
CE-18	...C.....C.....	..T.....
CE-19	...C.....T.....
CE-20	...C.....T.....
CE-21	C...CA.TAC	...CC...	T.....	..A.A.....	..C.....	..TA.....	...G...T..
CE-22	C...CA.TAC	...CC...	T.....	..A.A.....	..C.....	..TA.....	...G...T..
CE-23	C...CA.TAC	...CC...	T.A.....	..A.A.....	..C.....	..TA.....	...G...T..
CE-24	C...CA.TAC	...CC...	T.....	..A.A.....	..C.....	..TA.....	...G...T..
CE-25A.....	..A.A.....	..C.....	..TA.....	...G...T..
CE-26A.....	..A.A.....	..C.....	..TA.....	...G...T..
CE-27	...CA...-	..C..CC..	..A...T..	T..A...A..TA.....	...G...T..
CE-28	...CA...-	..C..CC..	..A...T..	T..A...A..TA.....	...G...T..
CE-29	...CA...-	..C..CC..	..A...T..	T..A...A..TA.....	...G...T..
CN	..C...C...	..T.....	..A...G..T..	..T.A.....	...G...T..



	220	230	240	250	260	270	280
CE-01	TGTGCTAGGA	CATGC-ATGT	ATAACAGTAC	ATGAGTT-AG	CGTATAGGAC	ATATTATGTA	TAATAGTACA
CE-02
CE-03
CE-04
CE-05A.....	..G.....
CE-06A.....	..G.....
CE-07
CE-08	..A.....
CE-09	..A.....
CE-10C.....
CE-11
CE-12C.....
CE-13A.....	..G.....
CE-14C.....
CE-15A.....	..C.....C.....T.....
CE-16A.....	..C.....C.....T.....
CE-17
CE-18
CE-19G.....T.....
CE-20G.....A.....	..T.....
CE-21	..AT.....	..A.....
CE-22	..AT.....	..A.....
CE-23	..AT.....	..A.....
CE-24	..AT.....	..A.....
CE-25	..AT.....	..A.....
CE-26	..AT.....	..A.....
CE-27	..A.....	..A.....
CE-28	..A.....	..A.....
CE-29	..A.....	..AT.....
CN	..CATC.A..	..A.T.....	..CGG.....	...AACCG..	TA.....	...C.....	..CGG.....



	290	300	310	320	330	340	350
CE-01	TAAATTAATG	TATTAAGACA	TATT-ATGTA	TAATAGTACA	TTATATTATA	TGCCCCATGC	TTATAAGCAT
CE-02
CE-03
CE-04
CE-05	...C...	...G...G
CE-06	...C...	...G...G
CE-07G...
CE-08C.G...
CE-09C.G...
CE-10	...C...C...
CE-11C...
CE-12C...
CE-13
CE-14C...
CE-15	...C...	...G...
CE-16	...C...	...G...A.....
CE-17G...	...C.T...
CE-18G...	...C.T...C.....
CE-19G...	...C...G.....
CE-20G...	...C...C.....
CE-21
CE-22
CE-23
CE-24C
CE-25C
CE-26C
CE-27A.....
CE-28A.....
CE-29A.....
CN-30GA...

4th copy

5th copy

	360	370	380	390	400	402
CE-01	GTACTTCTCA	CTATCTGAAG	TACATAGTAC	A-TAATGTTG	TTCATCGTAC	AT
CE-02
CE-03
CE-04
CE-05	...T...G...	..
CE-06	...T...G...	..
CE-07C...A...
CE-08C...A...
CE-09A...
CE-10	...T...	...C...T.A...A...G...
CE-11	...G.T...	...C...T.A...G...
CE-12	...G.T...	...C...T.A...A...G...
CE-13	...G.T...	...C...T.A...G...
CE-14	...G.T...	TC...T.A...
CE-15	...G.T...	T...TCA...
CE-16	...G.T...	T...TCA...
CE-17	...TCT.	T.G.T.A...
CE-18	...TCT.	T.G.T.A...
CE-19	...T...	T...T.A...G...A	...T...	..
CE-20	...TC..	T...T.A...G...A	...T...	..
CE-21	...TC..	T.G.T.AC..G...
CE-22	...TC..	T.G.T.AC..G...
CE-23	...TC..	T.G.T.AC..G...
CE-24	...T.T.	...G.T.AC..G...
CE-25	...T.T.	...G.T.AC..G...
CE-26	...T.T.	...G.T.AC..G...
CE-27	...TCT.	T...T.AT..G.	...G...
CE-28	...TCT.	T...T.AT..G...
CE-29	...TCT.	T...T.AT..G...
CN-30	...T..TCT.	T...AT..G...CA	...T...	..

6th copy