Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*)

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Abstract

In order to understand the origin, phylogeny, and phylogeography of the species *Cervus elaphus*, we examined the DNA sequence variation of the mitochondrial cytochrome *b* gene of 51 populations of deer from the entire distribution area of *Cervinae* with an emphasis on Europe and Asia. Several methods, including maximum parsimony, maximum likelihood, and nested clade analysis, revealed that red deer originated from the area between Kyrgyzstan and Northern India. We found two distinct groups of red deer: a western group consisting of four subgroups and an eastern group consisting of three subgroups. Our mtDNA data do not support the traditional classification of red deer as only one species nor its division into numerous subspecies. The discrepancies between the geographical pattern of differentiation based on mtDNA cytochrome *b* and the existing specific and subspecific taxonomy based on morphology are discussed.
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1. Introduction

Red deer (*Cervus elaphus*) is the most widespread and best known deer species in the world. Today there are up to 22 known subspecies (Table 4) in the Holarctic (Geist, 1999; Trense, 1989; Whitehead, 1972). Although much is known about the phylogeny of the species (Geist, 1999; Kuwayama and Ozawa, 2000; Mahmut et al., 2002; Polziehn and Strobeck, 1998, 2002; Randi et al., 1998, 2001; Trense, 1989), there are still some disagreements. Whereas the division into the two species *C. elaphus* and *Cervus canadensis* is widely accepted (Bryant and Maser, 1982; Cockerill, 1984), their subdivision into many subspecies is questioned (Cronin, 1992; Nowack, 1991) as is the assignment of the Central Asian subspecies to the two lineages *elaphus* and *canadensis* (Geist, 1999; Polziehn and Strobeck, 2002; Mahmut et al., 2002; Trense, 1989). Since cladistic analyses detected many cases of parallelism and convergence in the evolution of morphological traits among Cervidae (Groves and Grubb, 1987; Janis

and Scott, 1987), the understanding of their pattern of character evolution requires the drawing of a phylogeny independent from those traits (Randi et al., 1998). The present classification of the numerous subspecies is mostly based on morphological characters such as body and antler size (Dolan, 1988), antler shape (e.g., coronate or acoronate) or cranial measurements (Geist, 1991, 1992) which are all considerably affected by nutrition (Geist, 1999). Recent morphological studies have concentrated more on nutrition-independent characters such as the hair coat of social organs, social signals (Geist, 1999) and postcranial measurements (Pfeiffer, 2002). These studies as well as the most recent studies on mitochondrial DNA (Mahmut et al., 2002; Polziehn and Strobeck, 2002; Randi et al., 2001) query the number of subspecies and favor the classification of red deer into two different species. The mitochondrial protein-coding gene for cytochrome *b* has been proven to be useful for resolving phylogenetic patterns among various artiodactyls within evolutionary time frames shorter than 25 million years (Stanley et al., 1994; Tanaka et al., 1996).

In this study we analyzed the phylogeny of 50 populations of most living species and subspecies of the genus *Cervus* by comparing complete mtDNA

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cytochrome *b* sequences. The aim of this study was to answer the following questions: Does the red deer represent only one species (*C. elaphus*) with numerous subspecies distributed all over the Holarctic (Geist, 1999; Whitehead, 1972), or are there two different species (*C. elaphus* and *C. canadensis*) consisting of an eastern and a western group (Kuwayama and Ozawa, 2000; Mahmut et al., 2002; Polziehn and Strobeck, 1998, 2002; Randi et al., 1998, 2001)? Do the currently named subspecies agree with phylogenetic and phylogeographic knowledge based on genetic data? Does the origin of *C. elaphus* lie in Central Asia as has been assumed by Geist (1999) and Mahmut et al. (2002)? We also discuss the paths of distribution of this widespread species.

2. Materials and methods

2.1. Sample collection and laboratory procedures

Samples of tissue or antlers were obtained from 37 wild populations of red deer (*C. elaphus*) across most of the species range (Fig. 1), with 1–15 individuals from each population being sampled (Table 1). Additional three sequences of red deer were obtained from the NCBI nucleotide data bank for comparison and verification of our data. The following species were included in the study to determine species status: Sika deer (*Cervus nippon*) with five different subspecies (four from the NCBI nucleotide data bank), two populations of Thorold's white lipped deer (*Cervus albirostris*), as well as two populations of sambars (*Cervus unicolor* and *Cervus timorensis*) and one of the hog deer (*Axis porci-*

nus). This means a total of 50 populations of *Cervinae* or 415 sampled individuals (Table 1). One sequence of the fallow deer *Dama dama* (AJ000022), one of *Bos taurus* (J01394), and one of *Moshus moschiferus* (AF026883) were taken from the NCBI nucleotide data bank and were used as outgroups in the phylogenetic part of the work.

Tissue samples were preserved in 95% ethanol and stored together with the antlers at $-20\,°C$. DNA extraction from tissue samples was performed using standard proteinase-K phenol–chloroform protocols (Sambrook et al., 1989). Antler DNA was extracted by a method established by Kuehn et al. (2001). The complete cytochrome *b* gene (1140 bp) was amplified by polymerase chain reaction (PCR) (Saiki et al., 1985). The primer sequences designed for this study were cerni cytoB A1 (GAAAAACCATCGTTGTCATTCA) and cerni cytoB B2 (GGAGGTTGGTAGCTCTCCTTTT). Each PCR (total of $25\,\mu l$) was composed of $1\times$ PCR buffer ($10\,mM$ Tris–HCl, pH 8.3, $50\,mM$ KCl), $3\,mM$ $MgCl_2$, $0.2\,mM$ dNTPs, $0.2\,\mu M$ of each primer and $1\,U$ of *Taq* DNA polymerase. The PCR amplification consisted of an initial denaturing at $94\,°C$ for 3 min followed by 35 cycles of denaturing at $94\,°C$ for 45 s, annealing at $54\,°C$ for 45 s, and extension at $72\,°C$ for 70 s with a final extension period of 3 min at $72\,°C$. PCR products were purified using a NucleoSpin Extract Kit (MACHEREY-NAGEL), and cycle-sequenced from both ends, using either PHARMACIA Dye Primer kits (PCR primers with Cy 5.0 labeled 5′-tails) or PHARMACIA Cy 5.0 Dye Terminator kits; resulting fragments were analyzed in a PHARMACIA ALF Express II automated sequencer.
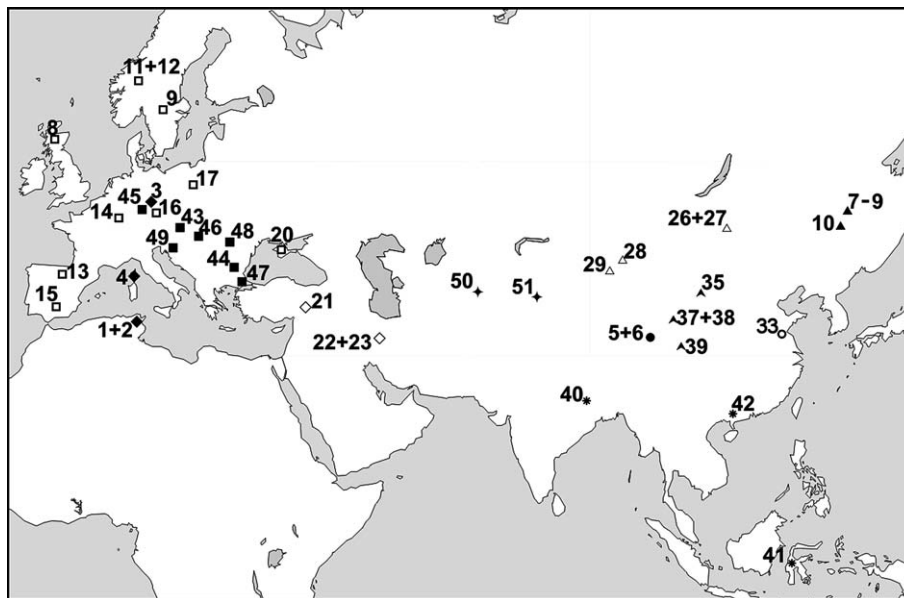

Fig. 1. Map showing approximate sample collection sites. Numbers next to sites are equivalent to numbers in Table 1. Symbols for sites are identical to group symbols in Table 1 and Fig. 4. Only populations with known geographical origin are shown. Population 24 from Montana/USA is not shown on this map due to inappropriate map size.

Table 1
Samples of *Cervinae* analyzed in this study

Nr.	PopID	*N*	Species name	Common name	Geographical origin	Group	Acc. No.
1	barba1	11	*Cer. el. barbarus*	Barbary Red Deer	Tunisia, Tunis	◆ Africa	AY070222
2	barba2	12	*Cer. el. barbarus*	Barbary Red Deer	Tunisia, Tunis	◆ Africa	AY070222
3	enclo1	11	*Cer. el.*	Red Deer	Germany, enclosure	◆ Africa	AY118198
4	corsic	3	*Cer. el. corsicanus*	Sardinian Deer	Sardinia	◆ Africa	AY244489
5	albir1	13	*Cer. albirostris*	Thorold's Deer	China, Qinghai	● Albi	AY044863
6	albir2	16	*Cer. albirostris*	Thorold's Deer	China, Qinghai	● Albi	AF423202
7	alasha	1	*Cer. el. alashanicus*	Ala Shan Red Deer	China	▲ East-Asia	AY070224
8	xanth1	10	*Cer. el. xanthopygus*	Isubra	Russia, Anjui	▲ East-Asia	AY070224
9	xanth2	10	*Cer. el. xanthopygus*	Isubra	Russia, Amur	▲ East-Asia	AF423197
10	anonym	10	*Cer. el. xanthopygus*	Isubra	China, Sinkiang	▲ East-Asia	AY244490
11	atlan1	9	*Cer. el. atlanticus*	Red Deer	Norway, Hitra	□ W-Europe	AY070226
12	atlan2	1	*Cer. el. atlanticus*	Red Deer	Norway, Hitra	□ W-Europe	AY070221
13	enclo2	15	*Cer. el. hispanicus*	Spanish Red Deer	Spain, enclosure	□ W-Europe	AY044859
14	france	10	*Cer. el. hippelaphus*	Middle-European Red Deer	France	□ W-Europe	AY244491
15	hispan	6	*Cer. el. hispanicus*	Spanish Red Deer	Spain, La Garganta	□ W-Europe	AF489281
16	kreuth	10	*Cer. el. hippelaphus*	Middle-European Red Deer	Germany, Kreuth	□ W-Europe	AY044858
17	poland	11	*Cer. el. hippelaphus*	Middle-European Red Deer	Poland, Massuria	□ W-Europe	AY044860
18	scotic	1	*Cer. el. scoticus*	Scottish Red Deer	Scotland	□ W-Europe	AB021099[a]
19	swedis	4	*Cer. el. elaphus*	Red Deer	Sweden	□ W-Europe	AY070226
20	ukrain	2	*Cer. el. brauneri*	Krim Red Deer	Ukraine/Krim	□ W-Europe	AY148966
21	boluot	2	*Cer. el. maral*	Maral	Turkey, Bolu	◇ Middle-East	AY118199
22	maral1	3	*Cer. el. maral*	Maral	Iran	◇ Middle-East	AF489280
23	maral2	4	*Cer. el. maral*	Maral	Iran	◇ Middle-East	AF489280
24	canad1	10	*Cer. el. canadensis*	American Wapiti	North America	△ Asia/Amer	AF423198
25	canad2	1	*Cer. el. canadensis*	American Wapiti		△ N-Asia/Amer.	AB021096[a]
26	sibir1	15	*Cer. el. sibericus*	Siberian Wapiti	China, Mongolia	△ N-Asia/Amer.	AY044862
27	sibir2	14	*Cer. el. sibericus*	Siberian Wapiti	China, Mongolia	△ N-Asia/Amer.	AF423199
28	songa1	15	*Cer. el. songaricus*	Tien Shan Wapiti	China, Tien Shan	△ N-Asia/Amer.	AY035871
29	songa2	12	*Cer. el. songaricus*	Tien Shan Wapiti	China, Tien Shan	△ N-Asia/Amer.	AY044856
30	nipcen	1	*Cer. nip. centralis*	Sika Deer		○ Nippon	AB021094[a]
31	nipmag	1	*Cer. nip. mageshima*	Sika Deer		○ Nippon	AB021092[a]
32	nipnip	1	*Cer. nip. nippon*	Sika Deer		○ Nippon	AB021093[a]
33	nipsic	15	*Cer. nip. sichuanicus*	Sika Deer	China, Sichuan	○ Nippon	AY035876
34	nipyes	1	*Cer. nip. yesoensis*	Sika Deer		○ Nippon	AB021095[a]
35	kansu1	10	*Cer. el. kansuensis*	Kansu Red Deer	China, Dong Da Shan	▲S-Asia	AY070223
36	kansu2	1	*Cer. el. kansuensis*	Kansu Red Deer		▲South-Asia	AB021098[a]
37	neill1	13	*Cer. el. macneilli*	M'Neill's Deer	China, Qinghai	▲South-Asia	AY035875
38	neill2	15	*Cer. el. macneilli*	M'Neill's Deer	China, Qinghai	▲South-Asia	AY070223
39	wallic	13	*Cer. el. wallichi*	Shou	China, Tibet	▲South-Asia	AY044861
40	axispo	12	*Axis porcinus*	Hog Deer	India	*Sambar	AY035874
41	timore	14	*Cer. tim. macassanicus*	Rusa Deer	Indonesia, Celebes	*Sambar	AF423200
42	unicol	15	*Cer. uni. cambojensis*	Sambar	China, Yunan	*Sambar	AF423201
43	austri	12	*Cer. el. hippelaphus*	Middle-European Red Deer	Austria	■ Balkan	AY044857
44	bulgar	13	*Cer. el. hippelaphus*	Middle-European Red Deer	Bulgaria	■ Balkan	AF423195
45	enclo3	1	*Cer. el. hippelaphus*	Red Deer	Germany, enclosure	■ Balkan	AF423196
46	hungar	15	*Cer. el. hippelaphus*	Middle-European Red Deer	Hungary	■ Balkan	AF489279
47	istan1	2	*Cer. el. hippelaphus*	Middle-European Red Deer	Turkey, Istanbul	■ Balkan	AY118197
48	montan	1	*Cer. el. montanus*	Eastern Red Deer	Romania	■ Balkan	AY070225
49	yugosl	11	*Cer. el. hippelaphus*	Middle-European Red Deer	Yugoslawia, Delibrat.	■ Balkan	AY070225
50	bactri	2	*Cer. el. bactrianus*	Bactrian Red Deer	Tadzikistan	✦ Tarim	AY142327
51	yarkan	3	*Cer. el. yarkandensis*	Yarkand Red Deer	China, Ürümki	✦Tarim	AY142326
52	damda	1	*Dama dama*	Fallow Deer			AJ000022[a]

W-Europe, Western-Europe; N-Asia/Amer., North-Asia/America; PopID, population ID; *N*, number of individual samples of population.
[a] Sequences obtained from the NCBI data bank with unknown origin.

Sequences were visually inspected and corrected using ALFwin Sequence Analyser version 2.10 (PHARMACIA); sites or segments from which sequences could not be unambiguously scored after three attempts (independent PCR and sequencing reactions) were treated as missing information and excluded from the analysis. Three individual samples of every population were sequenced unless they were not available (Table 1). If these three sequences were equal, the population was treated as one haplotype. If the sequences showed differences, all individual samples (N) of the population were sequenced and the haplotypes found (n) included in the data (Table 1). All in all 125 deer sequences of 43 populations were obtained. In total 44 haplotypes of 50 populations of the genus *cervus* and three outgroup sequences were used for calculation.

2.2. Data analysis

Sequences were aligned using ClustalX version 1.83 (Thompson et al., 1997) and checked visually. Initial sequence comparisons and measures of variability were performed using Mega version 2.1 (Kumar et al., 2001). Transition/transversion ratios and the α-parameter of the γ distribution of rate variation among sites were estimated using TreePuzzle version 5.0 (Schmidt et al., 2002). To determine the appropriate model of sequence evolution and statistically compare successively nested more parameter-rich models for this data set, the program MODELTEST version 3.06 (Posada and Crandall, 1998) was used. The chosen model was applied to the data matrix to produce maximum likelihood (ML) estimates using PAUP* version 4.10b (Swofford, 2003). PAUP* was used to compute consensus trees from ML trees found by heuristic searches (with replicated random sequence addition for each replicate) of 500 bootstrap (Felsenstein, 1985) replicates of the sequences.

Dates of evolutionary separation between different groups of populations were estimated using conventional molecular clock calibrations with the fossil record of *Bos* and *Dama* (Vrba and Schaller, 2000). Population divergence times were estimated using a molecular clock based on net genetic distance values (δ_c) to correct within population variation (Avise, 1994): $\delta_c = \delta_{AB} - 0.5(\delta_A + \delta_B)$, where δ_{AB} is the mean pairwise genetic distance between individuals in populations A and B, and δ_A and δ_B are nucleotide diversities (mean genetic distances between individuals) within the two populations. This procedure assumes that group diversity in the ancestral population was similar to the mean values in extant populations.

Population genetic parameters, such as gene diversity (the probability that two randomly chosen mtDNA sequences are different in the sample (Nei, 1987)) were estimated from the mtDNA data set using ARLEQUIN version 2.0 (Schneider et al., 2000). To estimate the proportion of genetic variation at different hierarchical levels, using information from the geographical distribution of haplotypes and the pairwise distance between them, an analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed. The statistical significance of the estimated F_{ST} values was tested using 100,000 permutations as implemented in ARLEQUIN. We also applied the nested cladistic analysis (NCA) proposed by Templeton et al. (1995) to draw phylogeographic inferences from our mtDNA data set, using GEODIS version 2.0 (Posada et al., 2000) and 10,000 permutations to test the significance of alternative historical scenarios. Accordant cladograms were created with TCS version 1.13 (Clement et al., 2000) with 95% parsimoniously plausible connections between haplotypes (Templeton and Sing, 1993) and nested by hand. Reliability of the correlation of genetic and geographical distances was assessed by a Mantel randomization test as implemented in R-Package version 4.0 (Casgrain, 2001). The geographical distance matrix needed for this test was computed with R-Package from the approximate latitudes and longitudes of the geographical origin of the sampled animals. For interpretation of the GEODIS output, we used the latest Inference Key for the Nested Haplotype Tree Analysis of Geographical Distances (Templeton, 2001).

3. Results

3.1. Characterization of sequence variation

A total of 52 (eight from the NCBI nucleotide data bank; Accession Nos. AB021092–AB021096, AB021098, AB021099, and AJ000022) out of 125 mitochondrial cytochrome *b* sequences (1140 nucleotides each), representing 51 populations, 45 haplotypes and 30 *Cervinae* taxa (according to Geist's (1999) classification), were included in the calculation (Table 1). Sequence identity was observed only among individuals originating from the same or adjacent geographical regions but for all individuals from the same population with one exception only (population of *Cervus elaphus atlanticus* split into two haplotypes atlan1 ($n = 9$) and atlan2 ($n = 1$)). The full-length sequences (Appendix A) were deposited in the NCBI nucleotide data bank (Accession Nos. AF423195–AF423202, AF489279–AF489281, AY035871, AY035874–AY035876, AY044856–AY044863, AY070221–AY070227, AY118197–AY118199, AY142326 and AY142327, AY148966, AY244489–AY244491). Sequences of a cow (*B. taurus*) and a musk deer (*M. moschiferus*), were extracted from the NCBI nucleotide data bank and were used as outgroups (Accession Nos. J01394 and AF026883). A total of 365 (32.0%) sites were variable, of which 235 (20.6%) were parsimony-informative. Within red deer (*C. elaphus*

and *C. canadensis*) there were 144 (12.6%) variable sites, of which 114 (10.0%) were parsimony-informative. The transition/transversion parameter was estimated to be 16.1 with and 18.4 without outgroups. Substitution rates varied among sites, resulting in an overall α-value of 0.20 with and 0.09 without outgroups. Base composition was biased with a deficiency of guanine ($A = 31.6\%$, $C = 26.8\%$, $G = 13.1\%$, $T = 28.5\%$).

3.2. Phylogenetic analysis

MODELTEST (Posada and Crandall, 1998) determined that the GTR model (Rodrígez et al., 1990) with γ-distributed rates (GTR + G) is the statistically appropriate model for the data set with outgroups. The maximum likelihood search found one tree with $\ln L$ of -4900.09461. This tree clearly shows eleven distinct groups of *Cervinae* (Fig. 2). They were designated as Western-Europe (W-Europe), Balkan, Middle-East, Africa, Tarim, North-Asia/America (N-Asia/Amer.), South-Asia (S-Asia), East-Asia (E-Asia), Nippon, Albirostris (Albi), and a Sambar group including the hog deer *A. porcinus* (which is not a Sambar). For red deer only, the HKY85 model (Hasegawa et al., 1985) with γ-distribution rates (HKY + G) was determined as the appropriate model by MODELTEST (Posada and Crandall, 1998). PAUP* produced one tree with $\ln L$ of -2721.14176. The same eight groups of red deer as in the *Cervinae*-tree form three big clusters which were designated as Western Red Deer, Eastern Red Deer, and Tarim (Fig. 3). To verify these results, especially the large gap between eastern (Asia and North America) and western (Africa, Middle-East, and Europe) species, an analysis of molecular variance (AMOVA) was conducted. The AMOVA was performed three times:

(1) Three populations with Western Red Deer (Africa, Middle-East, Balkan, and Western-Europe), Eastern Red Deer (East-Asia, South-Asia, and North-Asia/America), and Tarim. This structure showed a variation of 79.26% among the groups, 17.34% among populations within groups, and 3.40% within populations. The corresponding F_{ST}-value of 0.96602 was significant ($p < 0.05$) by 100,000 permutations. (2) Two populations with Western Red Deer (including the Tarim cluster) and Eastern Red Deer. This analysis revealed a variation of 75.21% among groups, 21.35% among populations within the groups, and 3.22% within populations. The corresponding F_{ST}-value of 0.96778 was significant ($p < 0.05$) by 100,000 permutations. (3) Two populations with Western Red Deer and Tarim incorporated into Eastern Red Deer. This structure showed a variation of 68.40% among groups, 28.14% among populations within groups, and 3.46% within populations. The corresponding F_{ST}-value of 0.96537 was significant ($p < 0.05$) by 100,000 permu-

tations. A structure in accordance with the present classification into the denominated subspecies (Trense, 1989) did not lead to significant results. To compare the eleven groups of deer identified in this study the values for gene diversity, basepair- and pairwise differences are shown in Table 2. Pairwise distances (pd) in this table are calculated with the appropriate model HKY + G (Hasegawa et al., 1985). The mean distances between Eastern and Western Red Deer (0.057 ± 0.006) as estimated by PAUP* ver. 4.10b (Swofford, 2003) under the HKY + G model (Hasegawa et al., 1985) and the distances between each of them and the three sister groups (sika deer (0.060 ± 0.006 to Western and 0.041 ± 0.004 to Eastern), Thorold's white lipped deer (0.060 ± 0.007 to Western and 0.048 ± 0.006 to Eastern), and the Sambar group (0.062 ± 0.007 to Western and 0.055 ± 0.006 to Eastern)) support a classification into two species. The mean distances within the groups are considerably lower (0.014 ± 0.002 for Western Red Deer and 0.013 ± 0.002 for Eastern Red Deer). The same applies to the Sambar group, which consists of three distinct species (0.013 ± 0.002), and to the Nippon group (0.027 ± 0.003), a group rich in subspecies. The two populations of Thorold's white lipped deer from different locations show a mean distance of 0.005 ± 0.002.

3.3. Molecular clock

The measure of nucleotide divergence (d_{xy}) (Saitou and Nei, 1987) based on a GTR + G model (Rodrígez et al., 1990) of nucleotide substitution between cow and deer sequences (mean $d_{xy} = 0.1966 \pm 0.0151$) was used to estimate a molecular clock for deer mtDNA sequences. However, lineage separation in a gene tree normally predate population-level splits (Avise, 2000), so a correction is needed for within-*cervus* nucleotide diversity. To estimate the substitution rate of the analyzed mtDNA, the net genetic distance (δ_{xy}) (Avise, 1994) between the deer sequences was computed ($\delta_{xy} = 0.0539 \pm 0.0017$). The standard equation $\delta_{xy} = 2T\mu$ (where μ is the substitution rate and T is time (Saitou and Nei, 1987)) and a conservative fossil record calibration of 25 million years (MY) for the most recent common ancestor (MRCA) of cervids and bovids (Vrba and Schaller, 2000) produced a substitution rate of approximately 0.39% ($\mu = 0.00393 \pm 0.00031$) per site per million years (PMY). We calculated an estimated age of 6.1–7.6 MY for the coalescence of the ancestors of the recent red deer mtDNA haplotypes. Albirostris split approximately between 5.1 and 6.1 million years ago (MYA) from red deer and Nippon between 2.9 and 3.4 MYA. The separation of *D. dama* from the *Cervus* group at about 12.6–7.6 MYA corresponds to the known fossil records (Vrba and Schaller, 2000).
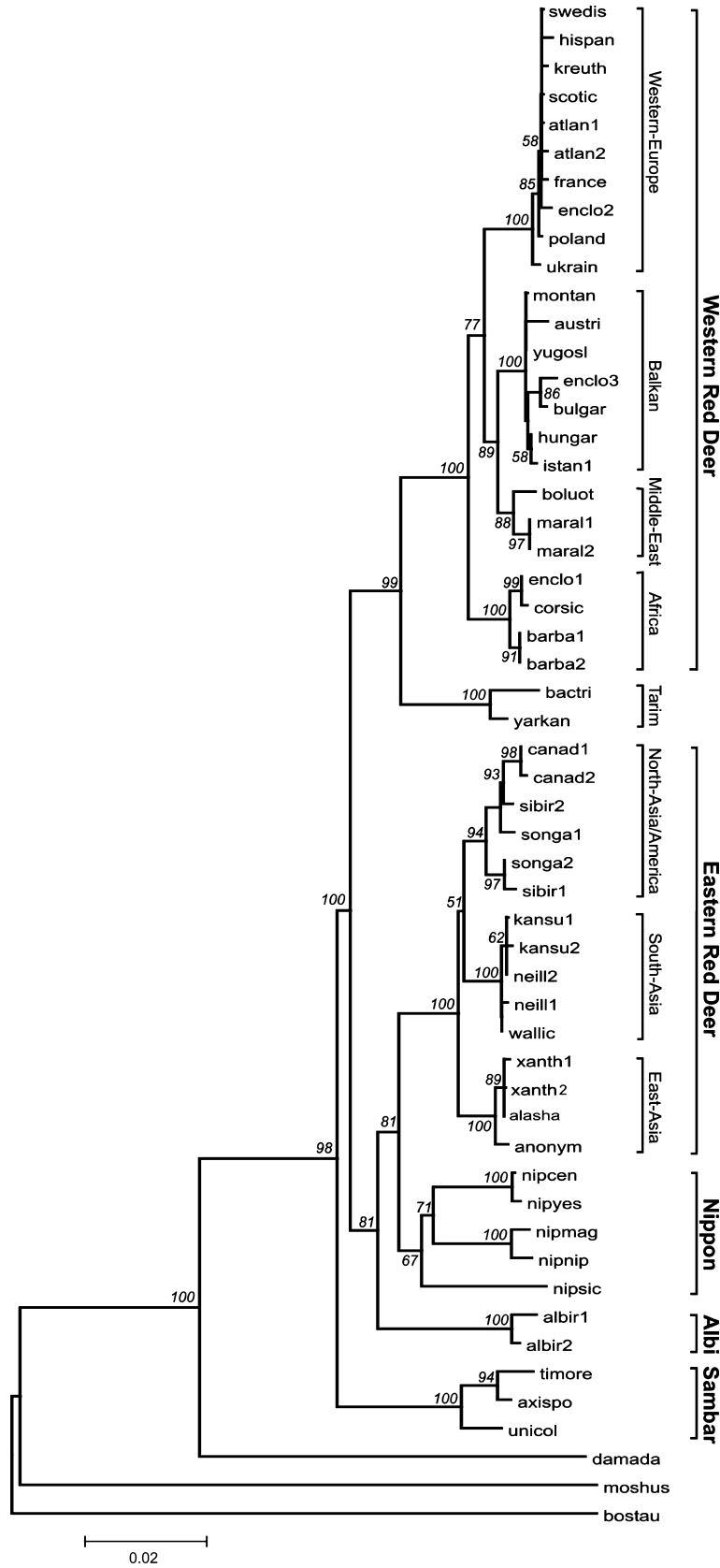
Fig. 2. Phylogeny of *Cervinae* constructed using maximum-likelihood with GTR + G model (Rodrígez et al., 1990) with a γ shape parameter of α = 0.2005 for complete sequences from the cytochrome *b* of mitochondrial DNA. Bootstrap (Felsenstein, 1985) support indices that were upheld in over 50% of the 1000 bootstrap replicates are shown above each branch.
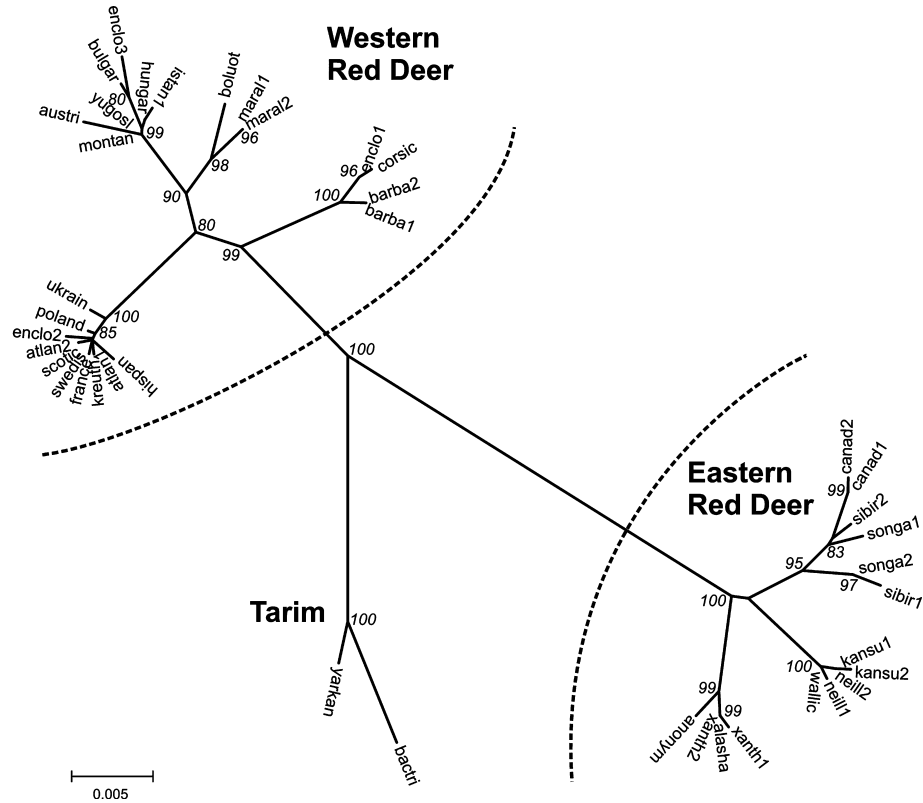
Fig. 3. Phylogeny of red deer constructed using maximum-likelihood with HKY + G model (Hasegawa et al., 1985) with a γ shape parameter of α = 0.0898 for complete sequences from the cytochrome b of mitochondrial DNA. Bootstrap (Felsenstein, 1985) support indices that were upheld in over 80% of the 1000 bootstrap replicates are shown at the inherent nodes.

Table 2
Measures of mitochondrial DNA diversity observed in the 11 groups of red deer identified in this study

Group	N	n	bp differences	Gene diversity	Pairwise difference (%)	Mean pd (%)
Western Red Deer	26	21	91	0.9815 ± 0.0164	0–4.86	1.86 ± 0.22
Western Europe	10	8	10	0.9333 ± 0.0773	0–0.44	0.19 ± 0.07
Balkan/Middle-East	10	8	27	0.9556 ± 0.0594	0–1.60	0.77 ± 0.15
Balkan	7	6	12	0.9524 ± 0.0955	0–0.88	0.38 ± 0.11
Middle-East	3	2	7	0.6667 ± 0.3143	0–0.62	0.41 ± 0.15
Africa	4	3	5	0.8333 ± 0.2224	0–0.35	0.28 ± 0.12
Tarim	2	2	12	1.0000 ± 0.5000	1.06	1.06 ± 0.30
Eastern Red Deer	15	13	43	0.9810 ± 0.0308	0–2.33	1.27 ± 0.20
Noth-Asia/America	6	6	17	1.0000 ± 0.0962	0.09–1.15	0.68 ± 0.17
South-Asia	5	4	3	0.9000 ± 0.1610	0–0.26	0.12 ± 0.08
East-Asia	4	3	5	0.8333 ± 0.2224	0–0.44	0.22 ± 0.09
Nippon	5	5	60	1.0000 ± 0.1265	0.18–4.39	2.77 ± 0.30
Albirostris	2	2	6	1.0000 ± 0.5000	0.53	0.53 ± 0.22
Sambar	3	3	23	1.0000 ± 0.2722	0.79–1.69	1.36 ± 0.27
Total	51	44	217	0.9937 ± 0.0054	0–6.92	5.54 ± 0.34

N is the number of populations, n the number of haplotypes per group, and bp stands for basepairs. Pairwise distances (pd) are estimated using HKY + G model (Hasegawa et al., 1985).

3.4. Nested clade analysis

Enclo2 and enclo3 (Table 1) were not included into the Mantel randomization test with R-Package (Cas-grain, 2001) due to their uncertain origin (samples were obtained from enclosures in Germany). The value for the correlation of the genetic and geographical distance estimated by the Mantel-Test was $r = 0.63$ with
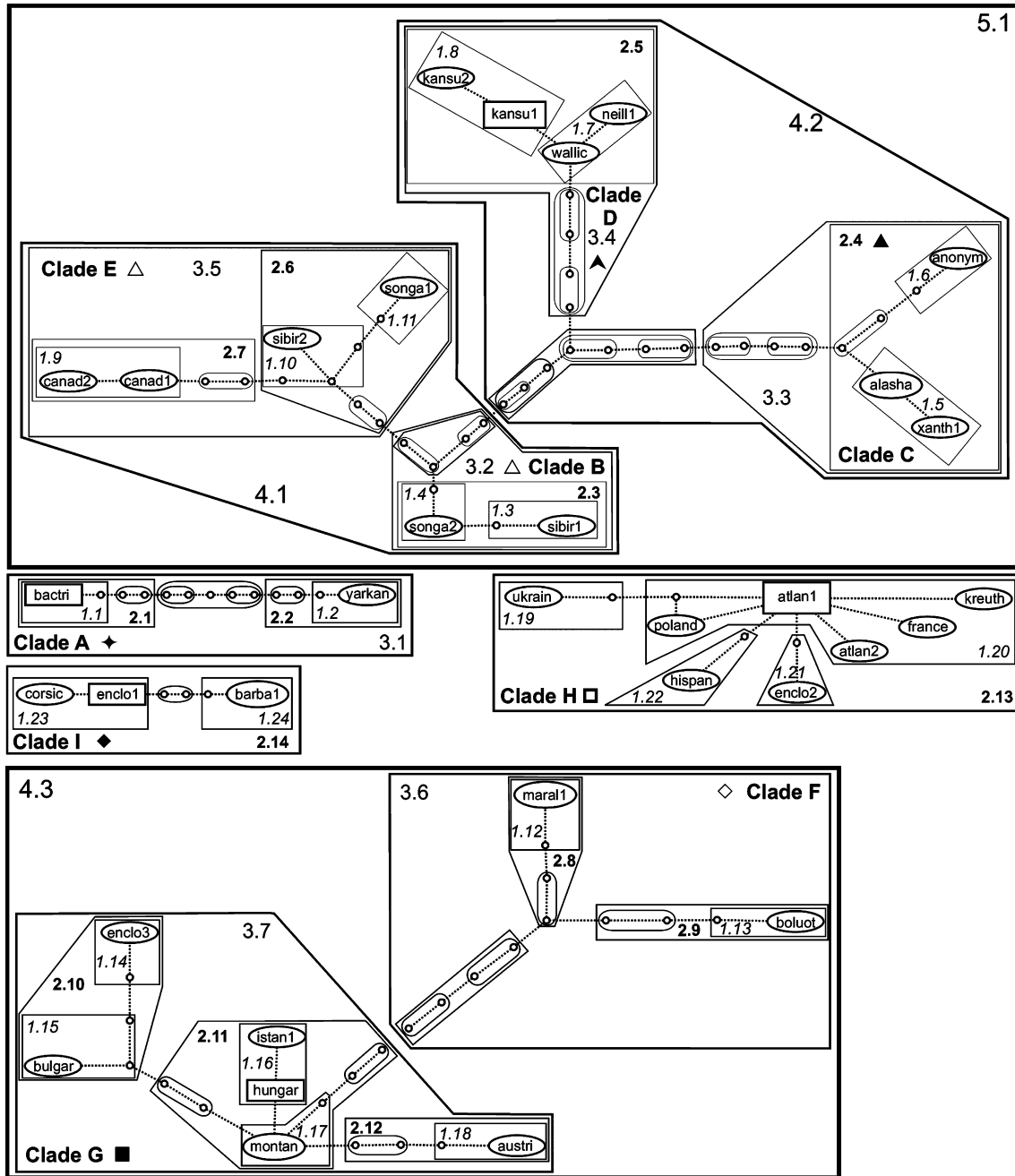
Fig. 4. The nested haplotype networks for red deer clades A–I. Haplotype connections (⩽14 substitutions) are based on a ⩾0.95 probability of being parsimonious. Dotted lines connecting haplotypes represent single substitutions. Circles represent unsampled haplotypes. Hierarchical nesting levels are denoted by boxes and numbered clades. Haplotypes and associated specimens are summarized in Table 1 and geographically depicted by clade-symbol in Fig. 1.

$p \leqslant 0.001$. Supported by this significant correlation, a nested clade analysis was performed (Fig. 4). Based on the 95% connection probability (⩽14 steps) calculated by TCS (Clement et al., 2000), haplotypes of Western Red Deer were subdivided into Western-Europe (H, $n = 10$), Africa (I, $n = 4$) and a third network (South-Europe) consisting of Balkan and Middle-East (FG, $n = 10$). The Eastern Red Deer, on the other hand,

formed a single network (BCDE, $n = 15$). Since the Albi group consists of only two individuals and the Sambar and Nippon groups were subdivided into several networks, these groups were not included into the nested clade analysis. Networks generally correspond with the relationships among well supported clades revealed by maximum parsimony (MP) and ML analysis (Figs. 2 and 3). The obtained cladograms were nested by hand

Table 3
Results of the nested clade analysis

Network	Nested clades	Inclusive clades	D_c	D_n	Inference chain; population inference
A	3.1 (A)	2.1 (Interior)	0	610	1-2-11-17-Inconclusive
		2.2 (Tip)	0	**621[L]**	Past fragmentation?
		I–T	0	**−11[S]**	
BCDE	2.4 (C)	1.5 (Tip)	0	**141[S]**	1-2-11-17-4-No; Restricted Gene Flow
		1.6 (Interior)	0	**431[L]**	with Isolation by Distance
		I–T	0	**290[L]**	
	3.5 (E)	2.6 (Interior)	389	7646	1-2-11-17-4-9-10-Yes; Allopatric
		2.7 (Tip)	0	**7015[S]**	Fragmentation
		I–T	389	**631[L]**	
	4.2 (CD)	2.4 (Tip)	214	1137	1-2-3-4-9-No; Past Fragmentation
		2.5 (Interior)	973	1294	
		I–T	*760[L]*	157	
	5.1 (BCDE)	2.3 (Tip)	569	2694	1-2-3-5-15-16-Yes; Allopatric
		2.4 (Interior)	**214[S]**	3196	Fragmentation
		2.5 (Interior)	2265	2873	
		2.6 (Tip)	389	2289	
		2.7 (Tip)	**0[S]**	**9641[L]**	
		I–T	1125	−1872	
FG	3.6 (F)	2.8 (Interior)	**0[S]**	259	1-2-11-12-No; Contiguous Range
		2.9 (Tip)	0	510	Expansion
		I–T	**0[S]**	−251	
	3.7 (G)	2.10 (Tip)	**626[L]**	630	1-2-11-12-No; Contiguous Range
		2.11 (Interior)	418	420	Expansion
		2.12 (Tip)	0	438	
		I–T	1	−145	
	4.3 (FG)	3.6 (Tip)	342	**1778[L]**	1-2-11-12-No; Contiguous Range
		3.7 (Interior)	**483[S]**	**763[S]**	Expansion
		I–T	141	**−1015[S]**	
H	2.13 (H)	1.19 (Interior)	0	**2015[L]**	1-2-11-12-No; Contiguous Range
		1.20 (Interior)	**802[S]**	**891[S]**	Expansion
		1.21 (Tip)	0	1655	
		1.22 (Tip)	0	1888	
		I–T	**701[S]**	−740	

Clades within each nested clade and their associated interior or tip designation are listed. Values for clade distance (D_c) and nested clade distance (D_n) in kilometers are included. Interior versus tip contrasts (I–T) are reported. Significantly large ([L]) or small ([S]) values of D_c or D_n are shown in bold. The inference chain and population inference follow from the inference key of Templeton (2001).

and the received clades were used to create an input file for GEODIS (Posada et al., 2000) with the corresponding locations of the populations as longitudes and latitudes. The ancient haplotypes derived from the MP tree were chosen as interior populations for the corresponding clade (Posada et al., 2000). Clade I was excluded from the analysis due to its small sample size. The Inference Key for the Nested Haplotype Tree Analysis (Templeton, 2001) was used to identify population-level processes; the results are summarized in Table 3. The key shows an allopatric fragmentation for the Eastern Red Deer (Clade 5.1) as well as for the N-Asia/America branch (Clade 3.5), whereas it presumes restricted gene flow with isolation by distance for East-Asia (Clade 2.4). Between East-Asia and South-Asia (Clade 4.2) past fragmentation was determined. In the case of the Western Red Deer (FG, H, and I) the outcome for all networks appeared to be the result of contiguous range expansion. Based on the results of the NCA, the data of the molecular clock as well as fossil and geographic data (Agustí and Antón, 2002), the approximate colonization routes of red deer were retraced and are shown in Fig. 5.

4. Discussion

4.1. Species or not species?

The phylogenetic trees obtained from the sequence data from the cytochrome *b* gene of mtDNA, support the classification of Western Red Deer and Eastern Red Deer as individual species. The integration of sika deer and Thorold's white lipped deer as sister taxa in the phylogenetic analysis clearly identify Western and Eastern Red Deer as two monophyletic groups (Fig. 2). The fallow deer (*D. dama*) showed a distance between 0.111 and 0.126 to the examined groups. This confirms the state of *Dama* as a distinct genus as formerly described (Douzery and Randi, 1997; Randi et al., 2001). Recent studies of the control region of *Cervidae* (Polziehn and Strobeck, 1998, 2002; Randi et al., 2001;
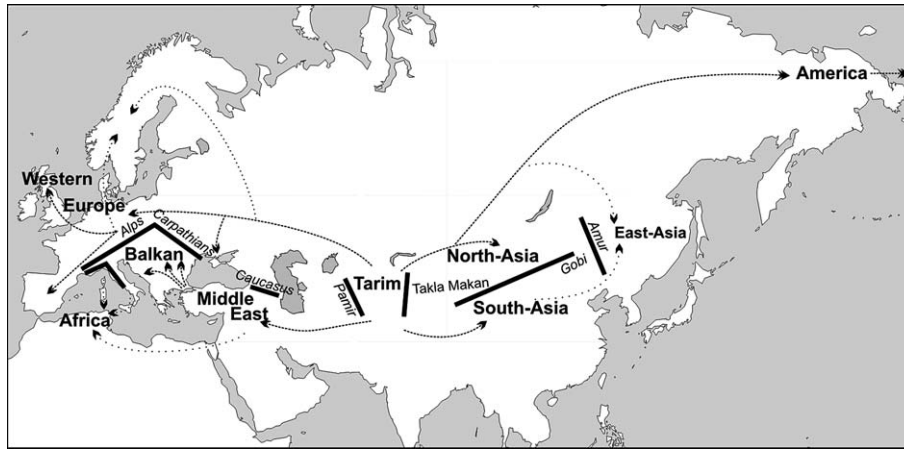
Fig. 5. Colonization routes of red deer in Asia and Europe based on cytochrome *b* sequences. Clade-names (bold) are the same as in Fig. 2. Geographic (genetic) barriers (italic) are marked by bars and named with geographic realities of today. Dashed arrows show definite routes of colonization and dotted arrows show possible ones.

Mahmut et al., 2002) yielded similar results and confirm our assumption that Western and Eastern Red Deer are two distinct species (Polziehn and Strobeck, 2002). Whereas Randi assigned *A. porcinus* to the genus *Axis* based on mtDNA data of the control region (Randi et al., 2001), our data assign it to the genus *Cervus*. This confirms Geist's postulation (1999) that hog deer (*A. porcinus*) may not be as closely related to *Axis axis* as hitherto assumed because of the antler structure with a preorbital gland that, as in sambars, is everted during agonistic or sexual arousal. Additionally, unlike chital (*A. axis*), hog deer have no rutting call. Apart from this, Randi's and our data match very well.

Therefore we do not support the current classification of red deer into one superspecies (Geist, 1999) nor its subdivision into numerous subspecies (Trense, 1989). The AMOVA supports the division into four Western and three Eastern groups with the Tarim group being at the basis, as it shows the highest and most significant *F*-values for this classification.

Based on the results of this study, Western Red Deer can be subdivided into four subgroups: Western-Europe, Balkan, Middle-East, and Africa. Former studies based on coloration and morphology (Groves and Grubb, 1987) yielded similar results. The barrier between the Balkan and the Middle-East group is clearly defined by the Bosphorus in Turkey. The Alps and Carpathians seem to form the barrier between Western Europe and the Balkan. Our data show that the red deer of Western Europe cannot be subdivided into several subspecies. The subspecies *C. atlanticus*, *C. brauneri*, *C. elaphus*, *C. hippelaphus*, *C. hispanicus*, and *C. scoticus* in Western Europe as well as *C. hippelaphus* and *C. montanus* in the Balkan group could not be differentiated by the mtDNA cytochrome *b* sequence data (Figs. 3 and 4). On the other hand, the subspecies *C. hippelaphus* is assigned to both Western Europe and

the Balkan group by morphological classification (Table 1). This is inconsistent with the large genetic gap between these two groups. Subspecies status of the African barbary deer, the maral and the two subspecies of the Tarim group (*C. bactrianus* and *C. yarkandensis*) can be confirmed (Table 4).

The three subgroups of the Eastern group (North Asia/America, South-Asia, and East-Asia) are clearly defined. The existence of further subspecies is unlikely according to our data. Thus, the current classification into subspecies has to be reconceived thoroughly. This can be seen in the N-Asia/Amer. group with *Cervus el. songaricus* and *sibericus* as well as in the S-Asia group with *Cervus el. kansuensis* and *macneilli* which we were unable to differentiate (Figs. 3 and 4). The barriers for speciation are more difficult to identify than those in the Western Group. In light of the fact that Siberian and American wapitis are more or less identical, the isolated position of the Isubra (*Cervus elaphus xanthopygus*) inhabiting the Amur mountains is not easy to understand. The South Asian group is isolated by the Takla Makan- and the Gobi-Deserts and evolved sympatrically with Thorold's white lipped deer, although their area of circulation is separated by altitude. The primordial Tarim group stands at the base of the red deer and is geographically isolated by the Takla Makan Desert to the East and the Pamir Mountains to the West. Genetically it is more related to the Western group, whereas its habitat is close to the N-Asia/Amer. group.

Sika deer and Thorold's deer form one group with Eastern Red Deer. The branching pattern of the subspecies of *C. nippon* complies with that described for the control region (Kuwayama and Ozawa, 2000), the mainland subspecies *C. n. sichuanicus* standing at the base of the entire sika group. The high divergence among the sika deer probably results from recent drift due to the island distribution of the populations, which

Table 4
Twenty-two currently known subspecies of red deer (Geist, 1999) in comparison to 10 haplotype-lineages found in this study

Nr.	Subspecies name	Common name	Geographical origin	Haplotype name
1	*Cer. el. barbarus*	Barbary Red Deer	Tunisia	*Cer. el. barbarus*
2	*Cer. el. corsicanus*	Sardinian Deer	Sardinia	*Cer. el. barbarus*
3	*Cer. el. hippelaphus*	Middle-European Red Deer	Bulgaria	*Cer. el. hippelaphus*
4	*Cer. el. montanus*	Eastern Red Deer	Romania	*Cer. el. hippelaphus*
5	*Cer. el. alashanicus*	Alashan Red Deer	China	*Cer. can. xanthopygus*
6	*Cer. el. xanthopygus*	Isubra	Russia	*Cer. can. xanthopygus*
7	*Cer. el. maral*	Maral	Iran	*Cer. el. maral*
8	*Cer. can. nelsoni*	Rocky Mountain Wapiti	North America	*Cer. canadensis*
9	*Cer. canadensis*	American Wapiti	North America	*Cer. canadensis*
10	*Cer. el. sibericus*	Siberian Wapiti	China, Mongolia	*Cer. can. sibericus*
11	*Cer. el. songaricus*	Tien Shan Wapiti	China, Tien Shan	*Cer. can. sibericus*
12	*Cer. el. Hanglu*	Kashmir Red Deer	India	ns
13	*Cer. el. kansuensis*	Kansu Red Deer	China, Dong Da Shan	*Cer. can. kansuensis*
14	*Cer. el. macneilli*	M'Neill's Deer	China, Qinghai	*Cer. can. kansuensis*
15	*Cer. el. wallichi*	Shou	China, Tibet	*Cer. can. kansuensis*
16	*Cer. el. bactrianus*	Bactrian Red Deer	Tadzikistan	*Cer. el. bactrianus*
17	*Cer. el. yarkandensis*	Yarkand Red Deer	China	*Cer. el. yarkandensis*
18	*Cer. el. atlanticus*	Red Deer	Norway	*Cer. el. elaphus*
19	*Cer. el. brauneri*	Krim Red Deer	Ukraine/Krim	*Cer. el. elaphus*
20	*Cer. el. elaphus*	Red Deer	Sweden	*Cer. el. elaphus*
21	*Cer. el. hispanicus*	Spanish Red Deer	Spain	*Cer. el. elaphus*
22	*Cer. el. scoticus*	Scottish Red Deer	Scotland	*Cer. el. elaphus*

Note. ns, not sampled.
Nomenclature of haplotype-lineages follows most common subspecies names.

was also verified by microsatellite and mtDNA data (Cook et al., 1999; Goodman et al., 2001). Their genetic proximity to Eastern Red Deer may point to hybridization as described in recent studies (Goodman et al., 1999; Willard et al., 1998). However, our phylogenetic tree lacks a mosaic form, which would be a hint to hybridization in mtDNA (Kuwayama and Ozawa, 2000). In addition, there are no hybridization zones with the Japanese subspecies. Therefore, it is likely that Eastern Red Deer and Sika deer shared a common ancestor which split off from Western Red Deer.

4.2. Time of divergence

The calculated time of divergence between Eastern and Western Red Deer of about 7 million years confirm the data of Randi (2001) for the mtDNA control region. In contrast to our and Randi's study, Polziehn and Strobeck (2002) as well as Kuwayama and Ozawa (2000) calculated a divergence time of less than one million years for red deer, wapiti, sika deer and Thorold's white lipped deer based on mtDNA cytochrome *b* data. The calibration point of 1.6 MY for the last common ancestor of red deer and fallow deer, which they had taken from Kurtén (1968), is incorrect. Kurtén described only the first appearance of fallow deer in the Pleistocene, but not their very first appearance. If Polziehn and Kuwayama had used newer fossil records like Vrba and Schaller (2000) or Agustí and Antón (2002), their unusually high substitution

rate of 3.5%/MY for the cytochrome *b* (Randi reported a substitution rate of 1.11–1.13%/MY for the highly variable control region) would decrease to 0.38%/MY; a result similar to ours.

4.3. Reasons for speciation

Extensive changes took place in the early and middle Miocene. When the African and Indian continental plates crashed into the Eurasian continent about 40 MYA, the orogenesis of the Himalayas and the Alps was initiated. The first cervoids appeared at the changeover from the Oligocene to the Miocene about 25 MYA in the region of today's Hindukush (Agustí and Antón, 2002). In those days they were archaic artiodactyls of medium size (like moshoids today). Their ancestors were Oligocene survivors such as *Dremotherium* and *Bedenomeryx*, which experienced a quick diversification into a number of genera like *Cetinensis* with its hypsodont check-teeth. About 15 MYA a high-latitude cooling event began (Flower and Kennet, 1994; Miller et al., 1991), causing a sea-level decline (Haq et al., 1987) by growth of the Eastern Antarctic ice sheets. This is the reason why the ancient sea Tethys in the western part of Eurasia dried and became vast grassland. The first ancestors of the genus *Dama* appeared (Agustí and Antón, 2002), which corresponds with our data of the molecular clock. Then the Arabian plate stopped the circulation of warm deep-water between the Mediterranean and the

Indo-Pacific (Axelrod and Raven, 1978). This causes a climatic trend to cooler winters and decreased summer rainfall (Axelrod, 1975), followed by a spread of grasses over large stretches of Europe and Asia between 8 and 7 million years ago (Cerling et al., 1997), enabled large and fast moving grazers such as cervids to radiate. In accordance with our data, this is the timeframe when the ancestors of today's red deer separated. After the split, they were isolated by distance. In regions of contingency the contact was interrupted by several Ice Ages with huge glaciers as well as by insurmountable barriers, such as mountains, deserts, and the sea. Therefore the populations were able to establish their species status. At the Pliocene/Miocene boundary of about 5 MYA, another sea-level fall started, which was related to glaciation. This led to the diversification of deer during early Pliocene and the first appearance of the genus *Cervus* (*C. perrieri, C. cusanus*). At the beginning of the Pleistocene of about 2 MYA, the ancestors of modern species started their speciation assisted by recurring glaciations.

4.4. Ways of differentiation

The nested clade analysis showed different models for the separation of the denominated groups. Allopatric or past fragmentation for the Eastern group is easy to understand by the geographical barriers such as deserts and high mountains, which came into being in the according timeframes (Fig. 5). In the case of the American wapiti and its close relationship to the North Asian red deer we have to take into account that the barrier, in the form of the Bering Sea, only appeared 9000 years ago. This timeframe can be neglected with mtDNA data. It seems that exchange took place between the American and North Asian wapitis until the Bering Land Bridge disappeared. The colonization route of the Isubra (*C. el. xanthopygus*) and the Ala Shan Red Deer (*Cervus. el. alashanicus*) and their isolated position is hard to explain. The nested clade analysis supports the colonization from the South (Fig. 4 and Table 3), whereas it is undeterminable whether South Asian populations are descendants of former East Asian populations or vice versa.

Due to the geographical distribution of the subgroups within the Western Red Deer, contiguous range expansion for the separate groups seems likely. Contiguous range expansion between the Balkan and the Middle-East is also possible due to the former land bridge across the Bosphorus. In contrast, the relatively large gap between Southern Europe and Western Europe is difficult to explain, because the Danube watersides should, for example, offer ways of exchange. On the other hand, the Alps and Carpathians form quite immense barriers. Therefore, the European populations should be investigated more thoroughly. Since the

African and Sardinian red deer are geographically isolated, they were probably subjected to recent gene drift, which provides an explanation for their high differentiation from the other subgroups. Thus, an overestimation of the time of divergence (~2.2 MYA) is possible. Due to the existence of a temporary Pleistocene land bridge between Europe and Africa (Agustí and Antón, 2002), it is possible that the ancestors of the Africa group moved to their current habitats from the north. Colonization from the East with a former habitat comprising the whole of Northern Africa is possible as well (Fig. 5). This would mean that today's African and Sardinian deer represent a relict endemic group of this former African population, which was depleted by the appearance of the Sahara desert.

5. Conclusion

Sequence deviation and the difference in the mtDNA cytochrome *b* gene in the various groups of the genus *Cervus*, appears to be informative and may be used as a marker to describe species boundaries, to appoint subspecies and to find the geographical origin of unidentified samples. Due to our results the present classification of red deer into a large number of subspecies has to be reconceived. This study shows a very high probability for the existence of two different species of red deer with three subspecies in Asia and America (Eastern Red Deer) and four subspecies in Eurasia (Western Red Deer) and additional one or two primordial subspecies in Central Asia (Tarim group). The origin of the genus *Cervus* seems to be in Central Asia near today's Hindukush.

Appendix A

Mitochondrial DNA cytochrome *b* haplotypes identified in this study. Nucleotide positions showing variation among haplotypes are depicted; numbers (vertical) refer to the aligned site in our 1140 bp data set.

```
Population    Nucleotide positions
                                              1          1111111111  1111111111  1111122222  2
              1111          1222333455   6666778890   0001111222  2333456666  7778900011  1
              4567890128    9178069847   0367581792   5681457016  9568762589  1499814706  9

bostau        ACTAACATTG    TCACAATTCC   CTAGACTACT   CCCGAACCCC  ACCCAACCAT  CCCCTCTCCC  G
albir1        .TCC.AT.CA    A..T...CTT   .C...T.C.C   .TA....T..  .T.....T.C  TTT.C...T.  C
albir2        ..C..T..CA    A..T...CTT   .C...T.C.C   .TA....T..  .T.....T..  TTT.C...T.  C
anonym        .TC..ATACA    A..T...CTT   .C..GT.C.C   ..AA..TT.T  .T.....T..  .TTTC...TT  C
atlan1        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
atlan2        ..C.....CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
austri        ..CC.T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
axispo        ..C..T..CA    AT.T.G.C.T   .C...T.C.C   .TAA..TT..  ..A.G..T..  .TTT.....T  C
bactri        TAC..T..CA    A......CTT   .C...T.C.C   ..A..GTT..  .......T..  TTTTC...TT  C
barba1        ..C..T..CA    A......CTT   .C...T.C.C   A.AA.G.T..  .....G.T..  TTT.C...TT  C
barba2        ..C..T..CA    A......CTT   .C...T.C.C   A.AA.G.T..  .....G.T..  TTT.C...TT  C
boluot        ..C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
bulgar        ..C..AT.CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
canad1        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  .TTTC...TT  C
canad2        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  .TTTC...TT  C
corsic        ..C..T..CA    A..G...CTT   .C...T.C.C   A.AA.G.T..  .....G.T..  TTT.C...TT  C
damada        .TC..T..CA    AT.TG.CCTT   TC...T.CTC   ..AA..T.T.  ..........  TTTTC.....  C
enclo1        ..C..T..CA    A..G...CTT   .C...T.C.C   A.AA.G.T..  .....G.T..  TTT.C...TT  C
enclo2        ..C..T..CA    A......CTT   .C.A.T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
enclo3        ..C.TATACA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
france        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
hispan        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
hungar        ..C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
istan1        T.C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
kansu1        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  ATTTC...TT  T
kansu2        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  ATTTC...TT  T
kreuth        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
maral1        ..C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
maral2        ..C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
montan        ..C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
moshus        ..C..T..CA    ...T......   .C....CC.C   ..AAC.....  T..A..T.C.  T.TT.TCT..  T
neill1        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  ATTTC...TT  T
neill2        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  ATTTC...TT  T
nipcen        ..C..T..CA    A..T...C.T   .CC..T.C.C   ..AA..TT..  ...T...T..  T.TTC...TT  C
nipmag        ..C.....CA    A..T...C.T   .CC..T.C.C   .TAA..TT..  .......T..  ..T.C...TT  C
nipnip        ..C.....CA    A..T...C.T   .CC..T.C.C   .TAA..TT..  .......T..  ..T.C...TT  C
nipsic        .TC..T..CA    A.GT...CTT   .CC..T.C.C   .TAA..TT..  .T.....T..  T.TTC....T  C
nipyes        .....T..CA    A..T...C.T   .CC..T.C.C   ..AA..TT..  ...T...T..  T.TTC...TT  C
poland        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
scotic        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
sibir1        .TC..T.CCA    A..T....TT   .C..GT.C.C   .TAA..TT..  .T.....T..  .TTTC...TT  C
sibir2        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  .TTTC...TT  C
songa1        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  .TTTC...TT  C
songa2        .TC..T.CCA    A..T....TT   .C..GT.C.C   .TAA..TT..  .T.....T..  .TTTC...TT  C
swedis        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
timore        ..C..T..CA    A..T.G.C.T   .C...T.C.C   .TAA..TT..  ..A.G..T..  .TTT.....T  C
ukrain        ..C..T..CA    A......CTT   .C...T.C.C   ATAA.G.T..  .....G.T..  TTT.C...TT  C
unicol        ..C..T..CA    A..T.G.C.T   .C...T.C.C   .TAA..TT..  ..A....T..  .TTT.....T  C
wallic        .TC..T.CCA    A..T...CTT   .C..GT.C.C   .TAA..TT..  .T.....T..  ATTTC...TT  T
xanth1        .TC..T.CCA    A..T...CTT   .C..GT.C.C   ..AA..TT.T  .T.....T..  .TTTC...TT  C
alasha        .TC..T.CCA    A..T...CTT   .C..GT.C.C   ..AA..TT.T  .T.....T..  .TTTC...TT  C
xanth2        .TC..T.CCA    A..T...CTT   .C..GT.C.C   ..AA..TT.T  .T.....T..  .TTTC...TT  C
yarkan        TAC..T..CA    A......CTT   .C...T.C.C   ..AA..TT..  .......T..  TTTTC...TT  C
yugosl        ..C..T..CA    A.G....CTT   .C...T.C.C   ATAA...T..  .....G.T..  TTT.C...TT  C
```

```
Population   Nucleotide Positions

             2222222222  2222222222  2223333333  3333333333  3333333333  3333444444  4
             2223344455  6677788888  9990000112  2223334455  5566666788  9999001333  4
             2584736958  1703902458  1473469581  4783692512  4701349545  0367257258  0

bostau       CCCCCCACCA  TGTTCTAATG  CAACTATGTC  TTCAAATTAA  CTGCCAACTA  ACCCAAAACC  C
albir1       TTTTTT...G  AA.C.C.TCA  T...CGCAAT  ...G..CC..  .CATTGT.CG  .T..GG...T  .
albir2       TTTTTT...G  AA.C.C.TCA  T...CGCAAT  ...G..CC..  .CATTGT.CG  .T..GG...T  .
anonym       TTTTT....G  AA.C.C.T.A  T...C.C.AT  ...G..C...  .CATT.T.CG  .T.....G.T  .
atlan1       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
atlan2       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
austri       TT.TTT...G  AA.C.C.TCA  T.G.CGCAAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
axispo       TT..TT...G  AA.CTC.T.A  ....CGCAA.  ...G..CC..  .CATT.T.CG  .T.......T  .
bactri       ...TTT..TG  AA.CTC.TCA  T...CGCAAT  ...G..CC..  T.ATT.T.CG  GT.......T  .
barba1       TT.T.....G  AA.CTCGT.A  T...CGCAAT  ...GG..C.G  T.ATT.T.CG  GT.......T  .
barba2       TT.T.....G  AA.CTCGT.A  T...CGCAAT  ...GG..C.G  T.ATT.T.CG  GT.......T  .
boluot       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...G..CC.G  T.ATT.T.CG  GT......TT  .
bulgar       TT.TTT...G  AA.C.C.TCA  T.G.CGCAAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
canad1       TTTTT....G  AA.C.C.TCA  T...C.C.A.  ...G..CC..  .CATT.T.CG  .T.......T  .
canad2       TTTTT....G  AA.C.C.TCA  T...C.C.A.  ...G..CC..  .CATT.T.CG  .T.......T  .
corsic       TT.TT....G  AA.CTCGT.A  T...CGCAAT  ...GG..C.G  T.ATT.T.CG  GT.......T  .
damada       T.T.T.G...  AAC..C.TCC  TT.TC.CAA.  ...G.GCC..  TCATT.TTCG  C.......TT  .
enclo1       TT.TT....G  AA.CTCGT.A  T...CGCAAT  ...GG..C.G  T.ATT.T.CG  GT.......T  .
enclo2       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...G..CC.G  T.ATT.T.CG  .T........  .
enclo3       TT.TTT...G  AA.C.C.TCA  T.G.CG.AAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
france       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
hispan       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
hungar       TT.TTT...G  AA.C.C.TCA  T.G.CG.AAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
istan1       TT.TTT...G  AA.C.C.TCA  T.G.CG.AAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
kansu1       TTTTT....G  AA.C.C.T.A  T...C.C.AT  ..AG..CC..  .CATT.T.CG  .T.......T  .
kansu2       TTTTT....G  AA.C.C.T.A  T...C.C.AT  ..AG..CC..  .CATT.T.CG  .T.......T  .
kreuth       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T.T......  .
maral1       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...G..CC.G  T.ATT.C.CG  GT.......T  .
maral2       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...G..CC.G  T.ATT.C.CG  GT.......T  .
montan       TT.TTT...G  AA.C.C.TCA  T.G.CGCAAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
moshus       .T.TT..TT.  AAC..CGT.A  T.....CAA.  AC....CCC.  .CAT....C.  .T..T.T...  .
neill1       TTTTT....G  AA.C.C.T.A  T...C.C.AT  ..AG..CC..  .CATT.T.CG  .T.......T  .
neill2       TTTTT....G  AA.C.C.T.A  T...C.C.AT  ..AG..CC..  .CATT.T.CG  .T.......T  .
nipcen       .TTT.....G  AA.C.C.TCA  T...CGCAAT  ...G..CC..  TCATT.T.CG  .T.......T  .
nipmag       TTTTT....G  AA.C.C.TCA  ...TCGCAAT  ...G..CC..  TCATT.C.CG  ..........  .
nipnip       TTTTT.....  AA.C.C.TCA  ...TCGCAAT  ...G..CC..  TCATT.C.CG  .T.......T  .
nipsic       TTTTT....G  AACC.C.TCA  T...CGCAAT  ...G..CC..  .CATT.T.CG  .T........  .
nipyes       .TTT.....G  AA.C.C.TCA  T...CGCAAT  ...G..CC..  TCATT.T.CG  .T.......T  .
poland       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
scotic       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
sibir1       TTTTT....G  AA.C.C.T.A  T...C.C.A.  ...G..CC..  .CATT.T.CG  .T.......T  .
sibir2       TTTTT....G  AA.C.C.TCA  T...C.C.A.  ...G..CC..  .CATT.T.CG  .T.......T  .
songa1       TTTTT....G  AA.C.C.TCA  T...C.C.A.  ...G..CC..  .CATT.T.CG  .T.......T  .
songa2       TTTTT....G  AA.C.C.T.A  T...C.C.A.  ...G..CC..  .CATT.T.CG  .T.......T  .
swedis       TT.TTT...G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
timore       TT..TT...G  AA.CTC.T.A  ....CGCAA.  ...G..CC..  .CATT.T.CG  GT.......T  .
ukrain       TT.TT....G  AA.CTC.TCA  T.G.CGCAAT  ...GG.CC.G  T.ATT.T.CG  .T........  .
unicol       TT..TT...G  AA.CTC.T.A  ....CGCAA.  C..G..CC..  .CATT.T.CG  GTT......T  A
wallic       TTTTT....G  AA.C.C.T.A  T...C.C.AT  ..AG..CC..  .CATT.T.CG  .T.......T  .
xanth1       TTTTT....G  AA...C.T.A  T...C.C.AT  ...G..C...  .CATT.T.CG  .T.....G.T  .
alasha       TTTTT....G  AA...C.T.A  T...C.C.AT  ...G..C...  .CATT.T.CG  .T.....G.T  .
xanth2       TTTTT....G  AA...C.T.A  T...C.C.AT  ...G..C...  .CATT.T.CG  .T.....G.T  .
yarkan       ...TTT..TG  AA.CTC.TCA  T...CGCAAT  ...G..CC..  T.ATT.T.CG  GT.......T  .
yugosl       TT.TTT...G  AA.C.C.TCA  T.G.CGCAAT  ...GG.CC.G  ..ATT.T.CG  GT.......T  .
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Population	Nucleotide positions 4444444444 4444555666 1478039258	4444444444 7777788889 1457803690	4455555555 9900112222 2814392358	5555555555 3334445566 1470362517	5555555555 6677778888 8904692589	5556666666 9990001113 4570362583	6 3 4
bostau	CCCTAACACC	CAATTACAAA	CCACCACCTC	ACCTCTCTTC	ATAATCACCC	ATCCCACCCT	T
albir1	..TCC.T.TT	...CC.....	.GCTT...G.	GT...CTCC.	GC.C...A.T	C..T.GA.TC	C
albir2	..TCC.T.TT	...CC.....	.GCTT...G.	GT...CTCC.	GC.C...A.T	C..T.GA.TC	C
anonym	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T.GA.T.	C
atlan1	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
atlan2	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
austri	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	CC.T..ATT.	C
axispo	..TCC.T.TT	T..CC.....	.ACTT...A.	.TT..C.C..	GCGCCT.A.T	C..T.GA.TC	C
bactri	..TCCGT.TT	T..CC.....	.GCTT...A.	.T...CTC..	GC.CC..A..	C....GATTC	C
barba1	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T.GATT.	C
barba2	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T.GATT.	C
boluot	..TCC.T.TT	A..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
bulgar	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T.GATT.	C
canad1	..TCC.T..T	...CC...G.	.ACTT...A.	.....CTC..	GC.CCT.A.T	C..T.GATTC	C
canad2	..TCC.T..T	...CC...GG	.ACTT...A.	.....CTC..	GC.CCT.A.T	C..T.GATTC	C
corsic	..TCC.T.TT	G..CC...G.	.GCT....A.	.T.C.CTC..	GC.CCT.A.T	C..T..ATT.	C
damada	.TTCC....T	T..CC.T...	.ACT...TAT	.....CTACT	GCGC.T.ATT	C.TT.GA.TC	C
enclo1	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
enclo2	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
enclo3	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T.GATT.	C
france	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
hispan	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..GTT.	C
hungar	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
istan1	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
kansu1	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T..A.TC	C
kansu2	..TCC.T..T	...CC.....	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T..A.TC	C
kreuth	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
maral1	..TCC.T.TT	A..CC.....	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
maral2	..TCC.T.TT	A..CC.....	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
montan	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
moshus	T.TCC.TC.T	TT.CCGTG..	TAC...A.C.	..TCTC.C.T	GC.CC.GTT.	C.T.T.A.TC	A
neill1	..TCC.T..T	...CC...G.	.ACTTC..A.	.T...CTC..	GC.CCT.A.T	C..T..A.TC	C
neill2	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T..A.TC	C
nipcen	...CC.T.TT	...CC...G.	.GC.T...A.	.T...CT...	GC.C.T.A.T	C..T.GA..C	C
nipmag	..TCC.T.TT	...CC...G.	.ACTT...A.	.T.C.CT...	.C.CCT.A.T	C..T.GA..C	C
nipnip	...CC.T..T	...CC...G.	.ACTT...A.	.T.C.CT...	.C.CCT.A.T	C..T.GA..C	C
nipsic	...CC.T..T	...CC.....	.GCTT...G.	.T...CT.C.	GC.CCT.A.T	C..T.GA.TC	C
nipyes	...CC.T.TT	...CC...G.	.GC.T...A.	.T...CT...	GC.C.T.A.T	C..T.GA..C	C
poland	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
scotic	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
sibir1	..TCC.T..T	...CC...G.	.AC.T...A.	.....CTC..	GC.CCT.A.T	C..T.GATTC	C
sibir2	..TCC.T..T	...CC...G.	.ACTT...A.	.....CTC..	GC.CCT.A.T	C..T.GATTC	C
songa1	..TCC.T..T	...CC...G.	.ACTT...A.	.....CTC..	GC.CCT.A.T	C..T.GATT.	C
songa2	..TCC.T..T	...CC...G.	.AC.T...A.	.....CTC..	GC.CCT.A.T	C..T.GATTC	C
swedis	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C
timore	.ATCC.T.TT	T..CC.....	.ACTTC..A.	..T..C.C..	GCGCCT.A.T	C..T.GA.TC	C
ukrain	..TCC.T.TT	G..CC...G.	.ACT....A.	.T...CTC..	GC.CCT.A.T	C..T.GATT.	C
unicol	..TCC.T.TT	T..CC.....	.ACTTC..A.	.TT..CTC..	GCGCCT.ATT	C..T.GA.TC	C
wallic	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T..A.TC	C
xanth1	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T.GA.T.	C
alasha	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T.GA.T.	C
xanth2	..TCC.T..T	...CC...G.	.ACTT...A.	.T...CTC..	GC.CCT.A.T	C..T.GA.T.	C
yarkan	..TCCGT.TT	T.GCC.....	.GCTT...A.	.T...CTC..	GC.CCT.A.T	C....GATTC	C
yugosl	..TCC.T.TT	G..CC...G.	.GCT....A.	.T...CTC..	GC.CCT.A.T	C..T..ATT.	C

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Population   Nucleotide positions

             6666666666 6666666666 6666667777 7777777777 7777777777 7777777777 7
             3344444556 6666777888 8999990000 0001111112 2222333444 4555666777 8
             6902348470 3679258147 8345790235 6891234570 1269056145 7069258167 0

bostau       CAGCGTCCAC CCTCTCTGCC TGGCCCTACA ATCAGCTCAA CTAACCGCCC CCCATCCCCC T
albir1       A.A..C..C. TT..C..AT. .CAT.ACT.G GA.CTTC... T...T..A.. ATT.C....A .
albir2       A.A..C..C. TT..C..AT. .CAT.ACT.G GA.CTTC... T...T..A.. ATT.CT...A .
anonym       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT...C.T..A .
atlan1       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT......A C
atlan2       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT......A C
austri       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
axispo       A....CTTT. TT.....AT. .CATTACTA. GA.CTTCT.. T...T.AG.. GT..C....A .
bactri       A..T.CT.C. TT.....ATT .CATTACT.. GA.CTTCT.G .......A.. AT..C....A C
barba1       A....C..CT TT.T...ATT .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
barba2       A....C..CT TT.T...ATT .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
boluot       AG...C..CT TT.....AT. .TATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
bulgar       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
canad1       A....C..C. .T...G.AT. .TATTACT.. .A.CTTC... T...T..AT. GT..C.T..A .
canad2       A....C..C. .T...G.AT. .TATTACT.. .A.CTTC... T...T..AT. GT..C.T..A .
corsic       A....C..CT TT.TC..ATT .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
damada       A..T..TTCT T...C..ATT .CAT.A.C.. T..CTTCT.. AC...T.A.T GT..CA.T.A .
enclo1       A....C..CT TT.TC..ATT .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
enclo2       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT......A C
enclo3       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
france       A....C..CT TT.T...AT. .CAT.ACT.T GA.CTTCT.. T...T..A.. ATT......A C
hispan       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT.....AA C
hungar       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
istan1       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
kansu1       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
kansu2       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
kreuth       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT......A C
maral1       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
maral2       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
montan       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
moshus       A...A....T ....C.CATT CTAT.A.... .CT.ATCT.. GC.G.TA... ATTGC.TT.A C
neill1       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
neill2       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
nipcen       AG...C..C. TT..C..AT. .CATTACT.. GA.CTTC... T...T..A.. GTT.C.T..A .
nipmag       AG...C..C. TT.....AT. CCATTACT.. GA.CTTC.T. TC..T..A.. GTT.C....A .
nipnip       AG...C..C. TT.....AT. CCATTACT.. GA.CTTC.G. T...T..A.. GTT.C....A .
nipsic       AG...CA.C. TT...T.AT. .CATTACT.. .A.CT.C... T......A.. GT..C.T..A .
nipyes       AG...C..C. TT..C..AT. .CATTACT.. GA.CTTC... T...T..A.. GTT.C.T..A .
poland       A....C..CT TT.T...AT. .CATTACT.G GA.CTTCT.. T...T..A.. ATT......A C
scotic       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT......A C
sibir1       A....C..C. TT.....AT. .TATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
sibir2       A....C..C. .T.....AT. .TATTACT.. GA.CTTC... T...T..AT. GT..C.T..A .
songa1       A....C..C. .T..C..AT. .TATTACT.. GA.CTTC... T...T..AT. GT..C.T..A .
songa2       A....C..C. TT.....AT. .TATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
swedis       A....C..CT TT.T...AT. .CATTACT.T GA.CTTCT.. T...T..A.. ATT......A C
timore       A....CTTT. TT.....AT. .C.TTACTA. GA.CTTCT.. T...T.AG.. GT..C....A .
ukrain       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. ATT......A C
unicol       A....CTTT. TT.....AT. .CATTACTA. GA.CTTCT.. T...T..G.. GT..C....A .
wallic       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
xanth1       A....C..C. TTA....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
alasha       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
xanth2       A....C..C. TT.....AT. .CATTACT.. GA.CTTC... T...T..A.. GT..C.T..A .
yarkan       A..T.CT.C. TT.....AT. .CATTACT.. GA.CTTCT.G ..G.T..A.. AT..C....A C
yugosl       A....C..CT TT.T...AT. .CATTACT.. GA.CTTCT.. T...T..A.. AT.......A C
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Population	Nucleotide positions						
	7777778888 8888990011 3469581403	8888888888 1122223334 6923890470	8888888888 4455567778 1925820340	8888888888 8888889999 2345781478	9999999999 0000000011 0134567925	9999999999 1122344555 6817925178	9 6 0
bostau	ACCCCTCCCG	ACCTTGCCAC	TACCCCAACC	AGCCTCTCAA	TCTGCTCACC	CAACACACAC	C
albir1	.........TA	.T.C......	C.TT...C..	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
albir2	.........TA	.T.C......	C.TT...C..	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
anonym	.......TTA	.T.C......	CGT....CT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
atlan1	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
atlan2	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
austri	......TTTA	...C.TG...	C.T....CT.	..T.CAC...	CTAAT..CGT	.TTAC.GT.T	.
axispo	....T.T.TA	.T.CC.....	C.T....C..	..T.CA.T..	CTAAT..CGT	.TCAC.G..T	.
bactri	......TTT.	GT.C...T..	C.T....CT.	..T.CAC...	CTAAT..CAT	.TTAC.G.CT	.
barba1	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CAT	.TTAC.G..T	.
barba2	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CAT	.TTAC.G..T	.
boluot	......TTTA	...C......	C.T....C..	..T.CAC...	CTAAT..CGT	.TTAC.G..T	.
bulgar	......TTTA	...C......	C.T....CT.	G.T.CAC...	CTAAT..CGT	.TTAC.GT.T	.
canad1	.......TTA	.T.C......	C.T...GCT.	.AT.CA....	CTGAT..CGT	.TTAC.G..T	T
canad2	.......TTA	.T.C......	C.T...GCT.	.AT.CA....	CTGAT..CGT	.TTAC.G..T	T
corsic	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CAT	.TTAC.G..T	.
damada	....T.TT.A	...C......	C.T.TTGGT.	..T.CAC.G.	C.AAT..TA.	TCCAC.G..T	T
enclo1	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CAT	.TTAC.G..T	.
enclo2	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
enclo3	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CGT	.TTAC.GT.T	.
france	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
hispan	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
hungar	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CGT	.TTAC.GT.T	.
istan1	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CGT	.TTAC.GT.T	.
kansu1	...T...TTA	.T.C......	CGT...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
kansu2	...T...TTA	.T.C......	CGT...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
kreuth	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
maral1	......TTTA	...C......	C.T....C..	..T.CAC...	CTAAT..CGT	.TTAC.G..T	.
maral2	......TTTA	...C......	C.T....C..	..T.CAC...	CTAAT..CGT	.TTAC.G..T	.
montan	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CGT	.TTAC.GT.T	.
moshus	.TATTATT.A	G.TC...TCT	C.TTT.....	..TT.AC...	.TAATCTCA.	..TA.T..C.	T
neill1	...T...TTA	.T.C......	CGT...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
neill2	...T...TTA	.T.C......	CGT...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
nipcen	.........TA	...C......	C.T....CT.	..T.CAC...	CTGAT..CGT	.TTGC.G..T	.
nipmag	G.......TA	.T.C......	C.T....CT.	..T.CAC...	C.GATC.CGT	.TTGC.G..T	.
nipnip	G.......TA	.T.C......	C.T....CT.	..T.CA....	C.GATC.CGT	.TTGC.G..T	.
nipsic	.........A	...C......	C.T....CT.	..T.CA....	CTGAT..CGT	TTTGC.G..T	.
nipyes	.........TA	...C......	C.T....CT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
poland	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
scotic	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
sibir1	.......TTA	.T.C......	C.T...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
sibir2	.......TTA	.T.C......	C.T...GCT.	.AT.CA....	CTGAT..CGT	.TTGC.G..T	.
songa1	.......TTA	.T.C......	C.T...GCT.	.AT.CA....	C.GAT..CGT	.TTGC.G..T	.
songa2	.......TTA	.T.C......	C.T...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
swedis	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
timore	......T.TA	.T.CC.....	C.T....C..	.AT.CA.T..	CTAAT..CGT	.TCAC.G..T	.
ukrain	......TTTA	.T.C......	C.T....CT.	..T.CAC..G	CTAAT..CGT	.TTAC.G..T	.
unicol	......T.TA	.T.CC.....	C.T....C.T	..T.CA....	CTAAT.TCGT	.TCAC.G..T	.
wallic	...T...TTA	.T.C......	CGT...GCT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
xanth1	.......TTA	.T.C......	CGT....CT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
alasha	.......TTA	.T.C......	CGT....CT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
xanth2	.......TTA	.T.C......	CGT....CT.	..T.CA....	CTGAT..CGT	.TTGC.G..T	.
yarkan	.......TT.	GT.C......	C.T....CT.	..T.CAC...	CTAAT..CAT	.TTAC.G..T	.
yugosl	......TTTA	...C......	C.T....CT.	..T.CAC...	CTAAT..CGT	.TTAC.GT.T	.

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Population   Nucleotide positions
                         11111111    1111111111   1111111111   1111111111   1111111111
             9999999999  9900000000  0000000000   0000000000   0000111111   1111111111
             6677788899  9901223334  4444455666   6777888888   9999000000   0111222224
             3902901234  7954393580  1346709568   9478013679   0235123456   8249256890
bostau       CCCAGCCCCC  CGCTACCCAA  TTCCCCATGC   CCTCCCCCCA   GGCAAACGGC   GCAACATACA
albir1       ....AT.T..  .A...T..CT  ...TT.....   T..T.AT.T.   AC.T..TCA.   A....TCC..
albir2       ....AT.T..  .A...T..CT  ...TT.....   T..T.AT.T.   AC.T..TCA.   A....TCC..
anonym       ....ATTT..  .A..GTT.CT  ..TTTT....   T..T.AT...   .C.T..TTA.   A....CCC..
atlan1       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
atlan2       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
austri       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
axispo       .T..AT.T..  .A.C.TT.CT  C..TTT..A.   T..T.AT..G   .C.T.GTCA.   A....CCC..
bactri       ....AT.T..  TA.C.TTTCT  ...TTT..A.   TT.T.AT...   .C.C..TCA.   A....CCC..
barba1       ....AT.T..  TA.C.TTTCT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
barba2       ....AT.T..  TA.C.TTTCT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
boluot       ....AT.TT.  TATC.TT.CT  ...TTT..A.   TTCT.AT...   .C.C..TCA.   A....CCC..
bulgar       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
canad1       ....AT.T..  .A..GTT.CT  ..TTTT..A.   T..T.AT...   .C.T..TTA.   A...TCCC..
canad2       ....AT.T..  .A..GTT.CT  ..TTTT..A.   T..T.AT...   .C.T..TTA.   A...TCCC..
corsic       ....AT.T..  TA.C.TTTCT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
damada       T.T..T....  .ATC..TTTT  ..T..T..A.   TT...ATT..   .C.T.G.CA.   A..GTCCT..
enclo1       ....AT.T..  TA.C.TTTCT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
enclo2       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
enclo3       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
france       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
hispan       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
hungar       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
istan1       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
kansu1       ....AT.T..  .A..GTT.CT  ..TTTT....   ...T.AT...   .C.T..TTA.   A...TCCC..
kansu2       ....AT.T..  .A..GTT.CT  .CTTTT....   ...T.AT...   .C.T..TTA.   A...TCCC..
kreuth       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
maral1       ....AT.TT.  TATC.TT.CT  ...TTT..A.   TTCT.A....   .C.C..TCA.   A....CCC..
maral2       ....AT.TT.  TATC.TT.CT  ...TTT..A.   TTCT.A....   .C.C..TCA.   A....CCC..
montan       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
moshus       ....ATTTTT  T.T..T....  ...TT..CAT   A...T.T...   .AA.GGTA..   ATG..TCCT.
neill1       ....AT.T..  .A..GTT.CT  ..TTTT....   T..T.AT...   .C.T..TTA.   A...TCCC..
neill2       ....AT.T..  .A..GTT.CT  ..TTTT....   ...T.AT...   .C.T..TTA.   A...TCCC..
nipcen       ....AT.T..  .A...TTTCT  ..TTTT..AT   ...T.AT...   .C.TG.TTA.   A...TCCC..
nipmag       ...GAT.T..  .A...TT.CT  ..TTTT....   ..CT.AT...   .C.TG.TCA.   A....CCC..
nipnip       ...GAT.T..  .A...TT.CT  ..TTTT....   ..CT.AT...   .C.TG.TCA.   A....CCC..
nipsic       ....AT.T..  .A...TT.CT  ..TTTT..A.   ...T.ATT.G   .C.CG.TCA.   A...TCCC..
nipyes       ....AT.T..  .A...TTTCT  ..TTTT...T   ...T.AT...   .C.TG.TTA.   A...TCCC..
poland       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
scotic       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
sibir1       ....AT.T..  .A..GT..CT  ..TTTT..A.   T..T.AT...   .C.T..TTA.   A...TCCC.G
sibir2       ....AT.T..  .A..GTT.CT  ..TTTT..A.   T..T.AT...   .C.T..TTAT   A...TCCC..
songa1       ....AT.T..  .A..GTT.CT  ..TTTT..A.   T..T.AT...   .C.T..TTA.   A...TCCC..
songa2       ....AT.T..  .A..GTT.CT  ..TTTT..A.   T..T.AT...   .C.T..TTA.   A...TCCC..
swedis       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
timore       .T..AT.T..  .A.C.TT.CT  CC.TTT..A.   T..T.AT..G   .C.T.GTCA.   A....CCC..
ukrain       T...AT.TT.  .ATC..T.CT  ...TTT....   TTCT.AT...   .C.C..TCA.   A....CCC..
unicol       ....AT.T..  .A.C.TT.CT  ..TTTT..A.   T..T.AT..G   .C.T..TCA.   A....CCC..
wallic       ....AT.T..  .A..GTT.CT  ..TTTT....   T..T.AT...   .C.T..TTA.   A...TCCC..
xanth1       ....ATTT..  .A..GTT.CT  ..TTTT....   T..T.AT...   .C.T..TTA.   A...TCCC..
alasha       ....ATTT..  .A..GTT.CT  ..TTTT....   T..T.AT...   .C.T..TTA.   A...TCCC..
xanth2       ....ATTT..  .A..GTT.CT  ..TTTT....   T..T.AT...   .C.T..TTA.   A...TCCC..
yarkan       ....AT.T..  TA.C.TT.CT  ...TTT..A.   TT.T.AT...   .C.C..TCA.   A....CCC..
yugosl       ....AT.TT.  TATC.TT.CT  ...TTTG.A.   TTCT.AT..G   .C.C..TCA.   A....CCC..
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