# Report

# Origin and Diffusion of mtDNA Haplogroup X

Maere Reidla,<sup>1</sup> Toomas Kivisild,<sup>1</sup> Ene Metspalu,<sup>1</sup> Katrin Kaldma,<sup>1</sup> Kristiina Tambets,<sup>1</sup> Helle-Viivi Tolk,<sup>1</sup> Jüri Parik,<sup>1</sup> Eva-Liis Loogväli,<sup>1</sup> Miroslava Derenko,<sup>2</sup> Boris Malyarchuk,<sup>2</sup> Marina Bermisheva,<sup>1,3</sup> Sergey Zhadanov,<sup>1,4</sup> Erwan Pennarun,<sup>1,5</sup> Marina Gubina,<sup>1,4</sup> Maria Golubenko,<sup>1,6</sup> Larisa Damba,<sup>1,4</sup> Sardana Fedorova,<sup>1,7</sup> Vladislava Gusar,<sup>1,8</sup> Elena Grechanina,<sup>8</sup> Ilia Mikerezi,<sup>9</sup> Jean-Paul Moisan,<sup>5</sup> André Chaventré,<sup>5</sup> Elsa Khusnutdinova,<sup>3</sup> Ludmila Osipova,<sup>4</sup> Vadim Stepanov,<sup>6</sup> Mikhail Voevoda,<sup>4</sup> Alessandro Achilli,<sup>10</sup> Chiara Rengo,<sup>10</sup> Olga Rickards,<sup>11</sup> Gian Franco De Stefano,<sup>11</sup> Surinder Papiha,<sup>12</sup> Lars Beckman,<sup>13</sup> Branka Janicijevic,<sup>14</sup> Pavao Rudan,<sup>14</sup> Nicholas Anagnou,<sup>15</sup> Emmanuel Michalodimitrakis,<sup>16</sup> Slawomir Koziel,<sup>17</sup> Esien Usanga,<sup>18</sup> Tarekegn Geberhiwot,<sup>19</sup> Corinna Herrnstadt,<sup>20</sup> Neil Howell,<sup>20</sup> Antonio Torroni,<sup>10</sup> and Richard Villems<sup>1</sup>

<sup>1</sup>Department of Evolutionary Biology, Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, Tartu, Estonia; <sup>2</sup>Genetic Laboratory, Institute of Biological Problems of the North, Magadan, Russia; <sup>3</sup>Institute of Biochemistry and Genetics, Ufa Research Center, Russian Academy of Sciences, Ufa, Russia; <sup>4</sup>Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; <sup>5</sup>Laboratoire d'Etude du Polymorphisme de l'ADN, Faculté de Médecine, Nantes, France; <sup>6</sup>Institute of Medical Genetics, Siberian Branch of the Russian Academy of Medical Sciences, Tomsk, Russia; <sup>7</sup>Vakut Scientific Center, Russian Academy of Medical Sciences, and Government of Republic Sakha (Yakutia), Yakutsk, Russia; <sup>8</sup>Center of Clinical Genetics and Prenatal Diagnostics, Kharkov, Ukraine; <sup>9</sup>Department of Biology, Faculty of Natural Sciences, Tirana University, Tirana, Albania; <sup>10</sup>Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; <sup>11</sup>Dipartimento di Biologia, Università "Tor Vergata," Rome; <sup>12</sup>Department of Human Genetics, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne; <sup>13</sup>Gotland University, Visby, Sweden; <sup>14</sup>Institute for Anthropological Research, Zagreb, Croatia; <sup>15</sup>Institute of Molecular Biology and Biotechnology and Department of Basic Sciences and <sup>16</sup>Department of Forensic Sciences and Toxicology, University of Crete School of Medicine, Heraklion, Greece; <sup>17</sup>Institute of Anthropology, Wroclaw, Poland; <sup>18</sup>Department of Haematology, University of Calabar, Calabar, Nigeria; <sup>19</sup>Birmingham and Solihull Teaching Hospital, Birmingham; and <sup>20</sup>MitoKor, San Diego, CA

A maximum parsimony tree of 21 complete mitochondrial DNA (mtDNA) sequences belonging to haplogroup X and the survey of the haplogroup-associated polymorphisms in 13,589 mtDNAs from Eurasia and Africa revealed that haplogroup X is subdivided into two major branches, here defined as "X1" and "X2." The first is restricted to the populations of North and East Africa and the Near East, whereas X2 encompasses all X mtDNAs from Europe, western and Central Asia, Siberia, and the great majority of the Near East, as well as some North African samples. Subhaplogroup X1 diversity indicates an early coalescence time, whereas X2 has apparently undergone a more recent population expansion in Eurasia, most likely around or after the last glacial maximum. It is notable that X2 includes the two complete Native American X sequences that constitute the distinctive X2a clade, a clade that lacks close relatives in the entire Old World, including Siberia. The position of X2a in the phylogenetic tree suggests an early split from the other X2 clades, likely at the very beginning of their expansion and spread from the Near East.

mtDNA and the nonrecombining part of the Y chromosome are widely used in archaeogenetic studies (Renfrew 2000; Cavalli-Sforza and Feldman 2003) that aim

Address for correspondence and reprints: Dr. Maere Reidla, Department of Evolutionary Biology, Tartu University, Estonian Biocentre, Riia 23, Tartu, 51010, Estonia. E-mail: mreidla@ebc.ee

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to reveal the human past. The uniparental inheritance and complete linkage of mutations in these two loci allow an unambiguous determination of the phylogenetic relationships between individual lineages. However, the putative genetic histories of the lineages that are obtained do not fully reflect the complex dynamics of ancient populations; thus, the data must be interpreted carefully. Phylogenetic clustering of mtDNA haplogroups has been found to be congruent with geography—there are haplogroups specific to African (Chen et

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**Figure 1** Maximum parsimony phylogenetic tree of complete mtDNA sequences belonging to haplogroup X. Five mtDNAs were selected for complete sequence analysis in the course of the present study (Dr09, Es39, Gm66, Go41, and Om1538), 11 coding region sequences were from the work of Herrnstadt et al. (2002), but their control region sequences have now been added, and 5 complete sequences (Lev2, MM, E18, Nav125, and Oj2) were taken from the literature (Levin et al.1999; Maca-Meyer et al.2001; Mishmar et al. 2003; Bandelt et al., in press). The diagnostic mutations relative to the revised reference sequence (Andrews et al. 1999) are indicated on the branches. Transversions are specified by suffixes, and underlined mutations appear more than once in the tree. For protein-coding genes, "ns" indicates nonsynonymous and "s" synonymous mutations. For coalescence-time estimates, a mutation rate of 1 mutation per 5,139 years in the coding region (nps 577–16023) was used (Mishmar et al. 2003). mtDNA sequence data are available at the Estonian Biocentre Web page.

al. 1995; Watson et al. 1997), Asian (Ballinger et al. 1992; Torroni et al. 1994; Kivisild et al. 2002), European/West Eurasian (Torroni et al. 1996; Macaulay et al. 1999), and Native American (Torroni et al. 1993) populations.

Haplogroup X is an exception to this pattern of limited geographical distribution. It is found, generally at low frequencies, in both West Eurasians (Richards et al. 2000) and some northern groups of Native Americans (Ward et al. 1991; Forster et al. 1996; Scozzari et al. 1997; Brown et al. 1998; Smith et al. 1999; Malhi et al. 2001), but, intriguingly, it is absent in modern north Siberian and East Asian populations (Brown et al. 1998; Starikovskaya et al. 1998; Schurr et al. 1999), which are genetically and geographically closest to those of Native Americans. Among Siberians, haplogroup X mtDNAs have only been detected in some Altaian populations of southwestern Siberia (Derenko et al. 2001).

When the sequence variation of the first hypervariable segment (HVS-I) of the control region is analyzed, haplogroup X mtDNAs from Europe and the Near East are found to yield similar coalescence times: 17,000–30,000 years before present (YBP) and 13,700–26,600 YBP, respectively (Richards et al. 2000). These estimates are consistent with a pre-Holocene origin and spread of this haplogroup into West Eurasia. For Native Americans, the relatively old presence of haplogroup X is confirmed by the analysis of ancient human remains (Stone and Stoneking 1999; Malhi and Smith 2002). Moreover, Native American haplogroup X mtDNAs form a clade dis-



tinct from that of West Eurasians and with coalescence time estimates varying widely depending on both the method of estimation and the number of assumed founders. Thus, the coalescence times ranged from 12,000– 17,000 YBP to 23,000–36,000 YBP, times that are consistent with both a pre- and a postglacial population diffusion (Brown et al. 1998).

To obtain further information about the extent of haplogroup X diversity, 5 mtDNAs (from 1 Druze, 1 Estonian, 2 Georgians, and 1 Omani) were completely sequenced and were compared with 16 previously published X sequences (fig. 1). These latter sequences included the 11 haplogroup X coding sequences published by Herrnstadt et al. (2002) that have now been completed with the sequencing of the control region.

A maximum parsimony tree of the 21 haplogroup X sequences revealed that one nonsynonymous (13966) and three synonymous (6221, 6371, and 14470) substitutions in the coding region, as well as three transitions in the control region (153, 16189, and 16278), distinguish haplogroup X from the root of superhaplogroup N. Moreover, haplogroup X is subdivided into two major subhaplogroups, designated "X1" and "X2." Subhaplogroup X1, represented by a single Druze mtDNA in figure 1, differs from the root of haplogroup X by eight coding and three control region transitions and lacks the two transitions (195 and 1719) that characterize X2. These two nucleotides are rather mutable (Finnilä et al. 2001; Herrnstadt et al. 2002); thus, it cannot be completely ruled out that X1 is indeed a subset of X2 that reverted at both nucleotide positions. However, this possibility appears very unlikely, especially when one considers the time depth and the distinct geographic distribution of X1 (see below).

In contrast with X1, X2 is well represented in the tree, and it is further subdivided into at least six major clades (X2a–X2f), which include clades X1 and X2 as defined by Herrnstadt et al. (2002). All 20 X2 sequences, including the 2 Native American X2a sequences, share transitions at nucleotide positions (nps) 195 and 1719. A recurrence of the nonsynonymous substitution at 13708 was observed (clades X2b and X2d). In addition, the nonsynonymous transition 13966 was found to have reverted in the Moroccan X2b sequence. These were the only recurrent mutations found among the 67 variable positions in the coding region sequences. The ratio of nonsynonymous to synonymous substitutions was 0.40 (18/45). Six mutations were located in RNA genes.

The data obtained from the analyses of complete mtDNA sequences belonging to haplogroup X were then used to survey 13,589 mtDNAs (21,682 when mtDNA data from the literature are included) from 66 populations of Eurasia and North Africa (table 1). A total of 175 mtDNAs were found to harbor the four coding region transitions-at 6221, 6371, 13966, and 14470that define haplogroup X. The four markers were always found in association and, by combination of the control and coding variation, all 175 mtDNAs could be apportioned to subhaplogroups X1 and X2 (table 2). No other main branches occur, and the root haplotype was not present among the sequences. The adenine at np 153 appears relatively conserved in the phylogenetic context of 376 complete human mtDNA sequences taken worldwide, exhibiting a change to guanine only in haplogroups A and X (Ingman et al. 2000; Finnilä et al. 2001; Maca-Meyer et al. 2001; Derbeneva et al. 2002; Mishmar et al. 2003). In contrast, this position shows a high level of variation in the background of haplogroup X as both the 153A and 153G alleles are present in its different subclades. It is possible that the  $A \rightarrow G$  transition at 153 arose only once in the haplogroup X ancestor and the recurrent reverse mutations in 11 branches in figure 2 bear witness to the process of favored fixation of the more stable A allele.

Subhaplogroup X1 was found to be largely restricted to the Afro-Asiatic–speaking populations of North Africa and neighboring areas, including Ethiopia, suggesting a possible geographic diffusion of X1 alongside the

Figure 2 Median-joining (MJ) network of 175 haplogroup X partial sequences. The MJ (Bandelt et al. 1999) algorithm was implemented within the Network 3111 program (A. Röhl; Shareware Phylogenetic Software Web site). Default settings were used for HVS-I (nps 16024-16383), HVS-II (nps 16518-310), and coding-region sequence variation as given in table 2. Highly variable (Hasegawa et al. 1993) positions of HVS-I (16093, 16126, 16129, 16187, 16189, 16223, 16234, 16278, 16292, 16293, 16311, 16325, 16355, and 16362) were assigned a weight of 1, other HVS-I and HVS-II sites were assigned a weight of 2, and coding-region sites were assigned a weight of 10. Areas of the circles are proportional to haplotype frequencies. Populations are indicated by the following abbreviations: ab = Abazin, ad = Adygei, alb = Abazin, ab = Abazin, Albanian, alg = Algerian, alt = Altaian, ar = Armenian, arb = Arab from Uzbekistan, be = Bengali, cz = Czech, dr = Druze, eg = Egyptian, es = Estonian, et = Ethiopian, ev = Evenk, fr = French, go = Georgian, gr = Greek, ho = Croat, hu = Hungarian, ir = Iranian, it = Italian, jo = Jordanian, kir = Kyrgyz, kr = Karachay, ku = Kumyk, kw = Kuwaiti, le = Lebanese, mo = Moroccan, ng = Nogay, os = Ossetian, sa = Saudi Arabian, sw = Swede, sy = Syrian, td = Tadjik, tu = Turk, and ukr = Ukrainian. Variant bases are numbered (Anderson et al. 1981) and are shown along links between haplotypes. Nucleotide changes are specified by suffixes only for transversions, and a "d" indicates a deletion. The node marked with a large asterisk ( $\star$ ) matches the root type of haplogroup X. The coalescence times of the clades are shown near the clades. Coalescence times of HVS-I clusters were calculated by means of  $\rho$ , the average mutational distance to the founder haplotype of the cluster, by using a mutation rate of 1 transition per 20,180 years in the segment between nps 16090 and 16365 (Forster et al. 1996). Standard deviations for  $\rho$  were calculated as in the work of Saillard et al. (2000), a procedure which ignores the variance due to molecular clock calibration. mtDNA sequence data are available at the Estonian Biocentre Web page.

### Table 1

Frequency	v and D	iversitv	of Subha	plogroups	5 X1 a	and X2	in Eurasian	and Afri	can Populations
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		VA	lue for Sui	BHAPLOGROUP <sup>b,c</sup>			
		X1		X2			
	SAMPLE	Mean Frequency [95% CR]		Mean Frequency [95% CR]			
REGION AND POPULATION <sup>a</sup>	Size	(%)	Diversity	(%)	Diversity		
North and northeastern Africa:	1,606	.8 [.5-1.4]	.936	.9 [.5–1.5]	.978		
Ethiopians <sup>1</sup>	270	.7 [.2–2.6]		0 [.0-1.1]			
Egyptians <sup>1</sup>	193	2.1 [.8–5.2]		.5 [.1–2.8]			
Libyans <sup>1</sup>	72	0 [.0-4.0]		0 [.0-4.0]			
lunisians	417	.2 [.1–1.3]		1.7[.8-3.4]	.952		
Algerians <sup>2</sup>	124	0 [.0-2.4]		1.6 [.3-3.7]			
Woot and couthern Africa.	527	1.1[.3-2.4]		.8 [.3-1.7]			
*Senegalese <sup>27</sup>	121	0 [.08]		0 [.08]			
*17 ethnic groups in Mozambique <sup>24,32</sup>	416	0 [0-7]		0 [0-7]			
Near East:	2.299	.5 [.39]	.564	2.9 [2.3–3.6]	.887		
Yemenis <sup>1</sup>	116	0 [.0-2.5]		.9 [.2–4.7]			
Omanis <sup>1</sup>	78	0 [.0-3.7]		1.3 [.3–6.9]			
Saudi-Arabians <sup>1</sup>	204	0 [.0-1.5]		1.5 [.5-4.2]			
Kuwaitis <sup>1</sup>	202	.5 [.1–2.7]		2.0 [.8-5.0]			
*Israeli Palestinians9,29	117	0 [.0-2.5]		3.4 [1.4-8.5]			
Israeli Druze <sup>17</sup>	45	15.6 [7.8-28.9]	0	11.1 [4.9–23.6]			
Jordanians <sup>1</sup>	202	.5 [.1–2.7]		1.5 [.5-4.3]			
Lebanese <sup>1</sup>	172	0 [.0-1.7]		5.8 [3.2-10.4]	.867		
Syrians <sup>1</sup>	219	0 [.0–1.4]		1.8 [.7-4.6]			
*Iraqis <sup>29</sup>	116	.9 [.2–4.7]		.9 [.2–4.7]			
Turks <sup>1</sup>	388	0 [.08]		4.4 [2.8–6.9]	.919		
Iranians <sup>1</sup>	440	.2 [.1–1.3]		3.0 [1.7–5.0]	.833		
Mediterranean Europe:	2,900	.0 [.02]		2.5 [2.0–3.2]	.859		
Cypriots <sup>-</sup>	1/9	0 [.0-1.7]		6.7[3.9-11.4]	.864		
Albanians <sup>1</sup>	2/3	0[.0-1.1]		4.4 [2.3-7.3]	.433		
Croats <sup>1</sup>	884	0 [.0-1.5]		2.3 [1.1–3.7] 9 [ 5_1 8]	786		
Italians from mainland and Sicily <sup>1,19,33</sup>	859	0 [0-3]		2 9 [2 0-4 3]	923		
*Basques <sup>3,4,29</sup>	147	0 [.0-2.0]		1.4 [ 4-4.8]	.725		
Spaniards <sup>5</sup>	118	0 [.0-2.5]		4.2 [1.9–9.5]			
Portugese <sup>23</sup>	241	.4 [.1–2.3]		1.7 [.7-4.2]			
Northwestern Europe:	1,775	0 [.02]		1.7 [1.2-2.5]	.665		
French <sup>1,30</sup>	398	0 [.07]		.8 [.3–2.2]			
*English <sup>12,26,28</sup>	334	0 [.0–.9]		.9 [.3–2.6]			
*Scots <sup>12</sup>	891	0 [.03]		1.6 [.9–2.6]	.692		
*Orkney inhabitants <sup>12</sup>	152	0 [.0–1.9]		7.2 [4.1–12.5]	.473		
Scandinavia:	1,271	0 [.02]		.9 [.5–1.5]	.491		
Swedes <sup>1</sup>	318	0 [.09]		.6 [.2–2.2]			
*Norwegians <sup>12,20,29</sup>	559	0 [.03]		.4 [.1–1.3]	0		
Icelanders	394	0 [.08]		1.8 [.9–3.6]	0		
Alps:	233	0[.0-1.2]		.8 [.2-2.8]			
Austrians <sup>22</sup>	101	0 [.0-1.9]		10[2-5.3]			
North-Central Europe:	1.419	0 [.0-2]		1.3 [.8-2.0]	948		
Poles <sup>1,18</sup>	547	0 [.05]		1.6 [.9–3.1]	.972		
*Germans <sup>13,16,25,28</sup>	532	0 [.06]		1.1 [.5–2.4]			
Czechs <sup>1</sup>	94	0 [.0-3.1]		2.1 [.7–7.4]			
Slovaks <sup>1</sup>	130	0 [.0-2.3]		0 [.0-2.3]			
Hungarians <sup>1</sup>	116	0 [.0-2.5]		.9 [.2-4.7]			
Northeastern Europe:	1,611	0 [.02]		1.1 [.7–1.8]	.902		
Finns <sup>10</sup>	192	0 [.0–1.5]		2.1 [.8–5.2]			
Estonians <sup>1</sup>	401	0 [.0–.7]		1.2 [.5–2.9]			
Latvians <sup>1</sup>	192	0 [.0–1.5]		.5 [ 0.1–2.9]			
Kussians <sup>1,10,21</sup>	826	0 [.04]		1.0 [.5-1.9]	.964		
Volga-Ural region:	1,037	0 [.03]		0 [.03]			
Komis <sup>2</sup>	136	0 [.0-2.2]		0 [.0-2.2]			
Marie <sup>2</sup>	101	0 [.0-2.9] 0 [ 0_2 2]		0 [.0-2.9] 0 [ 0-2 2]			
Mordvins <sup>2</sup>	102	0 [.0-2.2] 0 [ 0-2 9]		0 [.0-2.2] 0 [ 0-2 9]			
Nenets <sup>31</sup>	58	0 [.0-5.0]		0 [.0-5.0]			
Chuvashis <sup>2</sup>	55	0 [.0-5.2]		0 [.0-5.2]			
Tatars <sup>2</sup>	228	0 [.0–1.3]		0 [.0–1.3]			
Bashkirs <sup>2</sup>	221	0 [.0-1.3]		0 [.0–1.3]			

(continued)

### Table 1 (continued)

	VALUE FOR SUBHAPLOGROUP <sup>b,c</sup>						
		X1		X2			
	SAMPLE	Mean Frequency [95% CR]		Mean Frequency [95% CR]			
REGION AND POPULATION <sup>a</sup>	Size	(%)	Diversity	(%)	Diversity		
Southeastern Europe:	357	0 [.08]		2.0 [1.0-4.0]	.952		
Ukrainians <sup>1</sup>	357	0 [.08]		2.0 [1.0-4.0]	.952		
Northern Caucasus:	838	0 [.04]		2.7 [1.8-4.1]	.798		
Nogays <sup>1</sup>	213	0 [.0-1.4]		4.2 [2.3–7.8]	.722		
Adygeis <sup>1</sup>	159	0 [.0-1.9]		2.5 [1.0-6.3]			
Karachays <sup>1</sup>	106	0 [.0-2.8]		1.9 [.6–6.6]			
Abazins <sup>1</sup>	63	0 [.0-4.6]		6.3 [2.6–15.2]			
Kabardins <sup>1</sup>	66	0 [.0-4.4]		0 [.0-4.4]			
Kumyks <sup>1</sup>	111	0 [.0-2.6]		3.6 [1.5-8.9]			
Kalmyks <sup>1</sup>	120	0 [.0-2.4]		0 [.0-2.4]			
South Caucasus:	782	0 [.04]		4.3 [3.1-6.0]	.797		
Georgians <sup>1</sup>	340	0 [.09]		7.6 [5.3–11.0]	.738		
Southern Ossetians <sup>1</sup>	201	0 [.0-1.5]		.5 [.1–2.7]			
Armenians <sup>1</sup>	193	0 [.0-1.5]		2.6 [1.1-5.9]			
*Azeris <sup>29</sup>	48	0 [.0-5.9]		4.2 [1.3–14.0]			
Central Asia:	1,036	0 [.03]		.8 [.4–1.5]	.929		
Uighurs from Kazakhstan <sup>1</sup>	122	0 [.0-2.4]		.8 [.2–4.4]			
Kazakhs <sup>1</sup>	495	0 [.06]		.6 [.2–1.8]			
Kyrgyz <sup>1</sup>	105	0 [.0-2.8]		1.0 [.2–5.1]			
Tadjik <sup>1</sup>	77	0 [.0-3.8]		1.3 [.3-6.9]			
Uzbeks <sup>1</sup>	160	0 [.0-1.8]		.6 [.2–3.4]			
Arabs from Uzbekistan <sup>1</sup>	77	0 [.0-3.8]		1.3 [.3-6.9]			
India:	1,010	0 [.03]		.2 [.1–.7]			
Western Bengalis <sup>1</sup>	106	0 [.0-2.8]		.9 [.2–5.1]			
Gujaratis and Konkanastha Br. <sup>1</sup>	111	0 [.0-2.6]		0 [.0-2.6]			
Punjabis <sup>1</sup>	112	0 [.0–2.6]		0 [.0–2.6]			
Moors <sup>1</sup>	50	0 [.0-5.7]		0 [.0-5.7]			
Singhalese from Sri Lanka <sup>1</sup>	82	0 [.0–3.5]		0 [.0–3.5]			
Uttar Pradesh <sup>14</sup>	122	0 [.0–2.4]		.8 [.2–4.4]			
lelugus <sup>14</sup>	250	0 [.0–1.2]		0 [.0–1.2]			
Chenchu <sup>13</sup>	96	0 [.0–3.0]		0 [.0–3.0]			
Koya <sup>13</sup>	81	0 [.0–3.6]		0 [.0–3.6]			
Siberia:	2,949	0 [.01]		.4 [.2–.7]	.455		
Altaians <sup>1,7</sup>	481	0 [.06]		1.9 [1.0–3.5]	0		
Buryats'	105	0 [.0-2.8]		0 [.0-2.8]			
Tuvins <sup>1,7</sup>	314	0 [.09]		0 [.09]			
Koryaks''	105	0 [.0-2.8]		0 [.0-2.8]			
Evens'	65	0 [.0-4.4]		0 [.0-4.4]			
Evenks"	185	0 [.0-1.6]		1.1 [.3–3.8]			
Yakuts <sup>1,7</sup>	340	0 [.09]		0 [.09]			
Khakassians'	34	0 [.0-5.3]		0 [.0-5.3]			
Shors"	207	0 [.0-1.4]		0 [.0-1.4]			
Sojots	34	0 [.0-8.2]		0 [.0-8.2]			
Kets <sup>-</sup>	66	0 [.0_4.4]		0 [.0-4.4]			
Seikups <sup>-</sup>	120	0 [.0-2.4]		0 [.0-2.4]			
Nenets'	/9	0 [.0-3.7]		0 [.0-3.7]			
Dolgans	130	0 [.0-2.3]		0 [.0-2.3]			
INARAIS"	88	0 [.0-3.3]		0 [.0-3.3]			
Nomis <sup>-</sup>	/8	0 [.0-3.7]		0 [.0-3.7]			
Inganasans" Khantal	107	0 [.0-2.7]		0 [.0-2.7]			
Manaia <sup>1,6</sup>	233	0 [.0-1.2]		.4 [.1-2.2]			
IVIANSIS	138	0 [.0-2.1]		0 [.0-2.1]			

<sup>a</sup> An asterisk (\*) denotes frequencies deduced from published HVS-I sequences. Populations are divided into regions basically as in (Richards et al. 2000), with some changes: North African and South Caucasian populations were considered separately from populations of the Near East, and all European populations of the Mediterranean area were aggregated into a single Mediterranean European region. Sources are denoted as follows: <sup>1</sup>present study, <sup>2</sup>Bermisheva et al. (2002), <sup>3</sup>Bertranpetit et al. (1995), <sup>4</sup>Corte-Real et al. (1996), <sup>5</sup>Crespillo et al. (2000), <sup>6</sup>Derbeneva et al. (2002), <sup>7</sup>Derenko et al. (2001), <sup>8</sup>Dimo-Simonin et al. (2000), <sup>9</sup>Di Rienzo and Wilson (1991), <sup>10</sup>Finnilä et al. (2001), <sup>11</sup>Helgason et al. (2000), <sup>12</sup>Helgason et al. (2001), <sup>13</sup>Hofmann et al. (1997), <sup>14</sup>Kivisild et al. (1999), <sup>15</sup>Kivisild et al. (2003), <sup>16</sup>Lutz et al. (1998), <sup>17</sup>Macaulay et al. (1999), <sup>13</sup>Malyarchuk et al. (2002), <sup>19</sup>Mogentale-Profizi et al. (2001), <sup>20</sup>Opdal et al. (1998), <sup>22</sup>Parson et al. (1998), <sup>23</sup>Pereira et al. (2000), <sup>24</sup>Pereira et al. (2001), <sup>25</sup>Pfeiffer et al. (1999), <sup>26</sup>Piercy et al. (1993), <sup>27</sup>Rando et al. (1998), <sup>28</sup>Richards et al. (1996), <sup>29</sup>Richards et al. (2000), <sup>30</sup>Rousselet and Mangin (1998), <sup>31</sup>Saillard et al. (2000), <sup>32</sup>Salas et al. (2002), <sup>33</sup>Tagliabracci et al. (2001).

<sup>b</sup> The 95% credible regions (CR), calculated using software kindly provided by Vincent Macaulay, are shown in brackets.

<sup>c</sup> Haplotype diversities were calculated as in the work of Nei (1987), only for regions where more than six haplogroup X mtDNAs were available considering the sequence variation observed between nps 16090 and 16365.

### Table 2

Control-Region	and Coding-Region	n Variation of	Haplogroup	X mtDNAs
control-region	and counts-region	i variation of	riupiogroup	X III(D) 1/13

		CONTROL REGION	SEQUENCE <sup>a</sup>	Coding Region Sequence and RFLP Variation <sup>b</sup>							
Sequence <sup>c</sup>	Haplogroup	HVS-I <sup>4</sup>	HVS-II <sup>c</sup>	11111111111111111111111111111111111111							
cncf											
CRS <sup>1</sup> et 216	¥1*	169 189 213 223 278	146 152	G++G+A-TGATT-+AAAC+T+TAC+TCAT+TCA++-+CATA-TGAATGTTT+							
eg 96	X1a	109 139 213 223 278	146 152	G + G T ATC+ A T T A + +CATG+ GGGT CT							
eg 818	X1a	104 164 189 311	146 153 183	G + G T T T A +CATG+ GGAT CT							
et 146	X1a	104 189	146 153	G + T T + A T C A + + + G + GGAT CT							
ir 143	X1a	104 189	146 153	G + G T T T A +CATG+ GGAT CT							
jo 924	X1a	104 189	146 153	G + G T T T A +CATG+ GGAT CT							
kw 103	X1a X1a	104 189	146 153 195	G + G T ATC+ A T T A + +CATG+ GGAT CT							
mo 90E	X1a X1a	104 189 223	146 153	G + G T T T A +CGTG+ GGAT TT							
mo E99	X1a	104 189 223	146 153	G + GTG+ GG TT							
dr 09	X1b	126 189 223 278	146 153 256	G++A+A-CGACC++AAAC+T+TAC+TCGT+TCA++-+TACG+TAAATGTTC+							
eg 443	X1b	189 223 278	146 153 256	G + A T ATC + A T T T A + +TACG + AAAT TC							
mo C49	X1b	189 223 278	146 153 256	+ T T C +							
mo A/9	X1b X1b	189 223 278 360	146 153 256	G + A T T A TACG+ AA TC							
mo B64	X1b X1b	93 189 223 278 360	146 256	+ T T C +							
le 938	X2*	129 189 223 278 362	153 195 225 227	G G + +T T							
tu 47	X2*	129C 189 223 278	153 195	G+- + - A + A T + + A+ + G+ T							
ir 71	X2*	187 189 223 278 380	153 195 225	G - + - G $A$ $A + +T$ $T$							
alb 53	X2*	189 192 223 278 292	143 189 195 225 226 235	G - + - GA C+ A A C T+ A+ + + T T +							
tu 208	X2* X2*	189 192 223 278 292 189 192 4 223 278	143 195 225 226 235	G = + - GA + A T A C T + A + -+ G + T T + G + T C T + C + T C +							
be 50	X2*	189 223	195	G - A + A T + C + + G + T							
ir 161	X2*	189 223 234 278	195 225	G - + - G A CA + +T T							
ir 282	X2*	189 223 248 278	153 195	G - + - G A A + +T T							
it 173	X2*	189 223 248 278	153 195	G - + C + + T							
it 74	X2*	189 223 248 278	153 195	G G + T A C + +T T							
it 33 ar 175	X2* X2*	189 223 248 278	153 195	G - + A T + C + + G + T							
ng 227	X2*	189 223 248 278	153 195 198 225	+ + T							
ir 224	X2*	189 223 248 278	153 195 225	G - + - G TA A + +T T							
ng 167	X2*	189 223 248 278	153 195 225	G G + +T T							
ab 17	X2*	189 223 248 278	153 195 225	G - + - G $A$ $CA + +T$ $T$							
ab 31	X2*	189 223 248 278	153 195 225	- + - G A A + +T T							
ab 44 ad 67	X2* X2*	189 223 248 278	153 195 225								
ad 79	X2*	189 223 248 278	153 195 225	G A + +T T							
ng 2	X2*	189 223 248 278	153 195 225	- + A + + T							
ng 264	X2*	189 223 248 278	153 195 225	G - + + T							
ng 6	X2*	189 223 248 278	153 195 225	G - + A A + + T							
ir 240	X2*	189 223 248 278	16527 153 195	G - + - G A CA + +T T							
gr 34 tu 196	X2* X2*	189 223 248 278	1652/ 153 195 282	G = + + + +							
ev 4076	X2*	189 223 249 278	153 195 225 226 227	G = -G A $+ +T$ T							
it 4202	X2*	189 223 255 278	146 153 195 225 227	G A + A T T + C ++ + G+ T							
it 204	X2*	189 223 255 278	146 153 195 225 227	G G + A T TA $+ + + + G+T$ T							
gm 30	X2*	189 223 256A 266 274 278 304 (390)	195	G +- ATC+ T C T+ C + G+ GT							
tu 83	X2*	189 223 266 278	153 195 225	G+ + - G + A T A + + A++ + G+T T							
tu 131 le 376	X2* X2*	189 223 266 278	153 195 225 226	G = - + - GA + A T TA + CA++ + G+T T + C+ C							
er 70	X2*	189 223 274 278	153 195	G+- + - $G$ + AC T A + + A+ + G+T T							
alb 128	X2*	189 223 278	143 195 225 227	G+ GA C+ A A + + ++-+ +T T +							
ir 241	X2*	189 223 278	153 195	G - + - G A C + +T T							
tu 265	X2*	189 223 278	153 195	G+- + - $GA$ + A AC A + A + +T T							
ng 144	X2*	189 223 278	153 195 198 225	G A + + T							
tu 212 ir 267	X2* X2*	189 223 278	153 195 225	$G_{+-} - + - GA + A$ T TA $+$ $+$ $A_{++} + G_{+}T$ T $+$ $G_{-} - + - \Delta$ TAC $+$ $\pm T$ T							
fr 53	X2*	189 223 278	16527 153 195	G+- + GA + A + A + +T T							
ir 254	X2*	189 223 278	195	G - + - G T A A CA + G+T T							
le 547	X2*	189 223 278	195	G G A C + G+T T							
le 550	X2*	189 223 278	195	G - A C + + T							
tu 264	X2*	189 223 278	195	G + - GA + A T A C T+ C + G+T GT							
10 992 10 926	X2* V2*	189 223 278 293	146 153	G - G -TG T A A T A CC +CATG+TGAAT TTT							
ad 40	72° X2*	107 223 278 311 337 189 223 278 311G	155 175 225 227 153 195 198 249d	с с + +т Т с А + +т Т							
sy 289	X2*	189 223 278 335	195 196 2004	G + - + A T C T+ CA + + GT							
kw 148	X2*	189 223 278 344	153 195	G+- + - + A + + A + + T							
sa 844	X2*	189 223 278 344	153 195 225	G - + - T A + G + T							
it 154	X2*	189 223 278 344	153 195 225	G++-G + A $TA$ + $CA$ + + $T$ T							

## Table 2 (continued)

	Control Region Sequence <sup>a</sup>			CODING REGION SEQUENCE AND RFLP VARIATION <sup>b</sup>								
SEQUENCE	Haplogroup	HVS-I <sup>d</sup>	HVS-II*	112233344 7475855679 0113335230 9357111427 lckg w k	555666677 114233755 482268978 760193105 we	11 7788888900 5735579827 8990701359 5213253040 e e i	111111111111 011112223333 900890360377 228798903608 400724781645 u vant	1111111 3334444 889455 5766068 7965207 s	1111111 14445555 56783669 38661572 77710425 i			
i+ 4227	V2*	189 222 278 244	16510 152 105 225 226	<u> </u>	C   ]			C L T				
os 107	X2*	189 261 278	153 195 225 226	G	G + A G + A	T TA	+ + +	+T	T T			
kw 172	X2*	189 261 278	153 195 225	G +	GA + A	A	+ A + +	+T	Т			
le 1056	X2*	189 266 278	64 153 195 225 226	G – –	G		+	+T	Т			
kw 237	X2*	189 278	153 195 225	G+ + -	G + A	T TA +	+ A + +	+T	Т			
it 8 io 761	X2* X2*	189 278 69 157 186 189 223 278 352	153 195 225	G+ + - I	G + G	TA +	C + +	+ +T	T			
ir 404	X2*	86 162 179 189 223 278 352	153 195 225	G+ + - 1	G + A	T TA +	+ G + +	+T	Т			
ng 68	X2*	93 189 223 248 278	153 195 225	G – –	G	A	+	+ T	Т			
tu 145	X2*	93 189 223 278	153 195 225	G+ + -	G + A	T TA +	+ A++ +	G+T	Т			
ho 78	X2b X2b	129 189 223 278	153 195 225 226	A –	+AA		+ + -	+				
tu 42	X2b X2b	175 189 223 278	146 133 193 223 226	- G - A	++AA	- -T+T C	+ -	+	_			
it 13	X2b	179 181 189 223 278	153 195 225	G – – A	++ A	- +T C	+ ++ -	+	-			
ev 4107	X2b	189 223 234 278	153 195 225 226	G –			-	+				
alg R34	X2b	189 223 239 278 301	153 195 225 226 (357)	-		-	-	+				
mo B45	X2b X2b	189 223 248 278 294	153 195 225 226	- C - D	7 1177	- 		+	_			
es 15	X2b	189 223 278	153 195 225 226	G –	++AG	-T+	+ + -	G+	-			
sy 184	X2b	189 223 278	153 195 225 226	G – A	++AA	-T+T C	+ –	+	-			
alg R47	X2b	189 223 278 316T	153 195 225	-		-	-	+				
hu 16 ular	X2b X2b	189 278 355 365	153 195 225 226 275	G – A	++ A	- +T C	-	+	-			
mo D17	X2b X2b	93 189 223 278	153 195 198 225			-	_	+				
tu 77	X2b	93 189 223 278 292	146 153 195 225 226	G – A	++AA	-T+T C	+ + -	G+	-			
sw 44	X2c	108 189 223 255 278	153 195 225 227	G –		С	+	+				
es 39	X2c	108 189 223 255 278 298	153 195 225 227	G+G+A-T	GATC++AAA	AC+T+CAC+T	CAT+TCA++-+C	ATG+TG	ATGTTT+			
sw 18 kir 16m	X2c	108 189 223 255 278 511	153 195 225 227	G = G =		С	+	G+ +				
ng 250	X2c	189 223 255 278	153 195 198 225	G -		C	+	+				
es 314	X2c	189 223 255 278	153 195 225	G+	+ A	T C +	+ ++ +	G+				
alb 124	X2c	189 223 255 278 300 311	153 195 225	G – –	C+ A	С	+ ++ +	+				
arb 60 tu 144	X2c X2d	93 189 223 249 255 278	153 195 225 227	G - 2 -	A + C	C	+ + -	+ C+	G			
ir 347	X2d	133 189 223 278	195 204	A -	G + G	+ +		+	0			
mo C16	X2d	189 192 223 278	195 207		A			+				
it 350	X2d	189 192 223 278	195 207	A -	A + G	+C+		+				
it 80 it 82	X2d X2d	189 215 223 278	195	G -	A + G A +	C	+ -	+				
it 101	X2d	189 223 278	195 204 207	G -	G C+ G	+C+	- + -	+				
ad 37	X2d	189 223 278	195 204 207	G –	G			+				
kr 71	X2d	189 223 278	195 204 207	G -	G			+				
go 57 ab 21	X2d X2d	189 223 278	204 207	A -	G + G C	+C+		+				
ho 83	X2d X2d	189 223 278 299	195 204 207	G –	G + G	С	- + -	G+				
tu 162	X2d	189 223 278 326	195 204 207	A –	G + G	+C+	- + -	G+				
jo 958	X2d	189 223 278 352	195	G –	G	С	+ C -	+	Т			
ku 31 ar 31	X2d X2d	92 185 189 223 278	195 204 207	G -	G C C+ C	+C+		+				
ku 26	X2e	124 189A 223 278	153 195 225	G -	G CI G	TCT.	c +	+	GC			
ku 14	X2e	129 189 223 265 278	153 195	G –			T +	+	GC			
ku 39	X2e	172 189 223 278	153 195	G –			T +	+	GC			
go 3	X2e	189 223 234 250 278	153 195	G+-	A C+	T +	+ T + +	G+	GC			
go 78 go 28	X2e X2e	189 223 264 278	153 195	G+- G -	A C+ A + A	CT +	+ 1 + + T +	+	GC			
eg 596	X2e	189 223 278	153 195	G –	A		т +	+	GC			
kr 18	X2e	189 223 278	153 195	G –			T +	+	GC			
ir 184 ar 119	X2e	189 223 278	153 195	G -			T +	+	GC			
ar 118 alt 81	X2e	189 223 278	153 195	G -	A C+ A	ч.С.	т + Стт	+	GC			
alt 161	X2e	189 223 278	153 195	- G -			СТТ	+	GC			
alt 171	X2e	189 223 278	153 195	G –			СТ Т	+	G			
alt 188	X2e	189 223 278	153 195	-			СТТ	+	GC			
alt 208 om 168	X2e X2e	189 223 278	153 195	G - G+-	A C+	Ст +	υΤ Τ + Τ + -	+ G+	GC			
gm 42	X2e	189 223 278	153 195	G -	+	U 1 1	т +	+	GC			
gm 6	X2e	189 223 278	153 195	G	C+ A	AC	CTT +	+	GC			
go 111	X2e	189 223 278	153 195	G –	+ A	СТ	T +	+	GC			
go 60 alt 17	X2e X2a	189 223 278	153 195	G –	C+	C	T +	+	GC			
all 17	A20	10/ 223 2/0	133 173	G =			1 +	+	60			

### Table 2 (continued)

$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			CONTROL REGION SEQUENCE <sup>a</sup>			CODING REGION SEQUENCE AND RFLP VARIATION <sup>b</sup>							
go 6X2c189 223 278153 195G -C + A C T+ T + + G + GCgm 120X2c189 223 278153 195G -C + C CT + + + GCalt 43X2c189 223 278153 195 215-C T T+ GCalt 16X2c189 223 278153 195 225G -+ T C T + + + GCes 167X2c189 223 278153 195 225G -+ T C T + + + GCes 297X2c189 223 278153 195 225G -+ A + A + + T + T + + + + GCes 297X2c189 223 278195 207G -A + A A + T C T + T + + G + ACgm 164X2c189 223 278 316195G -C + A + A T C C + T + + G + ACgm 164X2c189 223 278 316195G C + T C C + T + + ACgm 66X2c189 223 278 316195G -A + A C T + T + + ACgm 66X2c189 223 278 316195G -T + T + + ACst 115X2c189 223 278 316195G -T + T + + ACst 115X2c189 223 278 316195G -T + T + + ACgr 5X2c189 223 278 310153 195G -T + T + + C + + C + C + C + C + C + C +	Sequence <sup>c</sup>	Haplogroup	HVS-I <sup>d</sup>	HVS-II°	1122333 74758556 01133352 93571114 lckg w	34455566667 67911423375 23048226897 42776019310 k we	778888 573557 899070 521325 e e	1111 890001 982790 135922 304040 j	.1111 .1112 00890 28798 00724 u	.1111 22233 3603 9036 17816 ygn	1111 3333 3778 5085 5457 nt	.11111 333444 389455 576606 796520 s	.11111111 44445555 56783669 588661572 )77710425 i
gm120X2e1891232781531956-CT++6Calt 43X2e189223278153195-CT++6Calt 64X2e189223278153195215-CT++6Ces 167X2e189223278153195225G-+T+++ <td< th=""><th>g0 6</th><th>X2e</th><th>189 223 278</th><th>153 195</th><th>G -</th><th>C+ A</th><th>СТ</th><th></th><th>+ T</th><th>' +</th><th>+</th><th>G+</th><th>GC</th></td<>	g0 6	X2e	189 223 278	153 195	G -	C+ A	СТ		+ T	' +	+	G+	GC
at 43X2e19213278133195-CCTTHGCah 16X2e189223278153195215-CTT+GCah 16X2e189223278153195225G-+TT+++GCes297X2e189223278153195225G-A+++	gm 120	X2e	189 223 278	153 195	G -	C+	c		т	,	+	+	GC
alt I6   X2   189 223 278   153 195 215   -   C   T   +   C   C   T   +   C     cs 167   X2e   189 223 278   153 195 225   G   -   A   +   T   C   T   +   +   C   c   s   6   6   -   A   +   A   T   +   6   AC     cs 297   X2e   189 223 278 316   195 207   G   -   A   +   A   T   +   6   AC     gm 64   X2e   189 223 278 316   195   G   -   +   T   C   T   +   A   AC   mt   AC	alt 43	X2e	189 223 278	153 195	_			С	тт	,		+	GC
n10012010010011001100 <td>alt 16</td> <td>X2e</td> <td>189 223 278</td> <td>153 195 215</td> <td>-</td> <td></td> <td></td> <td>c</td> <td>т т Т Т</td> <td>,</td> <td></td> <td>+</td> <td>GC</td>	alt 16	X2e	189 223 278	153 195 215	-			c	т т Т Т	,		+	GC
s 297   X2e   199 223 278   133 195 225   G+   A   ++ A   ++ T   T   ++ +   +   GC   +     ir 304   X2e   189 223 278 316   195 207   G   -   A   T   +   +   A   C   +   +   A   C   +   +   A   C   +   +   A   C   +   +   A   C   +   +   A   C   +   +   +   A   C   +   +   A   C   T   +   +   A   C   T   +   +   A   C   T   +   +   A   C   T   +   +   A   C   T   +   +   A   C   T   +   A   C   T   +   A   C   T   +   +   A   C   T   +   A   C   T   +   A   C   T   +   A   A   T   +   A   C   T   +   A   A   C   T	es 167	X2e	189 223 278	153 195 225	G -	+	т	C		,	+	+	GC
ir 304X2e189 223 278195 207G-ATHGACcz 55X2e189 223 278 316195G-AATCT++ACgm 64X2e189 223 278 316195G+TCT++ACgm 66X2e189 223 278 316195G-+TCT++ACgm 64X2e189 223 278 316195G-A+ACT++ACkw 225X2e189 223 278 316 319 368195 207G-A+ACT++ACgr 5X2e189 223 278 316 319 363153 195GT++CCgr 5X2e189 223 278 316 319 516537 153 195GT++CCgr 5X2e189 423 278153 1951633 151 195 225C+A+T++CCit 56X2e93 189 223 278153 195A-+AT+++CCit 57X2e93 189 223 278153 195 196G-A+AT+++CCit 56X2e93 189 223 278153 195 195 67G-A+AT+<	es 297	X2e	189 223 278	153 195 225	G+	A ++ A	+ +T	+	Т	. +	+	+	GC +
cz 55X22189 223 278 316195GA+ ATCTTT </td <td>ir 304</td> <td>X2e</td> <td>189 223 278</td> <td>195 207</td> <td>G -</td> <td>А</td> <td></td> <td></td> <td>т</td> <td>-</td> <td>+</td> <td>G+</td> <td>AC</td>	ir 304	X2e	189 223 278	195 207	G -	А			т	-	+	G+	AC
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	cz 55	X2e	189 223 278 316	195	G	A + A	т	С	T+ T	' +	+	G+	AC
gen 60X2e199 223 278 316195 $C + C + A - T C + T + + AC + TTA + + + - CAT + TGA T + CTT + Tgm 96X2e189 223 278 316195C -+TC + T++ACkw 225X2e189 223 278 316 319 368195 207G -A++ACAT++ACkw 225X2e189 223 278 325152 153 195G -T++ACAT++ACat 159X2e189 223 278 325152 153 195G -T++CCCT++ACgr 5X2e189 A 223 257 271 27816533 16537 153 195 225G +A+TT++CCgr 5X2e189 A 223 257 271 27816533 16537 153 195 225G +A+TT++CCgr 5X2e189 A 223 256 278153 195A-+T++CCCit 56x57X2e93 189 223 278 324 325153 195A-+T++CCCit 114X2eY3 189 223 278153 195 257G -C +A+T++CGCgo 105X2f104 189 223 278153 195 257G -C +A CT +++++GGGgo 110X2f104 189 223 278153 195 257G -<$	gm 164	X2e	189 223 278 316	195	G -	C+ A	T	Ċ	C+ T	' +	+	G+	AC
o my m<	gm 66	X2e	189 223 278 316	195	G+G+A	A-TGATC++AA	- AC+T+Ti	AC+TTA	C+TT	'A++-	-+CI	ATG+TC	AATACTT+
if 81X2191921327831619191910T++ACkw 225X2e189223278316319368195207G-A+ACT+++ACsa 1159X2e189223278325152153195G-T+++Cgr 5X2e18922327712781653316537153195G-T++CCgr 55X2e189223265278153195A-+AT+T++GCgr 57X2e93189223265278153195A-+AT+++GCgm 156X2e93189223278153195G-AA++++GCgo 105X2f104189223278153195157G-ACT++++GCgo 105X2f104189223278153195157G-ACT+++GCgo 110X2f104189223278153195257G-CAAC <td>9m 96</td> <td>X2e</td> <td>189 223 278 316</td> <td>195</td> <td>G -</td> <td>+</td> <td>Т</td> <td>C</td> <td>С+ Т</td> <td>-</td> <td>+</td> <td>+</td> <td>AC</td>	9m 96	X2e	189 223 278 316	195	G -	+	Т	C	С+ Т	-	+	+	AC
kw 225X2c189 223 278 316 319 368195 207G -A + AC T + T ++ACsa 1159X2c189 223 278 325152 153 195G -T +++Calt 61X2c189 223 278 38016533 16535 16537 153 195G -T +++Cgr 5X2c189 A 223 278 771 27816533 16535 16537 153 195G -A ++T ++C ++GCgr 5X2c189 A 223 278195 225G +A ++T ++GCGCit 56X2c93 189 223 265 278153 195A -+A T+T ++GCgm 156X2c93 189 223 278153 195A -+A C T +++GCgo 105X2f104 189 223 278153 195 196G -A C +A C T +++GCgo 105X2f104 189 223 278153 195 257G -A C +A C T +++GCgo 110X2f104 189 223 278153 195 257G -A C +A C T +++GCgm 172X2f136 189 223 278153 195 257G -A +A C T +++++go 110X2f189 223 278153 195 257G -A +A C T ++++go 110X2f189 223 278153 195 257G -A +A C T ++++go 123X2f189 223 278	ir 81	X2e	189 223 278 316	195	G -	А	-	-	т	,	+	+	AC
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$37$ $322$ $189 A 223 278$ $195 225$ $6^{+}$ $A + + + C T + C + + + + G^+$ $GC$ ir 56 $X2e$ $93 189 223 265 278$ $153 195$ $A - + A$ $T + T + + G^+$ $GC$ ir 57 $X2e$ $93 189 223 265 278$ $153 195$ $A - + A$ $T + T + + G^+$ $GC$ gm 156 $X2e$ $93 189 223 278$ $153 195$ $A - + A$ $T + + T + + G^+$ $GC$ tu 114 $X2e$ $93 189 223 278$ $215 3195 196$ $G - A + A$ $T + + T + + G^+$ $GC$ go 105 $X2f$ $104 189 223 278$ $153 195 257$ $G - C + T$ $+ + + G^+$ go 110 $X2f$ $104 189 223 278$ $153 195 257$ $G - A + A C T + + + + G^+$ go 17 $X2f$ $104 189 223 278$ $153 195 257$ $G - C + A G C T$ $+ + + G^+$ gm 172 $X2f$ $136 189 223 278$ $153 195 257$ $G - A + A C T + + + G^+$ gm 174 $X2f$ $189 223 278$ $153 195 257$ $G - A + A C T + + + G^+$ go 133 $X2f$ $189 223 278$ $153 195 257$ $G - A + A C T + + + + G^+$ go 133 $X2f$ $189 223 278$ $153 195 257$ $G - A + A C T + + + + G^+$ go 24 $X2f$ $189 223 278$ $153 195 257$ $G - A + A A C T + + + + G^+$ $at 101$ $X2f$ $189 223 278$ $153 195 257$ $G - C + A C T + + + + G^+$ $at 101$ $X2f$ $189 223 278$ $153 195 257$ $G - C + A C T + + + + G^+$ $at 101$ $X2f$ $189 223 278$ $153 195 257$ $G - C + A C T + C + + G^+$ <td>gr 5</td> <td>X2e</td> <td>189A 223 257 271 278</td> <td>16533 16535 16537 153 195 225</td> <td>G+</td> <td>A +</td> <td>ТТ</td> <td>+</td> <td>+ C</td> <td>: ++</td> <td>+</td> <td>G+</td> <td>GC</td>	gr 5	X2e	189A 223 257 271 278	16533 16535 16537 153 195 225	G+	A +	ТТ	+	+ C	: ++	+	G+	GC
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b   114   X2e   93 189 223 278 324 325   153 195 196   G -   A + A   + T   + T   + +   GC     go 105   X2f   104 189 223 278   153 195 257   G -   C+   T   +   +   +     go 110   X2f   104 189 223 278   153 195 257   G -   A C+   A AT   T   +   +   +   G+     go 41   X2f   104 189 223 278   153 195 257   G -   A C+   A AT   T   +   +   G+     go 41   X2f   104 189 223 278   153 195 257   G -   C + A GC T   + </td <td>gm 156</td> <td>X2e</td> <td>93 189 223 278</td> <td>153 195</td> <td>G+-</td> <td>A C+ A</td> <td>СТ</td> <td>+</td> <td>+ T</td> <td>. +</td> <td>+</td> <td>G+</td> <td>GC</td>	gm 156	X2e	93 189 223 278	153 195	G+-	A C+ A	СТ	+	+ T	. +	+	G+	GC
go 105   X2f   104 189 223 278   153 195 257   G -   C +   T   +   +     go 110   X2f   104 189 223 278   153 195 257   G -   A C + A AT T   +	tu 114	X2e	93 189 223 278 324 325	153 195 196	G -	A + A			+ T		+	+	GC
go 110   X2f   104 189 223 278   153 195 257   G -   A C + A AT T   + <td< td=""><td>go 105</td><td>X2f</td><td>104 189 223 278</td><td>153 195 257</td><td>G -</td><td>C+</td><td>т</td><td></td><td></td><td></td><td>+</td><td>+</td><td></td></td<>	go 105	X2f	104 189 223 278	153 195 257	G -	C+	т				+	+	
go 41   X2f   104 189 223 278   153 195 257   G+G+A-TGATC++AAAT+T+TAC+TCAT+TCA++++CATG+TGAATGTTT+     go 17   X2f   129 140 189 223 278   153 195 257   G -   C + A GC T   +   +   +   G+     gm 172   X2f   136 189 223 278   153 195 257   G -   A + AC T   +   +   G+     gm 140   X2f   189 223 278   153 195 257   G -   +   A + AC T   +	go 110	X2f	104 189 223 278	153 195 257	G -	A C+ A	- АТ Т		+	+	+	G+	
go 17   X2f   129 140 189 223 278   153 195 257   G -   C + A GC T   + + + + G+     gm 172   X2f   136 189 223 278   153 195 257   G -   A + AC T   + + + G+     gm 140   X2f   189 223 278   153 195 257   G -   + T   + +     le 208   X2f   189 223 278   153 195 257   G -   + AC T   + +     go 133   X2f   189 223 278   153 195 257   G -   + AC T   + +     go 24   X2f   189 223 278   153 195 257   G -   + AT   + +     go 24   X2f   189 223 278   153 195 257   G -   + A AC   + +   +     go 24   X2f   189 223 278   153 195 257   G -   + A AC   + +   +     go 24   X2f   189 223 278   153 195 257   G -   A AC   + +   +     tu 344   X2f   189 223 278   195 257   G -   C   T C   + +   +     tu 344   X2f   189 223 278   195 257   G -   C + A AC T   + C +   +   T <td>go 41</td> <td>X2f</td> <td>104 189 223 278</td> <td>153 195 257</td> <td>G+G+A</td> <td>A-TGATC++AA</td> <td>AT+T+T</td> <td>AC+TCA</td> <td>T+TC</td> <td>:A++-</td> <td>-+C7</td> <td>ATG+TC</td> <td>AATGTTT+</td>	go 41	X2f	104 189 223 278	153 195 257	G+G+A	A-TGATC++AA	AT+T+T	AC+TCA	T+TC	:A++-	-+C7	ATG+TC	AATGTTT+
gm 172   X2f   136 189 223 278   153 195 257   G -   A + AC T   + + + G+     gm 140   X2f   189 223 278   153 195 257   G -   + + T   + +     le 208   X2f   189 223 278   153 195 257   G -   + AC T   + +   +     go 133   X2f   189 223 278   153 195 257   G -   + AC T   + +   +     go 24   X2f   189 223 278   153 195 257   G -   + A T   + +   +     tu 344   X2f   189 223 278   153 195 257   G -   A + A C T   + +   +     tu 344   X2f   189 223 278   153 195 257   G -   A + A C   + +   +     tu 344   X2f   189 223 278   153 195 257   G -   C   T C   + +   +     tu 41m   X2f   189 223 278   195 257   G -   C   T C   + +   T     ar 101   X2f   189 223 278   195 257   G -   C   A C T   + C + +   G+   T     gm 143   X2f   86 189 223 278 <t< td=""><td>go 17</td><td>X2f</td><td>129 140 189 223 278</td><td>153 195 257</td><td>G -</td><td>C+ A</td><td>GC T</td><td></td><td>+</td><td>+</td><td>+</td><td>G+</td><td></td></t<>	go 17	X2f	129 140 189 223 278	153 195 257	G -	C+ A	GC T		+	+	+	G+	
gm 140   X2f   189 223 278   153 195 257   G -   +   T   +   +   +     le 208   X2f   189 223 278   153 195 257   G -   +   A AC T   +   +   +     go 133   X2f   189 223 278   153 195 257   G -   +   A AT   +   +     go 24   X2f   189 223 278   153 195 257   G -   +   A T   +   +     u 344   X2f   189 223 278   153 195 257   G -   +   A T   +   +     u 344   X2f   189 223 278   153 195 257   G -   A + A AC   +   +   +     u 344   X2f   189 223 278   153 195 257   G -   C   T C   +   +   +     u 344   X2f   189 223 278   195 257   G -   C   T C   +   +   +   +   +   +   +   +   +   +   +   +   +   +   +   +   +   T   ar 101   X2f   189 223 278   195 257 <t< td=""><td>gm 172</td><td>X2f</td><td>136 189 223 278</td><td>153 195 257</td><td>G -</td><td>A +</td><td>AC T</td><td></td><td>+</td><td>+</td><td>+</td><td>G+</td><td></td></t<>	gm 172	X2f	136 189 223 278	153 195 257	G -	A +	AC T		+	+	+	G+	
be 208   X2f   189 223 278   153 195 257   G -   + A AC T   +   +   +     go 133   X2f   189 223 278   153 195 257   G -   + A T   +   +     go 24   X2f   189 223 278   153 195 257   G -   + A T   +   +     u 344   X2f   189 223 278   153 195 257   G -   + A AC   +   +     u 344   X2f   189 223 278   153 195 257   G -   A AC   +   +     at 140   X2f   189 223 278   195 257   G -   C   T C   +   +     gm 143   X2f   189 223 278   195 257   G -   C   T C   +   +     gm 143   X2f   189 223 278   195 257   G -   C   +   +   +     gm 143   X2f   86 189 223 278   153 195 257   G -   C   +   +   +     go 39   X2f   93 189 223 278   153 195 257   G -   C + A AC T   +   +   +	gm 140	X2f	189 223 278	153 195 257	G -	+	Τ.				+	+	
go 133   X2f   189 223 278   153 195 257   G -   +   AT   +   +     go 24   X2f   189 223 278   153 195 257   G -   +   AT   +   +     u 344   X2f   189 223 278   153 195 257   G -   A   +   A   +   +   +     td 41m   X2f   189 223 278   153 195 257   G -   A   +   A AC   +   +   +     td 41m   X2f   189 223 278   195 257   G -   C   T C   +   +   T     ar 101   X2f   189 223 278   195 257   G -   C + A AC T   + C +   +   T     ar 140   X2f   189 223 278   153 195 257   G -   -   +   +   +     gn 143   X2f   86 189 223 278   153 195 257   G -   C + A AT T   +   +   +     go 39   X2f   93 189 223 278   153 195 257   G -   C + A AC T   +   +   +     go 39   X2f   93 189 223 278   153 195 257	le 208	X2f	189 223 278	153 195 257	G -	+ A	AC T		+		+	+	
go 24   X2f   189 223 278   153 195 257   G -   + A T   + +     tu 344   X2f   189 223 278   153 195 257   G -   A + A AC   + + +     tu 344   X2f   189 223 278   195 257   G -   C   T C + +   +     td 41m   X2f   189 223 278   195 257   G -   C   T C + +   T     ar 101   X2f   189 223 278   195 257   G -   C + A AC T   + C + +   G+   T     ar 140   X2f   189 223 278 311   153 195 257   G -   -   +   +   +     gm 143   X2f   86 189 223 278   153 195 257   G -   C + A AT T   +   +   +     go 39   X2f   93 189 223 278   153 195 257   G -   C + A AC T   +   +   +	go 133	X2f	189 223 278	153 195 257	G -	+	AT				+	+	
b 189 223 278 153 195 257 G - A + A + + +   td 41m X2f 189 223 278 195 257 G - C T C + + +   ar 101 X2f 189 223 278 195 257 G - C T C + + T   ar 140 X2f 189 223 278 311 153 195 257 G - C + + +   gm 143 X2f 86 189 223 278 153 195 257 G - + + +   go 39 X2f 93 189 223 278 153 195 257 G - - + + +	go 24	X2f	189 223 278	153 195 257	G -	+ A	т				+	+	
td 41m X2f 189 223 278 195 257 G - C T C + T   ar 101 X2f 189 223 278 195 257 G C + A AC T + C + + G + T   ar 140 X2f 189 223 278 311 153 195 257 G C + A AC T + C + + G + T   gm 143 X2f 86 189 223 278 153 195 257 G - C + A AT T + + + G +   go 39 X2f 93 189 223 278 153 195 257 G - A C T + + + G +	tu 344	X2f	189 223 278	153 195 257	G -	A + A	AC		+		+	+	
ar 101   X2f   189 223 278   195 257   G   C + A AC T   + C + + G + T     ar 140   X2f   189 223 278 311   153 195 257   G -   + +     gm 143   X2f   86 189 223 278   153 195 257   G -   -   + +     go 39   X2f   93 189 223 278   153 195 257   G -   C + A AT T   + + + G +	td 41m	X2f	189 223 278	195 257	G -		C		тс		+	+	т
ar 140   X2f   189 223 278 311   153 195 257   G -   + +     gm 143   X2f   86 189 223 278   153 195 257   G -   C+ A AT T   + + +     go 39   X2f   93 189 223 278   153 195 257   G -   C+ A AT T   + + +   G+	ar 101	X2f	189 223 278	195 257	G	C+ A	AC T		+ C	: +	+	G+	- T
gm 143 X2f 86 189 223 278 153 195 257 G - C+ A AT T + + + G+ go 39 X2f 93 189 223 278 153 195 257 G - A C+ A AC T + + + G+	ar 140	X2f	189 223 278 311	153 195 257	G -						+	+	-
go 39 X2f 93 189 223 278 153 195 257 G - A C + A AC T + + + G +	gm 143	X2f	86 189 223 278	153 195 257	G -	C+ A	ΑΤ Τ		+	+	+	G+	
	go 39	X2f	93 189 223 278	153 195 257	G -	A C+ A	AC T		+	+	+	G+	

<sup>a</sup> Two hypervariable segments of the control region were sequenced: HVS-I (16024-16383) and HVS-II (16518-310). Nucleotide change is specified for transversions; d = deletion. Length variation in the C stretch (16184-16193) is not shown. Mutations that were sequenced outside the specified range are shown in parentheses. Sequence analyses were performed using the Sanger dideoxy chain-termination method with the Amersham DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech) on an ABI PRISM 377 DNA Sequencer. Sequences were aligned and analyzed with the Wisconsin Package (GCG).

<sup>b</sup> Restriction-endonuclease sites are indicated as follows: c = DdeI, e = HaeIII, g = HinfI, i = MspI, j = MboI, k = RsaI, l = TaqI, m = BamHI, s = AccI, t = BstOI, u = MseI, w = MboII, y = BfmI. Haplogroup assignments are according to figure 2.

<sup>c</sup> Populations are indicated by the following letter code: ab = Abazin, ad = Adygei, alb = Albanian, alg = Algerian, alt = Altaian, ar = Armenian, arb = Arab from Uzbekistan, be = Bengali, cz = Czech, dr = Druze, eg = Egyptian, es = Estonian, et = Ethiopian, ev = Evenk, fr = French, gm and go = Georgian, gr = Greek, ho = Croat, hu = Hungarian, ir = Iranian, it = Italian, jo = Jordanian, kir = Kyrgyz, kr = Karachay, ku = Kumyk, kw = Kuwaiti, le = Lebanese, mo = Moroccan, ng = Nogay, os = Ossetian, sa = Saudi Arabian, sw = Swede, sy = Syrian, td = Tadjik, tu = Turk, ukr = Ukrainian.

<sup>d</sup> HVS-I nucleotide positions are -16000.

<sup>e</sup> All listed HVS-II sequences also harbored the transitions 16519, 73, and 263.

<sup>f</sup> Revised Cambridge reference sequence (Andrews et al. 1999).

Mediterranean Sea and the Red Sea (table 1). This subhaplogroup is subdivided into the two clades X1a and X1b, which are defined by two and five coding region mutations, respectively (fig. 2). Both clades share a recurrent transition at 146 in HVS-II. The coalescence time of the entire X1 subhaplogroup using HVS-I variation is 42,900  $\pm$  18,100 YBP, whereas the coalescence time of the X1a clade is 17,900  $\pm$  11,900 YBP.

Virtually all (97.2%) haplogroup X mtDNAs from the Near East, the South Caucasus, and Europe were found to belong to subhaplogroup X2, as did all (100%) of those from Siberia and Central Asia and some (36.8%) of those from North Africa (table 2). Thus, subhaplogroup X2 is characterized by a very wide geographic range but also by an infrequent occurrence. Indeed, it generally comprises <5% of the mtDNAs in West Eurasian and North African populations (table 1). Three exceptions include the Druze, the Georgians, and the Orkney Islanders, among whom the frequency of X2 reaches 11%, 8%, and 7%, respectively. The high frequencies of X2 in the Druze and the Orkney Islanders are combined with a low haplotype diversity (0.400 and 0.473, respectively), and the relatively high frequency in these populations is most likely due to genetic drift and founder events. Overall, it appears that the populations of the Near East, the Caucasus, and Mediterranean Europe harbor subhaplogroup X2 at higher frequencies than those of northern and northeastern Europe (P <.05) and that X2 is rare in Eastern European as well as Central Asian, Siberian, and Indian populations and is virtually absent in the Finno-Ugric and Turkic-speaking people of the Volga-Ural region. Coalescence time estimates based on HVS-I and coding region variation- $17,900 \pm 2,900$  YBP and  $21,600 \pm 4,000$  YBP, respectively (figs. 1 and 2)-are consistent with the range expansion of X2 around or after the last glacial maximum (LGM). It is intriguing that the estimated coalescence time for X2 alone is very close to that obtained from HVS-I data for the entire haplogroup X (20,200  $\pm$  3,100 YBP) (fig. 2). However, the latter is probably an underestimate due to both the higher proportion (>90%) of X2 mtDNAs included in the calculations and the fact that the HVS-I consensus sequence of X2 is completely identical to that of the overall haplogroup Х.

Two-thirds of the subhaplogroup X2 sequences fall into the five clades X2b-X2f (fig. 2). Two sequencesone from Lebanon and one from Georgia-lacked the transition at np 1719, suggesting either the presence of an early X2 branch or a reversion at that nucleotide position. The sister groups X2b and X2c (X1 and X2, respectively, in the work of Herrnstadt et al. 2002) encompass one-third of the European sequences (excluding the samples from the North Caucasus). It is of interest that some North African sequences (from Morocco and Algeria) belong to X2b as well. Subhaplogroup X2b shows a diversity that is consistent with a postglacial population expansion in both West Eurasia and North Africa. Clades X2e and X2f encompass the majority (87.1%) of the sequences from the South Caucasus area and show coalescence times  $(12,000 \pm 4,000 \text{ YBP})$  and  $10,800 \pm 5,000$  YBP, respectively) consistent with a Late Upper Paleolithic (LUP) origin and a subsequent spread in the region. We found significant differences between the haplogroup distribution between the North and the South Caucasian samples, a result that indicates a major geographical barrier between the two regions. The South Caucasian sample is enriched in mtDNAs belonging to clades X2e and X2f (P < .01), whereas the North Caucasian sample shows a higher proportion of sequences derived at nps 225 and 16248 (P < .01).

Clade X2e, defined by the synonymous substitution at 15310, encompasses all haplogroup X sequences in the Altaians (fig. 2). Among the nine Altaian X sequences, eight harbor the founder HVS-I motif of X2e, and seven of these eight also carry the HVS-II founder motif. As a result, a very low haplotype diversity of haplogroup X (0) in the Altaian region was obtained, making it significantly different from the haplotype di-

versities for haplogroups C and D (0.835 and 0.943, respectively) in the same region. Moreover, the nine Altaian mtDNAs do not harbor any nucleotide difference between nps 16090 and 16365. Therefore, under the assumption that these sequences are a random sample of the Altaian haplogroup X, an estimated  $\rho$  value <0.33 (P < .05) was obtained. This value corresponds to a time depth of <6,700 years (Forster et al. 1996), and it would suggest that Altaians have acquired haplogroup X2 only relatively recently. This scenario is supported by the concomitant presence in the Altaians of a wide range of other West Eurasian haplogroups (H, J, I, T, U1, U4, and U5) that comprise  $\sim 27\%$  of their mtDNAs. Indeed, any recent migration (for example, from the [southern] Caucasus region) that might have carried X2e mtDNA sequences to the Altai region would also explain the presence of the other West Eurasian mtDNA haplogroups in modern Altaians.

Further northeast of the Altai area, haplogroup X sequences were detected in the Tungusic-speaking Evenks, of the Podkamennaya Tunguska basin (Central Siberia). In contrast to the Altaians, the Evenks did not harbor any West Eurasian mtDNA haplogroups other than X. However, neither of the two Evenk X haplotypes showed mutations characteristic of the Native American clade X2a. Instead, one sequence was a member of X2b and the other of X2\* (fig. 2). Thus, one possible scenario is that several X haplotypes arrived in Siberia from western Asia during the Palaeolithic, but only X2a crossed Beringia and survived in modern Native Americans. Alternatively, the presence of two phylogenetically different haplogroup X mtDNA sequences in this particular subpopulation of Evenks might be due to recent gene flow.

The Native American-specific clade X2a appears to be defined by five mutations, three in the coding region (8913, 12397, and 14502) and two in the control region (200 and 16213) (fig. 1). The transition at np 200 was seen in virtually all previously analyzed Native American haplogroup X mtDNAs, whereas the transition at np 16213 was absent in some of the Ojibwa described by Brown et al. (1998). We surveyed our Old World haplogroup X mtDNAs for the five diagnostic X2a mutations (table 2) and found a match only for the transition at np 12397 in a single X2\* sequence from Iran. In a parsimony tree, this Iranian mtDNA would share a common ancestor with the Native American clade (fig. 2). Yet, the nonsynonymous substitution at np 12397 converting threonine to alanine cannot be regarded a conservative marker, as it has also been observed in two different phylogenetic contexts-in haplogroups J1 and L3e-among 794 complete mtDNA sequences (Finnilä et al. 2001; Maca-Meyer et al. 2001; Herrnstadt et al. 2002). Therefore, the scenario that the threonine to alanine change in the haplogroup X background is indeed due to recurrence appears most plausible.

These findings leave unanswered the question of the geographic source of Native American X2a in the Old World, although our analysis provides new clues about the time of the arrival of haplogroup X in the Americas. Indeed, if we assume that the two complete Native American X sequences (from one Navajo and one Ojibwa) began to diverge while their common ancestor was already in the Americas, we obtain a coalescence time of 18,000  $\pm$  6,800 YBP, implying an arrival time not later than 11,000 YBP.

The results of this study point to the following conclusions. First, haplogroup X variation is completely captured by two ancient clades that display distinctive phylogeographic patterns—X1 is largely restricted to North and East Africa, whereas X2 is spread widely throughout West Eurasia. Second, it is apparent that the Native American haplogroup X mtDNAs derive from X2 by a unique combination of five mutations. Third, the few Altaian (Derenko et al. 2001) and Siberian haplogroup X lineages are not related to the Native American cluster, and they are more likely explained by recent gene flow from Europe or from West Asia. Fourth, the split between "African" X1 and "Eurasian" X2 subhaplogroups of X is phylogenetically as deep as that within the branches of haplogroup U that also differ profoundly in their phylogeography. Thus, subhaplogroup U6 is largely restricted to North Africa (as X1), whereas subhaplogroup U5 is widespread in West Eurasia (as X2). The phylogeographic patterns and the coalescence times that we obtained here suggest that the basic phylogenetic structures of the mtDNA haplogroups in West Eurasia and North Africa are as ancient as the beginning of the spread of anatomically modern humans in this region. Finally, phylogeography of the subclades of haplogroup X suggests that the Near East is the likely geographical source for the spread of subhaplogroup X2, and the associated population dispersal occurred around, or after, the LGM when the climate ameliorated. The presence of a daughter clade in northern Native Americans testifies to the range of this population expansion.

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### **Electronic-Database Information**

Accession numbers and URLs for data presented herein are as follows:

- Estonian Biocentre, http://www.ebc.ee/EVOLUTSIOON/ mtDNA-X/ (for mtDNA sequence data)
- Shareware Phylogenetic Network Software, http://www .fluxus-engineering.com/sharenet.htm (for Network 3111)

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