Int J Legal Med (2004) 118 : 24–31
DOI 10.1007/s00414-003-0411-6

ORIGINAL ARTICLE

**François-Xavier Ricaut · Sergei Kolodesnikov
Christine Keyser-Tracqui · Anatoly Nikoyevich Alekseev
Eric Crubézy · Bertrand Ludes**

Genetic analysis of human remains found in two eighteenth century Yakut graves at At-Dabaan

Received: 4 July 2003 / Accepted: 14 October 2003 / Published online: 8 November 2003

Abstract We extracted DNA from three skeletons belonging to the Yakut population, which were excavated from the At-Dabaan site (dating back 300 years) in the Sakha Republic (Russia). Ancient DNA was analyzed by autosomal STRs (short tandem repeats) and by the sequencing of the hypervariable region 1 (HV1) of the mitochondrial DNA (mtDNA) control region. The results showed that these three skeletons were not close relatives but probably linked to the same clan structure. Comparison of their haplotypes with the haplotypes of 8,774 Eurasian individuals suggested a relative specificity and continuity of part of the Yakut mitochondrial gene pool during the last 3 centuries.

Keywords Siberia · Sakha · Ancient DNA · Mitochondrial DNA · Autosomal STRs

Introduction

Identification of ancient human remains by DNA analysis is a powerful tool in forensic and historical investigations (Jehaes et al. 2001). The methodology used in ancient DNA studies also allows to test the limits of commercial amplification kits to realise multiplex STR typing, as well as mtDNA protocols. Moreover, ancient DNA studies can be used to elucidate environmental factors that benefit DNA preservation.

The aims of this particular study were (i) to investigate the parentage relationships within Yakut burial groups and provide insight into their ancient funeral practice and (ii) to clarify the ancestor-descendant relationships between these ancient Yakut samples and the modern Sakha population.

The Sakha (also known as Yakuts) are an ethnic group living in north-eastern Siberia in the Sakha Republic, an autonomous republic within the Russian Federation. They differed completely in their traditional way of life (semi-nomadic cattle herders and horse breeders) and language (Turkic-speaking population) from their neighbors, who were Tungusic-speaking populations of reindeers herders or hunters (Balzer 1994; Forsyth 1996).

Archaeological and ethnohistorical data (Levin and Potatov 1964; Fondahl 1994; Alekseev 1996) as well as the recent genetic studies of modern Siberian/central Asian populations (Boeva 1988; Fefelova 1990; Pakendorf et al. 1999, 2002, 2003) suggested different hypotheses to explain the ethnogenesis of the Sakha. At present, it is generally accepted that the formation of the Sakha ethnic group could be the consequence of a Mongol or/and Turkish population migration northward from south Siberia/central Asia, around 800–1200 years ago. Genetic comparisons between the Sakha and other Siberian/central Asian populations did not, however, result in the clear determination of the origin and genetic evolution of this ethnic group. The difficulties encountered by molecular biologists could arise from many historic and demographic events since the formation of the Sakha population, such as the Russian colonization starting in the seventeenth century, and the high degree of isolation and genetic drift of the Siberian populations (Santos et al. 1999). These events could have obscured the ancestral Yakut gene pool. Consequently, a direct access to the gene pool of ancient Sakha individuals could lead to a better understanding of the genetic history of this Siberian population.

The excavation of two ancient Yakut graves from the eighteenth century located in central Yakutia are, to the

F.-X. Ricaut (✉) · C. Keyser-Tracqui · B. Ludes
Laboratoire d'Anthropologie Moléculaire,
Institut de Médecine Légale,
11 rue Humann, 67085 Strasbourg, France
Tel.: +33-39-0243348, Fax: +33-39-0243362,
e-mail: fx.ricaut@infonie.fr

F.-X. Ricaut · E. Crubézy · B. Ludes
Anthropobiologie, Université Paul Sabatier CNRS UMR 8555,
39 allées Jules Guesdes, 3100 Toulouse, France

S. Kolodesnikov
Institute of Archaeology and Ethnography,
Yakutsk State University,
Belinsky Street 58, 677891 Yakutsk, Russia

A. N. Alekseev
Yakutsk State University,
Belinsky Street 58, 677891 Yakutsk, Russia

best of our knowledge, the first opportunity to investigate the ancient Yakut gene pool. Two types of genetic markers were used to analyze these ancient human remains. Autosomal STRs allow determination of close genetic relationship within burial groups (Fily et al. 1998; Ricaut et al. 2003; Keyser-Tracqui et al. 2003) as well as the detection of possible modern DNA contaminations of ancient samples (Hummel et al. 2000). The hypervariable region 1 of the mtDNA with its haploid and maternal mode of inheritance allows maternal lineages to be followed and gives information on the origin and evolution of populations (Richards et al. 2000).

Materials and methods

During the summer of 2002 a French-Yakut scientific expedition excavated two ancient frozen graves at a location referred to as "At-Dabaan". These two graves belong to a larger funerary complex presenting numerous tombs dispersed over several kilometres on a quaternary terrace. This site is located in the Yakut department of Khangalasse, near the village of Ulak-Aan, 30 km from Yakutsk on the right side of the Lena river. The At-Dabaan site conforms to the ancient Altaic tradition (Roux 1963), and more specifically to the Yakut tradition, of looking over water and mountains. Indeed, deceased Yakuts were buried on the boundaries of settlements, usually in an elevated site from which a summer settlement was visible (Kolodesnikov 2000). Verbal tradition confirms that this location was the site of a summer camp (from the beginning of June until September) until the middle of the twentieth century. The tombs were noticed from a small depression at the surface of the meadow, and during excavation remains of a log case were found, a structure which is characteristic of traditional Yakuts graves. The two graves (AD1 and AD3) were 2 m from each other, and 2–10 m from the bank of an oxbow lake of the Lena river.

The first tomb (AD1) was an individual grave (95 cm in depth) containing a wooden sarcophagus with the very well preserved body of an individual wearing winter clothes. The second tomb (AD3) grave was a double grave (160 cm in depth) containing a wooden sarcophagus with two skeletons (AD3s and AD3n) wearing summer clothes. Anthropomorphic analysis suggested that skeleton AD1 belonged to an adolescent (epiphysis of the long bones not fused, third molar not erupted) and that skeletons AD3s and AD3n were those of two males. The exact causes of death were unknown.

The absence of iron in the construction of wooden cases is in accordance with an inhumation date prior to the nineteenth century. The orientation perfectly east/west of skeletons AD3s and AD3n as well as the presence of a Christian medal around their neck evoked a Christian tradition. However, it was the cut of the clothes that allowed this grave to be dated more precisely to the late eighteenth century. Grave AD1 seemed to be prior to christianization. Indeed, the body was not oriented east/west and no Christian artefact was found in the tomb. The presence of two earrings typical for Yakut women from the beginning of the eighteenth century seemed to date this grave to this period (O. Safedoseva, personal communication).

DNA extraction and purification

Ancient DNA was extracted from a femoral fragment of skeleton AD1 and from several metatarsi of skeletons AD3s and AD3n. To eliminate surface contamination, the outer surface of the bones was abraded to a depth of 2–3 mm with a sanding machine (Dremel, Breda, Netherlands). Powdered bone was then generated by grinding bone either with a drill fitted with a surgical trepan or under liquid nitrogen in a 6800 Freezer Mill (Fisher Bioblock, Illkirch, France).

DNA was carefully extracted from the bone powder according to Fily et al. (1998). Aliquots of 2 g of bone powder were suspended in an extraction buffer (5 mM EDTA, 2%SDS, 10 mM Tris HCL pH 8.0, 0.3 M sodium acetate, 1 mg/ml proteinase K) and incubated for 16 h at 56°C. After an organic extraction (phenol/chloroform/isoamyl alcohol, 25:24:1, v/v/v), the aqueous phase was purified with the CleanMix kit (Talent, Mundolsheim, France). Samples were then concentrated to 40 µl employing Microcon-30 filters (Millipore, Mundolsheim, France).

Autosomal STR analysis

Autosomal STRs were amplified with the help of the AmpFlSTR Profiler Plus kit (PE Applied Biosystems, Courtaboeuf, France) which allows simultaneous amplification of nine STRs (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820) and the amelogenin locus (for sex determination).

PCR conditions were modified from the manufacturer's conditions to reduce the volume of DNA extract used in the PCR reaction. Each amplification was carried out with a 10 µl reaction mixture containing 3.82 µl PCR reaction mix, 2 µl primer set, 0.182 µl AmpliTaq Gold (PE Applied Biosystems, Courtaboeuf, France) and 1–4 µl of ancient DNA sample. Cycling parameters were 94°C for 11 min, followed by 37 cycles with 94°C 1 min, 59°C 1 min, 72°C 1 min, and a final delay of 45 min at 60°C. The PCR products were analyzed on an ABI Prism 3100 (PE Applied Biosystems, Courtaboeuf, France) automated DNA sequencer.

Mitochondrial DNA analysis

Mitochondrial DNA analyses were performed on the first hypervariable segment of the control region (HV1). The HV1 region was divided into two overlapping sub-regions (a and b) amplified with the L15989 (Gabriel et al. 2001) /H16239 (Ivanov et al. 1996), and L16190/H16410 (Gabriel et al. 2001) primers, respectively. For sub-region a), we also used the primer H16167 (5'-GGGTTTGATGTGGATTGGG-3') to resolve amplification problems linked to the polycytosine region located between nucleotide positions 16184–16193 (Szibor and Michael 1999).

PCR amplifications were carried out in 50 µl of reaction mixture containing 2–6 µl of the ancient DNA extracts, 10 mM Tris HCL pH 8.3, 50 mM KCL, 1.5 mM $MgCl_2$, 1 mg/ml BSA, 200 µM each dNTP, 0.25 µM each primer and 2 U of Taq Gold Star (Eurogentec, Seraing, Belgium). Cycling parameters were 94°C for 10 min, followed by 38 cycles with 94°C for 30 s, 30 s at 48°C for HVIa or 30 s at 51°C for HVIb, 72°C for 45 s and 72°C for 5 min.

Amplification products were checked on a 1% agarose gel and purified with Microcon PCR filters (Millipore, Molsheim, France). Sequence reactions were performed on each strand, with the same primers than those employed for PCR amplification, by means of ABI Prism BigDye Terminator cycle sequencing Ready Reaction kit (PE Applied Biosystems, Courtaboeuf, France) according to the manufacturer's conditions. The sequence reaction products were analyzed on an ABI Prism 3100 (PE Applied Biosystems, Courtaboeuf, France) automated DNA sequencer.

Contamination precautions

To minimize the risk of contamination of the ancient samples, during each step of sample preparation (excavation, abrasion, extraction and amplification) all classical precautions cited by O'Rourke et al. (2000) were followed as closely as possible. The samples were always handled wearing gloves, face masks and laboratory coats (including during excavation). The entire process of DNA extraction and PCR amplification was carried out in a dedicated ancient DNA laboratory, routinely sterilized by treatment with DNAse away (Molecular BioProducts, San Diego, CA) bleach and ultraviolet light irradiation (254 nm), and using dedicated reagents and equipment. Extraction and amplification blanks were used as negative controls and all persons involved in processing samples

were genetically typed and compared to the results obtained from the ancient bone samples. Moreover, at least three extractions and three amplifications by extraction were made from each bone sample to assess the reproducibility of the results.

Statistical analysis

We analyzed the distribution of the mitochondrial haplotypes obtained from the three ancient Yakut samples in Eurasian populations, using the Blast 2.0 program (http://www.ncbi.nlm.nih.gov). The mtDNA sequences of 8774 individuals were used for comparative analysis (Table 1).

Table 1 Eurasian populations compared in this study

Region/population (size)	Reference
Europe (2,804)[1]	Richards et al. 2000
East Europe/West Asia (1,746)	
Komi (16)	Voevoda et al.[2]
Saami (115)	Sajantila et al. 1995
Khant (10)	Voevoda et al.[2]
Mordvinian (8)	Voevoda et al.[2]
Finns (403)	Meinila et al. 2001
North-east Europe (407)	Sajantila et al. 1995, 1996; Richards et al. 1996, 2000
Caucasian (315)	Macaulay et al. 1999; Richards et al. 2000; Lebedeva et al.[2]
Bielorussia (55)	Belyaeva et al.[2]
Mansi (98)	Derbeneva et al. 2002
Russia (143)	Orekhov et al. 1999; Markina et al.[2]
Beringia/Asia (647)	
Eskimo Siberian (254)	Shields et al. 1993; Voevoda et al. 1994; Starikovskaya et al. 1998; Ingman et al. 2000; Simonson et al.[2]
Koryak (190)	Schurr et al. 1999; Derenko and Shields 1997
Itelmen (47)	Schurr et al. 1999
Chukchi (156)	Shields et al. 1993; Voevoda et al. 1994; Starikovskaya et al. 1998; Ingman et al. 2000
East Siberia (250)	
Yakuts (139)	Derenko and Shields 1997; Pakendorf et al. 2003
Evenk (41)	Torroni et al. 1993b; Ingman et al. 2000; Lebedeva et al.[2]
Udegey (3)	Torroni et al. 1993b
Evens (65)	Derenko and Shields 1997
Nivkh (2)	Torroni et al. 1993b
East Asia (1,880)	
Korean (335)	Torroni et al. 1993a; Lee et al. 1997; Ingman et al. 2000; Snall et al. 2002
Tibetan (41)	Yao et al. 2002b; Torroni et al. 1993a
Non-Han Chinese (285)	Yao et al. 2002b
Han (China) (431)	Ingman et al. 2000; Yao et al. 2002a; Oota et al. 2002
Thai (North Thailand) (32)	Yao et al. 2002b
Vietnam (35)	Oota et al. 2002
Han (Taiwan) (227)	Torroni et al. 1993a; Horai et al. 1996; Tsai et al. 2001
Han (Hongkong) (20)	Betty et al. 1996
Shanghai (120)	Nishimaki et al. 1999
Japan (354)	Horai et al. 1996; Ingman et al. 2000; Imaizumi et al. 2002; Oota et al. 2002
India (645)	Mountain et al. 1995; Kivisild et al. 1999
Central Asia (802)	
Altai (17)	Shields et al. 1993
Kirghiz (95)	Comas et al. 1998; Ingman et al. 2000
Kazakh (85)	Comas et al. 1998; Yao et al. 2000
Uighur (100)	Comas et al. 1998; Yao et al. 2000
Tuvinian (36)	Derenko et al. 2000
Buryat (296)	Derenko et al. 2000; Pakendorf et al. 2003; Shimada et al.[2]
Scyhtian (8)[3]	Clisson et al. 2002; Ricaut et al. 2003; Voevoda et al. 2000
Uzbek (1)	Ingman et al. 2000
Mongol (118)	Kolman et al. 1996; Yao et al. 2002b
Mongol (46)[3]	Keyser-Tracqui et al. 2003

[1]These 2,804 European HV1 sequences were compiled by Richards et al. 2001 (www.stats.ox.ac.uk/-macaulay/founder2000/index.html).
[2]Data only available on Genbank (http://www.ncbi.nlm.nih.gov).
[3]Archaeological samples.

Table 2 Allelic profiles obtained with the Profiler Plus kit from three ancient DNA samples

Samples	Amelo	D8S1179	D21S11	D7S820	D3S1358	D13S317	vWA	D18S51	D5S818	FGA
AD1	XX	12/13	28/30	8/9	15/15	11/11	14/19	12/14	7/12	23/24
AD3s	XY	13/14	32.2/32.2	10/12	15/15	11/11	14/19	15/21	11/12	21/21
AD3n	XY	11/14	29/29	9/10	15/15	10/11	17/18	13/15	11/13	22/24

Table 3 MtDNA sequences (between positions 16018 and 16383) of the three ancient DNA samples

[1]Numbered according to the published Cambridge Reference Sequence (Anderson et al. 1981).

Samples	Polymorphic positions [1]							
	16092	16172	16182	16183	16189	16223	16266	16362
CRS	T	T	A	A	T	C	C	T
AD1	C	C	C	C	C	T	T	C
AD3s	C	C	C	C	C	T	T	C
AD3n	C	C	C	C	C	T	T	C

To deduce putative genetic relationships between the three Yakuts skeletons from the allelic profiles, we used the DNA·VIEW program (http://www.dna-view.com). The Caucasian (200 subjects) and Asian (161 subjects) databases provided from the DNA VIEW program were used and the likelihood ratio (LR) was calculated assuming a prior probability of 0.5.

Results

STR analysis

For the three ancient Yakut subjects, autosomal STR typing gave complete allelic profiles (Table 2). To reduce ancient DNA STR genotyping errors and ensure the reliability of the results, only the amplified products from each sample and locus that were reproducible in at least five different amplification reactions were considered as authentic (Schmerer et al. 1999). This strategy allowed the identification of "artefact alleles" and false homozygosity, resulting from sporadic contaminations or amplification artefacts such as shadow bands and allelic dropout (Schmerer et al. 1999; Hummel et al 2000; Miller et al. 2002). Repeated amplifications showed that the degree of reproducibility of allele determinations was high, indicating the good quality of the DNA molecule present in the ancient bone samples.

Reproducible results obtained for the amplification of the amelogenin locus clearly identified the subjects AD3s and AD3n as two men, and the subject AD1 as a woman.

The genetic relationship investigated with the DNA·VIEW program allowed us to formally exclude a close parentage relationship between the three ancient subjects. Indeed, all close genetic relationship combinations were tested on three generations and a parent/child, grandparent/grandson, full siblings, etc., relationship can be unambiguously excluded (LR<0.01; probability<1%). A more distant kinship (first cousins, great uncle or aunt/half-nephew, half-siblings, etc.) can also be formally excluded (LR<0.1; probability<1%) except for a possible maternal uncle/nephew relationship between the AD3s and AD3n subjects (LR=0.264; probability=21%).

Mitochondrial DNA analysis

A 366 base pair (bp) segment of the mitochondrial DNA HV1 region was sequenced (positions 16018–16383 of the CRS, Cambridge Reference Sequence) (Anderson et al. 1981) for each of the three ancient Yakut samples (Table 3). Comparison with the CRS indicated the presence of eight variable nucleotide positions represented by six transitions and two transversions. These three HV1 sequences presented the same variable nucleotide positions (np) indicating a probable kinship through maternal lineage between the three Yakut subjects.

The substitutions at nucleotide positions 16223 C→T and 16362 T→C are characteristics of Asian haplogroup D (Torroni et al. 1993a). With the presence of additional sequence polymorphisms at np 16189 T→C and 16266 C→T we can safely conclude that these sequences belong to sub-haplogroup D5a (Yao et al. 2002a). The distribution of the ancient Yakut haplotype among the 8,774 individuals used for comparison, showed that it was shared only by 17 Yakut individuals (Pakendorf et al. 2003) and 1 Yili subject from Xinjiang (China) (Yao et al. 2002a).

Discussion

In this study, the skeletal remains analyzed were recovered from two graves located in a region where climatic conditions allowed the formation of permanent permafrost. The architectural structure of the two graves (e.g. bodies enclosed in a wooden sarcophagus) and the direct contact of the coffins with the permafrost could explain the very good preservation of the three bodies (all bones still connected, clothes perfectly preserved). These conditions might have played a crucial role in protecting the DNA from degradation (Leonard et al. 2000), and could explain the good quality and quantity of the DNA extracted from the ancient Yakut bones. Indeed, complete STR genetic profiles and HV1 sequence were determined from each of the three Yakut skeletons.

To obtain HV1 sequences from the three ancient Yakut samples without ambiguities, several amplifications were

Table 4 Allelic profiles obtained with the Profiler Plus Kit of all persons involved in processing samples

Team	Person	Amelo	D8S1179	D21S11	D7S820	D3S1358	D13S317	vWA	D18S51	D5S818	FGA
French team	1	XY	10/13	29/30	9/11	14/18	11/11	14/18	15/17	12/15	20/23
	2	XY	13/14	28/29	10/10	16/18	8/9	17/18	12/15	11/11	20/21
	3	XX	11/13	28/30	7/12	15/18	11/13	18/18	13/18	10/12	24/24
	4	XX	13/13	27/31	7/11	16/17	11/14	17/19	13/15	12/12	20/23
	5	XY	8/15	27/31.2	8/10	14/18	12/12	17/19	14/15	9/13	20/20
	6	XX	10/13	29/29	10/12	15/15	8/8	18/18	12/13	9/11	23/25
Yakut team	1	XY	13/16	29/30	8/11	19/19	8/11	16/17	16/21	11/11	21/24
	2	XX	10/13	29/30	9/11	17/17	10/11	17/18	13/14	12/13	21/23

necessary from three independent extractions. The thymine (T) to cytosine (C) transition at nucleotide position 16189, coupled with double adenine (A) to cytosine (C) transversion at nucleotide positions 16182 and 16183 led to the formation of a polycytosine region containing 12 cytosines. This polycytosine region produced a characteristic blurred sequence in nucleotides beyond the C-stretch, and notably did not allow the binding of primer H16239 and the subsequent amplification of the heavy strand of sub-region a. The use of primers F16190 and H16410 enabled both the L-strand and H-strand of the sub-region-b to be read correctly. For sub-region a, the design of primer H16167 which annealed before the polycytosine region enabled the heavy strand to be read correctly, and confirmed the T to C transition at nucleotide position 16092 for both strands. With this methodology, the proportion which could not be sequenced in both directions was kept to a minimum. The T to C transition at nucleotide position 16172 was only recorded as L-strand sequencing of the sub-region a, but it was confirmed by its reproducibility both within the same sample extract, and between different sample extracts (Parson et al. 1998).

The authenticity of the DNA extracted from the ancient human remains is usually a major concern mainly because of risk of contamination by exogen modern DNA (Handt et al. 1994). In this study the results obtained with STR analysis and HV1 region were fully reproducible from multiple extractions and amplifications, and were never found to correspond to someone involved in processing samples (Tables 4 and 5). The molecular and morphological sex determination of subjects AD3s and AD3n were in accordance, and in spite of the absence of a morphological sex determination, the molecular sex of subject AD1 was compatible with the type of clothes worn by this subject. Moreover, an inverse relationship between STR autosomal amplification efficiency and the length of the amplicons was observed (data not shown). All of these findings and the extensive precautions taken against contamination (as described in the Methods section) allowed us to consider it to be highly unlikely that our data arose from contaminating DNA, and attested to the authenticity of DNA extracts and amplified products.

The archaeological context with (i) the proximity of graves AD1 and AD3, (ii) the double inhumation in grave AD3, and (iii) the probable inhumation of all three bodies in the eighteenth century, could have suggested the possibility of a close parentage relationship between the three individuals. The STR data analysis however formally excluded a close kinship between them, but could not exclude with certainty a more distant familial relationship, in agreement with the subjects AD1, AD3n and AD3s belonging to the same maternal lineage. The inhumation site of At-Dabaan could therefore have been selected on familial criteria and precisely those of matrilinearity. Nevertheless, the absence of a close genetic relationship between the subjects is disturbing. This absence of a relationship between the woman (AD1) and the two men (AD3s and AD3n) could be explained by the chronology of the inhumations as well as by complex cultural practices (possible polygamy and exogamy) (Balzer 1994). Indeed, the AD1 subject had probably been buried two or three generations before subjects AD3s and AD3n, prior to christianization. However, the two men were buried at the same time, and with the traditional Yakut society practicing essentially patrilineal exogamy (Balzer 1994), it is surprising to find in the same grave two men belonging to the same maternal lineage but lacking a close kinship. An explanation could be that these two individuals belonged to the same clan structure in which this mitochondrial haplotype was very frequent, obscuring the genetic kinship determination. This hypothesis is in agreement with the relatively high frequency of this haplotype in the modern Yakut population (12.2%) (Derenko and Shields 1997; Pakendorf et al. 2003) and its historical presence in the At-Dabaan region. Indeed, the same HV1 sequence was also discovered in a woman buried during the seventeenth century in the Istekh Myrane site, around 3 km from our study site (authors' unpublished data). Therefore, the absence of a close kinship between the three subjects, despite belonging to the same maternal lineage, could be explained by the wide spread of this HV1 haplotype in the Yakut population of this region. This excludes neither the possibility of these three individuals belonging to the same clan structure nor the possible inhumation of two unrelated men together to minimize the time and preparations necessary for the burial.

Sequence determination of the HV1 region revealed that the three ancient Yakut subjects were linked by maternal lineage to Asian populations. The analysis of the distribution of the ancient Yakut HV1 haplotype among 8,774 Eurasian haplotypes (Table 1) showed that, except for 1 Han individual from Xinjiang (Yao et al. 2002a), this haplo-

Table 5 MtDNA sequences (between positions 16018 and 16383) of all persons involved in processing samples

Team	Person	Polymorphic positions [1]																				
		16039	16051	16092	16093	16126	16162	16172	16182	16183	16189	16221	16223	16232	16239	16249	16263	16266	16292	16294	16296	16298	16362
	CRS	G	A	T	T	T	A	T	A	A	T	C	C	C	C	T	T	C	C	C	C	T	T
French team	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	C	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	T	–	–	–	–	–	–	–	C	–
	4	A	–	–	–	C	–	–	–	–	–	–	–	–	–	C	–	–	T	T	T	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Yakut team	1	–	–	–	C	–	–	–	–	–	–	–	T	T	–	–	–	–	–	–	–	–	C
	2	–	G	–		–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[1]Numbered according to the published Cambridge Reference Sequence (Anderson et al. 1981).

type is exclusively shared by the present-day Yakut population (Pakendorf et al. 2003). The persistence of this mitochondrial haplotype in the Yakut population since the eighteenth century, suggests a relative stability of this maternal lineage in the Yakut mitochondrial gene pool during the last 3 centuries, in spite of the drastic reduction of the size and genetic diversity of the Yakut population (Levin and Potatov 1964; Forsyth 1996; Pakendorf et al. 2002). Moreover, the restrictive distribution of this ancient haplotype in the populations compared in this study could allow it to be considered as specific for the Yakut population.

It is likely that Y-chromosomal STR analyses might have contributed to solving the questions which arose in this study, but unfortunately the quantity of ancient bone material available did not allow the analysis of Y-chromosomal markers.

Conclusion

The present study revealed that, in spite of the Yakut society being patrilocal, the three subjects buried in At-Dabaan site were linked by their maternal lineage. Nevertheless, the absence of a close kinship between them and the high frequency of this mtDNA haplotype in the Yakut population suggest that the three ancient skeletons were linked to the same clan structure rather than to the same family group. Moreover, the geographical distribution of this mtDNA haplotype indicates the specificity and continuity of part of the Yakut mitochondrial gene pool during the last centuries.

Acknowledgments This work was supported by grants from the French Department of Research, and the program of the French/Sakha cooperation, funds from the Action Cooperation Integrated from the French Minister of the research "Le complexe spatial Altaï-Baïkal. Plaque tournante des flux géniques en Haute Asie de la période protohistorique à l'époque moderne".

References

Alekseev AN (1996) Ancient Yakutia: the Iron Age and the medieval epoch (in Russian). Izdatel'stvo Instituta Akheologii i Etnogrfii SO RAN, Novosibirsk

Anderson S, Bankier AT, Barrell BG et al. (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457–465

Balzer MM (1994) Yakut. In: Friedrich P, Diamond N (eds) Encyclopedia of world culture, vol I. Russia and Eurasia/China. Hall, New York, pp 404–407

Betty DJ, Chin-Atkins AN, Croft L, Sraml M, Easteal S (1996) Multiple independent origins of the COII/tRNALys intergenic 9 bp mtDNA deletion in aboriginal Australians. Am J Hum Genet 58:428–433

Boeva SB (1988) Anthropogenetic study of the indigenous population of the Yakut ASSR in connection with the ethnogenesis of the Yakuts. PH.D. thesis (in Russian), Moscow State University, Moscow

Clisson I, Keyser C, Francfort HP, Crubézy E, Samashev Z, Ludes B (2002) Genetic analysis of human remains from a double inhumation in a frozen kurgan in Kazakhstan (Berel site, Early 3rd Century BC). Int J Legal Med 116:304–308

Comas D, Calafell F, Mateu E et al. (1998) Trading genes along the silk road: mtDNA sequences and the origin of central populations. Am J Hum Genet 63:1824–1838

Derbeneva OA, Starikovskaya EB, Wallace DC, Subernik RI (2002) Traces of early Eurasians in the Mansi of northwest Siberia revealed by mitochondrial DNA analysis. Am J Hum Genet 70:1009–1014

Derenko MV, Shields GF (1997) Mitochondrial DNA sequence diversity in three north Asian aboriginal populations groups. Mol Biol (Mosk) 31:665–669

Derenko MV, Malyarchuk BA, Dambueva IK, Shaikhaev GO, Dorzhu CM, Nimaev DD, Zakharov AIA (2000) Mitochondrial DNA variation in two south Siberian aboriginal populations: implications for the genetic history of north Asia. Hum Biol 72: 945–973

Fefelova VV (1990) Participation of Indo-European tribes in ethnogeny of the Mongoloid population of Siberia. Am J Hum Genet 47:294–301

Fily ML, Crubézy E, Courtaud P, Keyser C, Ebrard D, Ludes B (1998) Paleogenetic analysis of the skeletons from the sepulchral cave of Elzarreko Karbia (Bronze Age, Basque Country). C R Acad Sci Paris 321:79–85

Fondahl G (1994) Evenki northern Tungus. In: Friedrich P, Diamond N (eds) Encyclopedia of world culture, vol VI-Russia and Eurasia/China. Hall, New York, pp 120–124

Forsyth J (1996) A history of the peoples of Siberia. Cambridge University Press, Cambridge

Gabriel MN, Huffine EF, Ryan JH, Holland MM, Parsons TJ (2001) Improved mtDNA sequence analysis of forensic remains using "mini-primer set" amplification strategy. J Forensic Sci 46:247–253

Handt O, Höss M, Krings M, Pääbo S (1994) Ancient DNA: methodological challenge. Experientia 50:524–529

Horai S, Murayama K, Hayasaka K et al. (1996) MtDNA polymorphism in east Asian populations, with special reference to the peopling of Japan. Am J Hum Genet 59:579–590

Hummel S, Bramanti B, Schultes T, Kahle M, Haffner S, Herrmann B (2000) Megaplex DNA typing can provide a strong indication of the authenticity of ancient DNA amplifications by clearly recognizing any possible modern contamination. Anthropol Anz 58:15–21

Imaizumi K, Parsons TJ, Yoshino M, Holland MM (2002) A new database of mitochondrial DNA hypervariable regions I and II sequence from 162 Japanese individuals. Int J Legal Med 116: 68–73

Ingman M, Kaessmann H, Pääbo S, Gyllensten U (2000) Mitochondrial genome variation and the origin of modern humans. Nature 408:708–713

Ivanov PL, Wadhams MJ, Holland MM, Weedn VW, Parsons TJ (1996) Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II. Nat Genet 12:417–420

Jehaes E, Toprak K, Vanderheyden N, Pfeiffer H, Cassiman JJ, Brinkmann B, Decorte R (2001) Pitfalls in the analysis of mitochondrial DNA from ancient specimens and the consequences for forensic DNA analysis: the historical case of the putative heart of Louis XVII. Int J Legal Med 115:135–141

Keyser-Tracqui C, Crubézy E, Ludes B (2003) Nuclear and mitochondrial analysis of a 2000-year-old necropolis in the Egyin Gol valley (Mongolia). Am J Hum Genet 73:247–260

Kivisild T, Bamshad MJ, Kaldma K et al. (1999) Deep common ancestry of Indian and western-Eurasian mitochondrial DNA lineages. Curr Biol 9:1331–1334

Kolman CJ, Sambuughin N, Bermingham E (1996) Mitochondrial DNA analysis of Mongolian populations and implications for the origin of new world founders. Genetics 142:1321–1334

Kolodesnikov S (2000) The person in traditional Yakut (Sakha) worldview. Archaeol Ethnol Anthropol Eurasia 39:42–79

Lee SD, Shin CH, Kim KB, Lee YS, Lee JB (1997) Sequence variation of mitochondrial region DNA control region in Koreans. Forensic Sci Int 87:99–116

Leonard JA, Wayne RK, Cooper A (2000) Population genetics of Ice Age brown bears. Proc Natl Acad Sci U S A 97:1651–1654

Levin MG, Potatov LP (1964) The peoples of Siberia. Chicago University Press, Chicago

Macaulay V, Richards M, Hickey E et al. (1999) The emerging tree of west Eurasian mtDNAs: a synthesis of control-region sequences and RFLPs. Am J Hum Genet 64:232–249

Meinila M, Finnila S, Majamaa K (2001) Evidence for mtDNA admixture between the Finns and the Saami. Hum Hered 52: 160–170

Miller CR, Joyce P, Waits LP (2002) Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160: 357–366

Mountain JL, Hebert JM, Bhattacharyya S, Underhill PA, Ottolenghi C, Gadgil M, Cavalli-Sforza L (1995) Demographic history of India and mtDNA-sequence diversity. Am J Hum Genet 56:979–992

Nishimaki Y, Sato K, Fang L, Ma M, Hasekura H, Boettcher B (1999) Sequence polymorphism in the mtDNA HV1 region in Japanese and Chinese. Legal Med 1:238–249

Oota H, Kitano T, Jin F et al. (2002) Extreme mtDNA homogeneity in continental Asian populations. Am J Hum Genet 118: 146–153

Orekhov V, Poltoraus A, Zhivotovsky LA, Spitsyn V, Ivanov P, Yankovsky N (1999) Mitochondrial DNA sequence diversity in Russians. FEBS Lett 445:197–201

O'Rourke DH, Hayes MG, Carlyle W (2000) Ancient DNA studies in physical anthropology. Annu Rev Anthropol 29:217–242

Pakendorf B, Spitsyn VA, Rodewald A (1999) Genetic structure of a Sakha population from Siberia and ethnic affinities. Hum Biol 71:231–244

Pakendorf B, Morar B, Tarskaia LA, Kayser M, Soodyall H, Rodewald A, Stoneking M (2002) Y-chromosome evidence for a strong reduction in male population size of Yakuts. Hum Genet 110:198–200

Pakendorf B, Wiebe V, Tarskaia LA, Spitsyn VA, Soodyall H, Rodewald A, Stoneking M (2003) Mitochondrial DNA evidence for admixed origins of central Siberian populations. Am J Phys Anthropol 120:211–224

Parson W, Parsons TJ, Scheithauer R, Holland MM (1998) Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequences: application of mtDNA sequence analysis to a forensic case. Int J Legal Med 111:124–132

Ricaut FX, Keyser-Tracqui C, Cammaert L, Crubézy E, Ludes B (2003) Genetic analysis and ethnic affinities from two Scytho-Siberian skeletons. Am J Phys Anthropol (in press)

Richards M, Corte-Real H, Forster P et al. (1996) Palaeolithic and neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:604–615

Richards M, Macauley V, Hickey V et al. (2000) Tracing European founder lineages in the near eastern mtDNA pool. Am J Hum Genet 67:1251–1276

Roux JP (1963) La mort chez les peuples altaïques anciens et médiévaux. Librairie d'Amérique et d'Orient Adrien-Maisonneuve, Paris

Sajantila A, Lahermo P, Anttinen T et al. (1995) Genes and languages in Europe: an analysis of mitochondrial lineages. Genome Res 5:42–52

Sajantila A, Salem AH, Savolainen P, Bauer K, Gierig C, Pääbo S (1996) Paternal and maternal DNA lineages reveal a bottleneck in the founding of the Finnish population. Proc Natl Acad Sci U S A 93:12035–12039

Santos FR, Pandya A, Tyler-Smith C et al. (1999) The central Siberian origin for Native American Y chromosome. Am J Hum Genet 64:619–628

Schmerer WM, Hummel S, Herrmann B (1999) Optimized DNA extraction to improve reproducibility of short tandem repeat genotyping with highly degraded DNA target. Electrophoresis 20:1712–1716

Schurr TG, Subernik RI, Starikovskaya YB, Wallace DC (1999) Mitochondrial DNA variation in koryaks and itel'men: population replacement in the Okhotsk sea-Bering sea region during the neolithic. Am J Phys Anthropol 108:1–39

Shields GF, Schmiechen AM, Frazier BL, Redd A, Voevoda MI, Reed JK, Ward RH (1993) mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern north America populations. Am J Hum Genet 53:549–562

Snall N, Savontaus ML, Kares S, Lee MS, Cho EK, Rinne JO, Huoponen AK (2002) A rare mitochondrial DNA haplotype observed in Koreans. Hum Biol 74:253–262

Starikovskaya YB, Subernik RI, Schurr TG, Kogelnik AM, Wallace DC (1998) MtDNA diversity in Chukchi and Siberian Eskimos: implications for the genetic history of ancient Beringia and the peopling of the New World. Am J Hum Genet 63: 1473–1491

Szibor R, Michael M (1999) Correct mitochondrial L-strand sequencing after C-stretches. Int J Legal Med 112:348–349

Torroni A, Schurr TG, Cabell MF et al. (1993a) Asian affinities and continental radiation of the four founding native American mtDNAs. Am J Hum Genet 53:563–590

Torroni A, Subernik RI, Schurr TG et al. (1993b) MtDNA variation of aboriginal Siberians reveals distinct genetic affinities with native Americans. Am J Hum Genet 53:591–608

Tsai LC, Lin CY, Chang JG, Linacre A, Goodwin W (2001) Sequence polymorphism of mitochondrial D-Loop DNA in the Taiwanese Han population. Forensic Sci Int 119:239–247

Voevoda MI, Avksentyuk AV, Ivanova AV et al. (1994) Molecular genetic study in a population of indigenous Chukotka inhabitants: an analysis of mitochondrial DNA polymorphism and genes of alcohol-metabolizing enzymes (in Russian). Sib Ecol Zhurnal 2:149–162

Voevoda MI, Romashchenko AG, Sitnikova VV, Shulgina EO, Kobsev VF (2000) a comparison of mitochondrial DNA polymorphism in Pazyrik and modern Eurasian populations. Archaeol Ethnol Anthropol Eurasia 4:88–94

Yao YG, Lu XM, Luo HR, Li WH, Zhang YP (2000) Gene admixture in the silk road region of China: evidence from mtDNA and melanocortin1 receptor polymorphism. Genes Genet Syst 75:173–178

Yao YG, Kong QP, Bandelt HJ, Kivisild T, Zhang YP (2002a) Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am J Hum Genet 70:635–651

Yao YG, Nie L, Harpending H, Fu YX, Yuan ZG, Zhang YP (2002b) Genetic relationship of Chinese ethnic populations revealed by mtDNA sequence diversity. Am J Phys Anthropol 118:63–76