Journal of Archaeological Science: Reports xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia

N.M. Moussa [a,*], V.I. Bazaliiskii [b], O.I. Goriunova [b,c], F. Bamforth [a], A.W. Weber [d,e]

[a] Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta T6G 2R7, Canada
[b] Department of Archaeology and Ethnography, Irkutsk State University, Karl Marx, Street 1, Irkutsk 664003, Russia
[c] Institute of Archaeology and Ethnography, Siberian Branch of the Russian Academy of Sciences, Academician Lavrentiev Ave. 17, Novosibirsk 630090, Russia
[d] Department of Anthropology, 13-15 H.M. Tory Building, University of Alberta, Edmonton, Alberta T6G 2H4, Canada
[e] Laboratoire Méditerranéen de Préhistoire Europe Afrique (LAMPEA) – UMR 7269, Aix-Marseille Université, 5 rue du Château de l'Horloge, B.P. 647, 13094 Aix-en-Provence Cedex 2, France

ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form 27 October 2016
Accepted 1 November 2016
Available online xxxx

Keywords:
Humans
Ancient DNA
Y-chromosome
mtDNA
Haplogroups
Sub-haplogroups of Q
Siberian population

ABSTRACT

The Lake Baikal region of Siberia was home to two temporally distinct populations from Early Neolithic, EN (*7500–7000 cal BP*) to Late Neolithic-Early Bronze Age, LN-EBA (*5570–3725 cal BP*). The EN group was separated from the LN-EBA group by a ~1500-year gap (hiatus), and during this hiatus no human remains have been recovered from the Lake Baikal area. Examination of the paternal lineage through Y-chromosomal polymorphisms is a novel approach to BAP and will facilitate the assessment of the paternal continuities and/or discontinuities within and between the EN and the LN-EBA groups, and complement the previously examined maternal data. Several new ancient DNA extraction and PCR amplification techniques were optimized to address the technical challenges during sample analysis. Each sample was extracted twice in duplicate on different occasions to authenticate the results. Thirteen Y-chromosomal Single Nucleotide Polymorphism (SNP) markers were examined via the SNaPshot multiplex PCR reaction to determine Y-chromosomal haplogroups of males. Results have been obtained from 16 males from the EN cemeteries Lokomotiv and Shamanka II representing haplogroups K, R1a1 and C3, and 20 males from the LN-EBA Ust'-Ida and Kurma XI cemeteries representing haplogroups Q, K and unidentified SNP (L914). For those males belonging to haplogroup Q, further experiments were obtained to examine sub-haplogroups of Q, and the results showed that those males belong to sub-haplogroup Q1a3. The paternal Y-chromosome results suggest a discontinuity between the EN and LN-EBA populations. The significance of this research lies on the utility of DNA analysis in making inferences about the pre-historic social structure.

1. Introduction

1.1. Background

Archaeological data indicate that the Cis-Baikal region of Siberia is the site of several large prehistoric cemeteries since the Early Upper Paleolithic period (Weber, 1995; Goebel, 1999). The Cis-Baikal area has four main micro-regions: the Angara River Valley, the upper Lena River Valley, the Little Sea (Ol'khon) on the northwest coast of Lake Baikal, and the South Baikal region (Fig. 1). The four micro-regions have been investigated by several Russian and Canadian scholars since 1990. The Baikal Archaeology Project (BAP), an international multidisciplinary initiative, aims to reconstruct the lifestyle of the hunter-gatherer groups inhabiting the area and buried in formal cemeteries during the Neolithic and Bronze Age periods. The area was home to two temporally distinct populations from Early Neolithic (EN) (Kitoi culture), *7500–7000 cal BP*, to Late Neolithic-Early Bronze Age (LN-EBA) (Serovo-Isakovo-Glazkovo culture), *5570–3725 cal BP*. Dates were acquired via radiocarbon dating. The EN group was separated from the LN-EBA group by a ~1500-year gap (hiatus) during which large mortuary sites are entirely absent (Weber et al., 2016).

Both the EN (Kitoi) and the LN-EBA (Serovo-Isakovo-Glazkovo) cultures had formal cemeteries, an area used repeatedly and more or less exclusively for disposal of the dead (e.g. (Goldstein, 1981)).

Earlier Russian craniometric studies suggested that the EN and the LN-EBA populations are genetically distinct (Gerasimova, 1992; Mamonova, 1973; Mamonova, 1980; Mamonova, 1983). Measuring the biological differentiation between the two cultures can also be achieved through their genetic signatures, which would give a strong verification of the genetic relationships between them.

Ancient DNA (aDNA) research of several hunter-gatherer individuals from Cis-Baikal was first conducted by Russian researchers analyzing mitochondrial DNA (mtDNA) composition (Naumova et al., 1997; Naumova and Rychkov, 1998). Ancient DNA analysis was continued by several researchers, who examined mtDNA polymorphisms of skeletal samples from different mortuary sites. These mortuary sites include Lokomotiv (EN cemetery) and Ust'-Ida (LN-EBA cemetery) (Mooder, 2004), Shamanka II (EN cemetery) (Thomson, 2006) and Khuzhir-Nuge XIV (LN-EBA cemetery) (Gustafson, 2007). Unfortunately,

* Corresponding author.
E-mail address: nmoussa@ualberta.ca (N.M. Moussa).

http://dx.doi.org/10.1016/j.jasrep.2016.11.003
2352-409X/

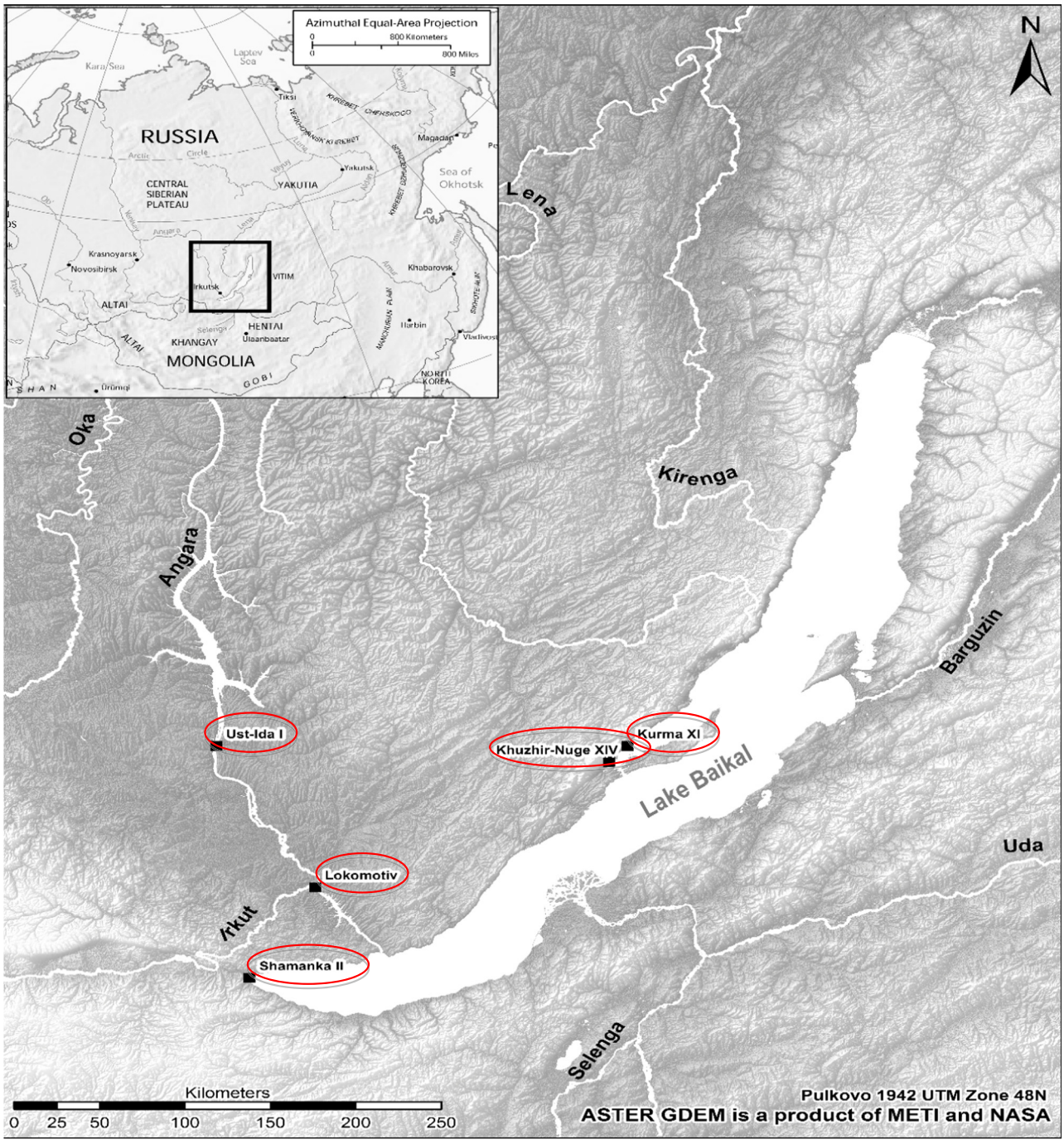

Fig. 1. Cis-Baikal cemeteries' and their locations-circled in red (Shamanka II and Lokomotiv-EN) and (Ust'-Ida, Khuzhir-Nuge XIV and Kurma XI-LN-EBA) (Lieverse et al., 2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Source: Adapted with permission from [Point taken: An unusual case of incisor agenesis and mandibular trauma in Early Bronze Age Siberia. A.R. Lieverse, I.V. Pratt, R.J. Schulting, D.M.L. Cooper, V.I. Bazaliiskii, A.W. Weber. International Journal of Paleopathology. 6. Copyright© 2014 Elsevier] (License number 3571161002487).

samples from Khuzhir-Nuge XIV, located on the Little Sea micro-region, were poorly preserved and mtDNA analysis was not possible.

No Y-chromosomal analyses were obtained previously on any of Lake Baikal's cemeteries.

1.2. Archaeological context

As a part of the archaeological research, estimation of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures in human bone is considered the most direct and reliable method to determine diet and subsistence strategies of prehistoric populations (Katzenberg, 2008). In BAP, the values of $\delta^{13}C$ and $\delta^{15}N$ isotopes from the individuals' osteological remains were compared to $\delta^{13}C$ and $\delta^{15}N$ isotopes' ratios of local fauna in different Cis-Baikal micro-regions to create a thorough descriptive reconstruction of prehistoric populations' diet and subsistence practices since the EN period to the LN-EBA period (Weber and Bettinger, 2010; Katzenberg and Weber, 1999; Katzenberg et al., 2010). Examination of different samples from Cis-Baikal micro-regions and the different periods provided clear evidence for the consumption of freshwater foods (e.g. fish and seal) in groups from Lake Baikal (Weber and Bettinger, 2010).

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

Mobility strategies are correlated with several aspects including social, cultural and environmental variables in hunter-gatherer societies. Mobility patterns can be addressed through dietary evidence, as carbon and nitrogen stable isotope ratios, and also through Strontium (Sr) isotope signatures. Strontium isotope signature is considered a more considerable method to measure mobility than carbon and nitrogen stable isotope signatures (Weber and Bettinger, 2010).

Strontium, a natural element of earth, is integrated into the skeletal tissues through the ingestion of food and water. The ratios of ^{87}Sr to ^{86}Sr detected in the skeletal tissues of a human or an animal is a reflection of the $^{87}Sr/^{86}Sr$ signatures found in the environment and directly related to the food and water consumed by the human or the animal from a specific region. The bone tissue undergoes a constant remodeling every 7 to 10 years during the individual's lifetime, but the tooth enamel development stabilizes after infancy and childhood (Lawenstam and Weiner, 1989). Thus, any difference in the Sr isotope ratios between bones and teeth would reveal the migratory history of an individual throughout a different geographical area, which might differ from the individual's residential location during childhood.

1.3. Burial sites

1.3.1. EN cemeteries

Lokomotiv cemetery is considered the largest Neolithic cemetery identified in northern Asia (87 graves have been recovered so far) (Bazaliiskiy and Savelyev, 2003; Bazaliiskii, 2010). The cemetery is located in the junction between the Irkut and the Angara rivers (in the Angara River Valley micro-region) in a park in Irkutsk city (Bazaliiskii, 2010).

Shamanka II cemetery located at the southwestern tip (on the South Baikal micro-region) of Lake Baikal is also considered a large cemetery (97 graves) (Weber et al., 2016).

Mortuary rituals and grave structure demonstrate similarities between the two EN cemeteries. For example, the usage of red ochre, as a mortuary ritual, is observed at both Lokomotiv and Shamanka II (Bazaliiskii, 2010). Both cemeteries have single, double, triple, and communal graves (Thomson, 2006; Bazaliiskiy and Savelyev, 2003; Mooder et al., 2005; Bazaliiskii, 2000a, 2000b). The extended-supine body position is common in both Lokomotiv and Shamanka II.

However, there is some variation in mortuary rituals. Many graves in Shamanka II cemetery had bear skulls, mandibles, canines and molars indicating an interesting bear mortuary ritual (Bazaliiskii, 2010), but none were found in Lokomotiv (Bazaliiskiy and Savelyev, 2003). Also, fire rituals were documented in Shamanka II implied by the existence of fire pits that often disturbed the graves (Bazaliiskii, 2010).

1.3.2. LN-EBA cemeteries

Ust'-Ida cemetery is situated on the Angara River (Angara River Valley micro-region) with graves scattered parallel to the river (Tiutrin and Bazaliiskii, 1996).

Kurma XI cemetery located on the northwest coast of Lake Baikal's Little Sea micro-region, is smaller, regarding number of graves (26 graves), than the other cemeteries. The cemetery was dated to the EBA (Sosnovskaia, 1996); however, six graves out of 26 were found to be EN according to archaeological and radiocarbon data (Weber and Goriunova, 2005).

The use of fire was evident at Ust'-Ida with occasional burials showing red ochre treatment (Tiutrin and Bazaliiskii, 1996; Weber et al., 2002). However, at Kurma XI, the use of fire mortuary ritual was completely absent, but still three burials were covered in red ochre (Goriunova et al., 2004).

Extended-supine body position is common in Ust'-Ida with the head oriented to the north, or sometimes the head was facing south and bodies either extended-supine or sideways (Mooder, 2004; Tiutrin and Bazaliiskii, 1996; Bazaliiskii, 2003). Also, two body positions were found at Kurma XI, one mostly extended-supine with the head pointing southwest, and the other was a sitting position (three cases only) (Weber et al., 2011a, 2011b, 2011c; McKenzie, 2010). Other mortuary variability include, single, double, triple and multiple graves in Ust'-Ida cemetery (Mooder, 2004; Tiutrin and Bazaliiskii, 1996; Bazaliiskii, 2003). At Kurma XI, all of the graves were single except for one double grave (Weber and Goriunova, 2011).

1.4. Mitochondrial DNA analysis

Previous studies on mtDNA from EN Lokomotiv (28 individuals sampled for aDNA), LN-EBA Ust'-Ida (29 LN and 10 EBA sampled for aDNA) and EN Shamanka II (21 individuals sampled for aDNA) have examined restriction fragment length polymorphisms (RFLP) and/or direct sequencing of the hyper-variable 1 region (HV1) of mtDNA to identify mtDNA population specific polymorphisms (haplogroups). Several Eurasian mtDNA haplogroups were identified with varying frequencies in the different cemeteries' samples (Mooder, 2004; Thomson, 2006; Mooder et al., 2005; Mooder et al., 2006).

Mitochondrial DNA analyses from Lokomotiv, Shamanka II and Ust'-Ida cemeteries showed that individuals from the two EN cemeteries (Lokomotiv and Shamanka II) both had higher frequencies of haplogroups D and F and lower frequencies of haplogroups A and C. According to Mooder et al. (2010), Fisher's exact test results suggested that Lokomotiv and Shamanka II people were maternally related (Mooder et al., 2010). The LN-EBA cemetery (Ust'-Ida) illustrated different mtDNA haplogroup frequencies with higher frequency of haplogroup A and lower frequencies of haplogroups D and F, and EBA Ust'-Ida shows higher frequency of haplogroup C, lower frequency of haplogroup A and the absence of haplogroups D and F (Mooder et al., 2006).

Mitochondrial DNA was also analyzed, through direct sequencing of the HV1 region, from 12 EBA Kurma XI individuals and two EN Kurma XI individuals. In addition, mtDNA was examined from ten additional EN Shamanka II individuals. EBA Kurma XI shows a high frequency of haplogroup D, a low frequency of haplogroup A, the absence of haplogroup C, and the appearance of haplogroup Z, which was not found in other Cis-Baikal cemeteries. Statistical analysis (Fisher's exact test) shows that EBA Kurma XI is maternally different from both LN Ust'-Ida and EBA Ust'-Ida (Moussa, 2015). Also, the additional Shamanka II mtDNA data, when added to the previously obtained Shamanka II results (Thomson, 2005), show that Shamanka II is maternally different from Lokomotiv, which contradicts the previous findings (Moussa, 2015). The new results from EBA Kurma XI and EN Shamanka II question the notion of maternal continuity/discontinuity between EN, LN and EBA populations of Cis-Baikal. Therefore, investigating the populations of the two EN cemeteries and the two LN-EBA cemeteries through the patrilineal lineage using Y-chromosomal polymorphisms is crucial to verify discontinuity or continuity between the EN and LN-EBA populations.

1.5. Y-chromosomal analysis

The goal of this study was to explore the paternal lineages of the Cis-Baikal prehistoric populations through the examination of Y-chromosomal polymorphisms. Examination of the patrilineal lineage is a novel approach to BAP not only to complement the matrilineal lineage data, but also to elucidate patrilineal continuities and/or discontinuities within and between the EN and the LN-EBA groups.

The Y-chromosome consists of two main regions, the non-recombining region (NRY) and the pseudoautosomal regions (PARs). Loci on the Y-chromosome's NRY are haploid, inherited paternally and have been shown to be useful in tracking the male lineage in populations (Wolf et al., 1992). Many DNA polymorphisms among different human populations that can be found on Y-chromosomal DNA, have led to a dramatic increase in studies of the paternally inherited Y-chromosome and helped in highlighting the important role of Y-chromosomal polymorphisms in evolutionary and population genetics

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

(Jobling and Tyler-Smith, 1995; Hammer and Zegura, 1996; Hammer et al., 1997; Hammer et al., 1998; Thomas et al., 1998; Perez-Lezaun et al., 1997; Perez-Lezaun et al., 1999). These polymorphisms include single nucleotide substitutions, small and large deletions, inversions and duplications. These polymorphisms have different mutation rates (Gusmao and Carraccedo, 2003). Some loci have low mutation rates, which provide the opportunity to use them in differentiating between ancestral branches on the human evolutionary tree (Hammer et al., 1997; Hammer et al., 1998; Hammer, 1994; Su et al., 1999). Y-chromosomal SNPs (Single Nucleotide Polymorphisms) have a low mutation rate (10^{-8} mutation/generation), making them appropriate for identifying stable paternal lineages that can be tracked back in time for thousands of years (Y Chromosome Consortium, 2002).

To determine the Y-chromosomal haplogroup distribution of individuals from EN and LN-EBA cemeteries, Y-chromosomal SNPs were examined. Certain Y-chromsomal SNPs were examined in this study that define Y-chromosomal haplogroups (e.g. C-M216, C3-M217, F-M89, K-M9, N3-Tat, O-M175, P-M45, Q-M242, Q3-M3, R1-M173 and R1a1-M17) distributed among East Asian populations and Siberian populations (Bouakaze et al., 2007). Regarding analyzing Y-chromosomal sub-haplogroups, haplogroup Q-M242 is identified by M242 SNP mutation (Karafet et al., 2008) and is widely distributed among different groups in Siberia (Karafet et al., 2002). There are several sub-haplogroups under haplogroup Q-M242. Only certain sub-haplogroups from the haplogroup Q-M242 tree are represented in the Siberian populations (Q1a*-MEH2, Q1a2-M25 and Q1a3-M346) (Malyarchuk et al., 2011).

2. Materials and methods

2.1. Contamination controls

Rigorous measures are required when working with ancient DNA to minimize the risk of exogenous contamination with modern DNA. Published recommendations (Cooper and Poinar, 2000) were followed in our laboratory. During DNA extraction and PCR amplification negative controls were introduced to detect any probability of contamination. All sample preparations, DNA extractions and preparation for PCR amplifications were conducted in a dedicated laboratory (clean room) for aDNA only. Prior to entering the clean room, researchers dressed in gowns, booties, sterile sleeves, masks and goggles. After entering the clean room another set of gloves was worn over the existing gloves and sprayed with 30% (v/v) bleach (sodium hypochlorite). The clean room was equipped with a separate workstation for sample preparation, and with a class II type A2 biological safety cabinet (Thermo Scientific™, USA) for all other procedures. The bone box was cleaned with undiluted industrial strength bleach (100% v/v) between each sample to avoid cross contamination. The biological safety cabinet was cleaned with 70% ethanol before and after usage and exposed to UV light for four hours after each usage. Racks, pipettes, and containers were cleaned with 10% (v/v) bleach before and after each single use. Sterile pipette tips (Rose Scientific, AB, Canada) were used. Reagents were decontaminated either by bleaching, UV irradiation and/or autoclaving prior to use depending on the nature of the material. PCR amplifications and data analysis were carried out in duplicate on two separate occasions. All personnel working in the laboratory gave consent to have their mtDNA and Y-chromosomal haplogroups determined.

2.2. DNA sampling

2.2.1. Sample selection from Lokomotiv, Ust'-Ida, Shamanka II and Kurma XI

Male samples confirmed by molecular sex analysis were selected for Y-chromosomal analysis from the four main cemeteries in the Cis-Baikal area, except for one Kurma XI individual, whose molecular sex could not be determined but has a morphological sex of probable male (KUR_2002.010). This Kurma XI individual was added only to increase the possibility of obtaining more Y-chromosomal results from Kurma XI cemetery. Two cemeteries (Lokomotiv and Shamanka II) belong to the EN period and two belong to the LN-EBA period (Ust'-Ida and Kurma XI).

The molecular sex of Lokomotiv and Ust'-Ida samples was determined previously through amelogenin analysis (Mooder et al., 2005). The male samples were selected for further Y-chromosomal analysis. The reliance was mostly on molecular sex results rather than morphological sex because there was occasionally discordance between molecular and morphological sexing (Mooder et al., 2006). For the analysis, 26 vertebral bones and/or teeth samples were available from 17 males from the Lokomotiv cemetery and 21 vertebral bones and/or teeth samples from 16 males from the Ust'-Ida cemetery (Table 1). Nine male samples were available for Y-chromosomal analysis from the Shamanka II cemetery. The molecular sex of five Shamanka II samples was previously determined by Thomson (2006), and the remaining four samples were analyzed as part of this study (Tables 1 and 3). For Kurma XI individuals, amelogenin analysis was conducted as part of this study, from sixteen vertebral bone and tooth samples. Seven males, confirmed through amelogenin analysis, were available for Y-chromosomal analysis from Kurma XI cemetery (Tables 1 and 3).

Vertebral bones were preferred for two main reasons. Vertebrae contain a high proportion of spongy bone tissues, which has a higher DNA yield compared with other types of bone tissue (Lee et al., 1991), and multiple vertebrae are available from one individual. Molars were the preferred teeth samples because there is more than one root available for DNA extraction for each molar.

2.2.2. Specimen preparation and decontamination

Bones and teeth were cleaned to ensure a full decontamination of the surface of the samples from exogenous modern DNA or bacterial DNA that would affect the final results. The method was a modification of what described by Mooder (2004) (Mooder, 2004).

Vertebral samples: A sterile saw was used to remove the processes from the body of the vertebra. The outer surface of the vertebra was removed using a sterile scalpel. The vertebra was immersed in 16.7% (v/v) bleach for five minutes. The bleach concentration was optimized according to a published protocol (Kemp and Smith, 2005).

Teeth samples: A sterile saw was used to separate the root from the crown. The root surface was removed using a sterile scalpel. The root was immersed in 50% (v/v) bleach for 10 min.

Following decontamination, all samples were rinsed in HPLC grade water.

The sample surface, for either bone or tooth samples, was exposed to UV light for one hour and then left to dry in a sterile container for two days in the Dead Air Box workstation. The samples were then frozen in liquid nitrogen, and pulverized using a sterile mortar and pestle. The powder was collected with sterile spatulas and stored in sterile containers at −20 °C until further analysis.

2.2.3. DNA extraction from bones and teeth

A modified silica-guanidium thiocyanate extraction protocol targeted for optimal extraction of DNA from ancient bones and teeth was used. The DNA extraction protocol was adapted from the published method (Rohland and Hofreiter, 2007) with modifications to reduce the risk of contamination. For reagent preparation, the extraction buffer was filtered using a sterile PVDF filter (0.22 μM, Millex- GV, Billerica, MA, USA). The extraction, the binding (before adding the guanidium thiocyanate), and the washing buffers (before adding the ethanol), were autoclaved for 20 min at 121 °C. All centrifugation times were doubled from the published method to assure a good separation between the precipitate and the supernatant. The last elution step was repeated twice to avoid transferring a large amount of silica, which can inhibit PCR amplification.

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

Table 1
Male individuals available for Y-chromosomal analysis from the four studies cemeteries (Lokomotiv, Shamanka II, Ust'-Ida and Kurma XI) with the individuals' sample type, and IDs, age of the individuals, period, morphological sex and archaeological age (Weber et al., 2011a, 2011b, 2011c).

No	Cemetery & master ID	Sample no	Type of sample	Morphological sex	Age of individual (years)	Archaeological age
Lokomotiv (EN)						
1	LOK_1980.004	2009.002.	Bone	Female	25–35	EN
2	LOK_1980.006	1995.100.	Bone	Male	20+	EN
3	LOK_1980.010.02	2009.007, 2001.504	Bone and tooth	Male	20–25	EN
4	LOK_1980.010.03	2009.008.	Bone	Female	50+	EN
5	LOK_1980.012	2009.010.	Bone	Female	18–22	EN
6	LOK_1981.013	2009.023, 2001.492	Bone and tooth	Male	25–35	EN
7	LOK_1980.014.03	2009.011.	Bone	Immature	10–11	EN
8	LOK_1980.016	2009.014, 2001.509	Bone and tooth	Male	45–55	EN
9	LOK_1980.017	2009.015.	Bone	Male	35–50	EN
10	LOK_1980.022.02	2009.020, 2009.021	Bone	Male	20+	EN
11	LOK_1981.024.01	2009.024.	Bone	Immature	11.5–15	EN
12	LOK_1984.027	2009.027, 2001.527, 2001.529	Bone and teeth	Male	15–18	EN
13	LOK_1985.031.02	2009.032, 2001.412	Bone and tooth	Male	25–30	EN
14	LOK_1988.038.01	2009.036.	Bone	Female	50+	EN
15	LOK_1990.042	1995.130, 2009.041, 2001.549	Bone and tooth	Male	40–50	EN
16	LOK_1990.044.01	2009.042.	Bone	Male	35–39	EN
17	LOK_1990.044.02	2009.043.	Bone	Male	30–39	EN
Shamanka II(EN)						
1	SHA_2001.012	2009.060.	Bone	Undetermined	25–35	EN
2	SHA_2001.013.03	2009.061.	Bone	Probable female	18–19	EN
3	SHA_2002.021.02	2002.257.	Tooth	Male	25–30	EN
4	SHA_2002.021.03	2009.070.	Bone	Undetermined	16–18	EN
5	SHA_2002.023.04	2001.221.	Bone	Undetermined	20+	EN
6	SHA_2004.052.01	2004.131.	Bone	Probable male	20–24	EN
7	SHA_2004.044.02	2004.031.	Bone	Undetermined	20+	EN
8	SHA_2005.059.01	2004.058.	Bone	Male	35–39	EN
9	SHA_2006.083.01	2009.109.	Bone	Male	20–22	EN
Ust'-Ida (LN-EBA)						
1	UID_1987.005	2009.177	Bone	Immature	7–9	LN
2	UID_1987.009	2009.179, 2001.546	Bone and tooth	Immature	4–7.5	LN
3	UID_1987.012	2001.490	Tooth	Male	50+	LN
4	UID_1988.016.01	2009.182	Bone	Male	25–35	LN
5	UID_1988.016.02	2009.183	Bone	Male	50+	LN
6	UID_1989.020.01	2009.185, 2001.418	Bone and tooth	Male	18–24	LN
7	UID_1989.026.01	2009.190, 2001.547	Bone and tooth	Immature	13–15	LN
8	UID_1989.029	1995.157, 2001.489	Bone and tooth	Male	50+	EBA
9	UID_1989.030	2009.194	Bone	Female	50+	LN
10	UID_1990.033.01	2009.196, 2001.513	Bone and tooth	Male	12–15	LN
11	UID_1991.038	2009.199	Bone	Male	45–60	LN
12	UID_1993.043	2009.201	Bone	Male	Mature	LN
13	UID_1993.044.03	2001.574	Tooth	Immature	11–12	LN
14	UID_1994.048	2009.202	Bone	Male	50+	EBA
15	UID_1994.053.02	2009.204	Bone	Immature	4–6	LN
16	UID_1994.055.02	2009.205	Bone	Male	15–18	LN
Kurma XI (EBA)						
1	KUR_2002.001	2002.106	Teeth	Male	25–30	EBA
2	KUR_2002.007.02	2002.116	Bone	Male	20–29	EBA
3	KUR_2002.010	2003.099	Tooth	Probable male	18–25	EBA
4	KUR_2002.015	2002.134	Bone	Probable male	17–18	EBA
5	KUR_2003.017	2003.013	Teeth	Probable male	20+	EBA
6	KUR_2003.018	2003.003	Tooth	Probable female	17–19	EBA
7	KUR_2003.019	2003.008, 2003.010	Tooth	Probable male	20–30	EBA

2.3. Mitochondrial DNA analysis

Mitochondrial DNA was amplified as previously described by Mooder et al., 2005 (Mooder et al., 2005) with slight modifications. The PCR reaction amplified the HV1 region of the mtDNA (bp 16,191 to 16,367), where most Asian specific polymorphisms were found (Schurr et al., 1999; Kivisild et al., 2002). L16211 and H16346 primers were designed to amplify a 176 bp sequence of mtDNA (Mooder et al., 2005) in a reduced number of PCR cycles from previously described (50 PCR amplification cycles (Mooder et al., 2005)) to a 40-cycle PCR amplification reaction. The PCR amplification reaction was performed using Eppendorf AG Thermocycler (Hamburg, Germany). Each 25 μl PCR reaction consisted of 1× PCR buffer (Invitrogen, NY, USA), 1.5 mM $MgCl_2$ (Invitrogen, NY, USA), 0.2 mM of each dNTP (Invitrogen, NY, USA), 0.4 mg/ml BSA (Roche, IN, USA), 1.0 μM of each primer (Invitrogen, NY, USA), and 1.25 U of Platinum Taq DNA Polymerase (Invitrogen, NY, USA). The amplification cycle started with a denaturing step at 95 °C for 2 min, and 40 cycles of 95 °C for 1 min, 56 °C annealing temperature for 1 min and 72 °C for 1 min. For PCR clean-up, the ExoSAP-IT Clean-up Kit (GE Healthcare, Life Sciences, NY, USA) was used at The Applied Genomic Core (TAGC, University of Alberta). Then, sequencing was performed using the BigDye® Terminator Kit v3.1 (Life Technologies, NY, USA) at TAGC (University of Alberta) with the same PCR amplification primers. For post-reaction clean-up of unincorporated dyes, nucleotides and the primers, the magnetic beads Agencourt CleanSEQ (Beckman Coulter, California, USA) was used on an automated Biomek 3000 work station (TAGC). The samples were loaded on the 3130xl Genetic Analyzer (Life Technologies, NY, USA) and analyzed using the Sequence Scanner v1.0 (Applied Biosystems, NY, USA). Sequence data were compared with the Cambridge reference

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

sequence (GenBank number: NC_012920) (Anderson et al., 1981; Andrews et al., 1999), and base substitutions in the HV1 region were used to assign mtDNA haplogroups.

2.4. Molecular sex assignment

Molecular sex determination was done as part of the Y-chromosome analysis. The sex of the Kurma XI and Shamanka II (four individuals only) samples were assigned by amelogenin analysis as part of this study (Table 3). The sex of Lokomotiv and Ust'-Ida samples were assigned previously by Mooder et al. (2006) (Mooder et al., 2006) (Table 3). Amelogenin, a gene involved in the formation of dental enamel, is found on both X and Y chromosomes (Nakahori et al., 1991). PCR primers for a region of intron 1 in the amelogenin gene gave a 106 bp product for females and 106/112 bp product for males (Sullivan et al., 1993). The PCR amplification was performed as stated above for mtDNA but with a modification of the annealing temperature to 62 °C. The amelogenin PCR amplification product was visualized via the Gel Doc™ EZ System (Bio-Rad, Ontario, Canada) after gel-electrophoresis on 12% polyacrylamide gel and ethidium bromide (10 mg/ml) staining.

2.5. Y-chromosome single nucleotide polymorphisms (SNPs) analysis

2.5.1. Multiplex PCR amplification and SNaPshot reaction

Specific Y-chromosomal SNPs representative of East Asian and Siberian paternal lineages were selected for this study (Fig. 2). Y-chromosomal SNPs were analyzed through the SNaPshot Multiplex kit (Life Technologies, NY, USA) via the ABI PRISM 3130xl Genetic Analyzer (Life Technologies, NY, USA) using POP-4® (Life Technologies, NY, USA). The method was optimized for aDNA by adaptation of a published protocol that designed primers suitable to amplify SNP sites with amplicon size ranged between 81 and 155 bp (Bouakaze et al., 2007). The 13 SNP markers were examined via two multiplex PCR amplification reactions. The modified PCR amplification was performed using an Eppendorf AG Thermocycler (Hamburg, Germany). Each 25 μl PCR consisted of 1× PCR buffer (Invitrogen, NY, USA), 4.0 mM $MgCl_2$ (Invitrogen, NY, USA), 400 μM of each dNTP (Invitrogen, NY, USA), 1.0 mg/ml BSA (Roche, IN, USA), 2.0 U of Platinum Taq DNA Polymerase (Invitrogen, NY, USA), and the two multiplex primers' mixtures (Invitrogen, NY, USA). The ExoSAP-IT PCR clean-up kit (GE Healthcare, Life Sciences, NY, USA) was selected for PCR clean-up. The SNaPshot results were analyzed on GeneMapper 4.0 software (Life Technologies, NY, USA). Y-chromosomal haplogroups were assigned according to the updated human Y-chromosomal haplogroup tree (Karafet et al., 2008).

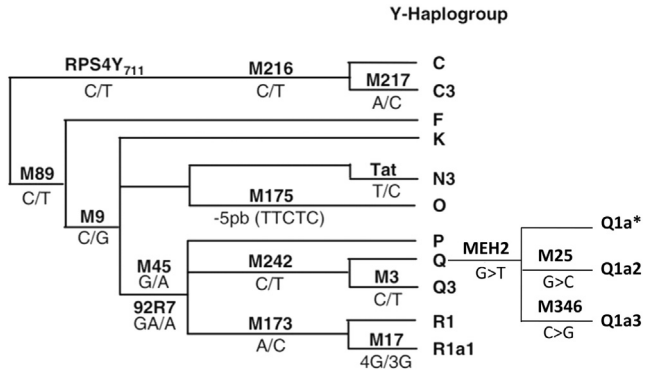

Fig. 2. Phylogenetic tree of the 13 Y-chromosomal SNPs analyzed in the multiplex/SNaPshot reactions and the three SNPs identifying the Q-M242 sub-haplogroups. SNP names and sequence variations indicated above and under the lines, respectively. The haplogroups associated with the specific variations designated at the end of the line (Bouakaze et al., 2007).
Source: Adapted with permission from [First successful assay of Y-SNP typing by SNaPshot minisequencing on ancient DNA. C. Bouakaze. International Journal of Legal Medicine. 121 (6). Copyright© 2007 Springer] (License number 3537380106957).

2.5.2. Y-chromosomal sub-haplogroups of Q-M242

For this study, primers suitable for amplifying the SNP sites, identifying Q-M242 sub-haplogroups, were designed using Primer3Plus software (Untergasser et al., 2012) (Table 2, Fig. 2). The amplicon size was <155 bp to match the criteria used with the SNaPshot multiplex PCR reaction. The PCR amplification reaction was identical to mtDNA analysis except that the annealing temperature was 58 °C.

2.6. Evaluation of authenticity

Authenticity of all results obtained during the study was established through multiple analyses of the same sample following published guidelines (Cooper and Poinar, 2000). Two separate SNaPshot reactions, multiplex I (7-plex) and multiplex II (6-plex), were performed on each sample, and results rejected if there was any discordance between the obtained results. Analysis of sub-haplogroups of Q-M242 was repeated twice for each sample to confirm the results. Because the peak threshold, on the electropherogram, is 100 relative fluorescence units (RFUs), any peak less than this value were rejected. Only females were working with the samples in this study, minimizing the risk of contamination from male DNA.

2.7. Statistical analysis

To test the null hypothesis for the Y-chromosomal haplogroup distributions, i.e. that the EN cemeteries (Lokomotiv and Shamanka II) were continuous to LN-EBA cemeteries (Kurma XI and Ust'-Ida), Fisher's exact test was applied with a two-by-two contingency table. The table was extended to the size described by the number of populations and the number of haplogroups examined in the study. Fisher's exact test is analogous to two-way contingency chi-square test, but Fisher's exact test is always chosen when dealing with a small sample size as in this study's case as two-way contingency chi-square test requires that any given cell have a minimum frequency of five (Gould and Gould, 2002), which was not obtainable in our study for some haplogroups. Fisher's exact test was performed using the **SAS/STAT®** software.

3. Results

3.1. Analytical results

Amelogenin analysis for Lokomotiv samples by Mooder et al. (2005) identified 15 males and four females (Mooder et al., 2005). Amelogenin analysis for Shamanka II samples by Thomson (2005) identified six males and one female (Thomson, 2005), and another four Shamanka II males and two females were identified by amelogenin analysis as part of this study. For Ust'-Ida, 18 males and eight females were identified by Mooder et al. (2006) through amelogenin analysis (Mooder et al., 2006). For Kurma XI, six males and five females were identified through amelogenin analysis as part of this study (Table 3). Table 3 included the male individuals only and their mtDNA haplogroups as this is the focus of this paper.

Authentic Y-chromosomal results were obtained from a total of 36 males from the four cemeteries are represented in Table 3. Only results meeting the authenticity criteria, stated in Section 2.6, are included. The Lokomotiv cemetery showed a low success rate for Y-chromosomal analysis (7/17 males, 41%). However, higher success rates were obtained from the other cemeteries, Kurma XI (6/7 males, 86%), Ust'-Ida (14/16 males, 87%) and Shamanka II (9/9 males, 100%). Eight individuals from Lokomotiv and two from Ust'-Ida did not give results. Three individuals (two from Lokomotiv and one from Kurma XI) did not have Y-chromosomal haplogroups assigned (marked "not assigned" in

Table 2
Primers designed to amplify sub-haplogroups of Q-M242.

SNP markers (sub-haplogroups)	PCR primer sequence (5′ → 3′)		Amplicon size (bp)	Annealing temperature
	Forward	Reverse		
MEH2 (Q1a*)	CAAATTTTGAGTAAGCCATCACC	TGGAAACACAACTGTTTGAAAAT	150	58 °C
M25 (Q1a2)	CACCCAGAGACACACAAAACA	TGTTGTAAGAATTCAGTAGGATTGATG	107	58 °C
M346 (Q1a3)	TTTGTCTCTGAGCTGACAAGGA	TCCACTCACTCTGCCTACCTG	125	58 °C

Table 3
Amelogenin results, mtDNA and Y-chromosomal haplogroups from the male individuals belonging to the four studied cemeteries (Lokomotiv, Shamanka II, Ust'-Ida and Kurma XI).

No	Cemetery & master ID	Molecular sex	Mitochondrial DNA haplogroup	Y-chromosome haplogroup- YSNP marker
Lokomotiv (EN)				
1	LOK_1980.004	XY[a]	D[a]	m.d.
2	LOK_1980.006	XY[a]	F[a]	R1a1-M17
3	LOK_1980.010.02	XY[a]	Other[a]	K-M9
4	LOK_1980.010.03	XY[a]	F[a]	Not assigned
5	LOK_1980.012	XY[a]	A[a]	m.d.
6	LOK_1981.013	XY[a]	D[a]	Not assigned
7	LOK_1980.014.03	XY[a]	U5a[a]	m.d.
8	LOK_1980.016	XY[a]	D[a]	K-M9
9	LOK_1980.017	XY[a]	A[a]	m.d.
10	LOK_1980.022.02	XY[a]	C[a]	K-M9
11	LOK_1981.024.01	XY[a]	F[a]	R1a1-M17
12	LOK_1984.027	XY[a]	D[a]	m.d.
13	LOK_1985.031.02	XY[a]	A[a]	C3-M217
14	LOK_1988.038.01	XY[a]	F[a]	m.d.
15	LOK_1990.042	XY[a]	G2a[a]	K-M9
16	LOK_1990.044.01	XY[a]	F[a]	m.d.
17	LOK_1990.044.02	XY[a]	F[a]	m.d.
Shamanka II (EN)				
1	SHA_2001.012	XY[b]	D	K-M9
2	SHA_2001.013.03	XY[b]	D[b]	K-M9
3	SHA_2002.021.02	XY[b]	G2a	K-M9
4	SHA_2002.021.03	XY[b]	A	K-M9
5	SHA_2002.023.04	XY[b]	D	K-M9
6	SHA_2004.052.01	XY	D	K-M9
7	SHA_2004.044.02	XY	C	K-M9
8	SHA_2005.059.01	XY	C	K-M9
9	SHA_2006.083.01	XY	C	K-M9
Ust'-Ida (LN-EBA)				
1	UID_1987.005	XY[a]	Other[a]	Other (L914 SNP)
2	UID_1987.009	XY[a]	A[a]	Q1a3-M346
3	UID_1987.012	XY[a]	D[a]	Q1a3-M346
4	UID_1988.016.01	XY[a]	F[a]	Q1a3-M346
5	UID_1988.016.02	XY[a]	Other[a]	Q1a3-M346
6	UID_1989.020.01	XY[a]	C[a]	Q1a3-M346
7	UID_1989.026.01	XY[a]	A[a]	Other (L914 SNP)
8	UID_1989.029	XY[a]	A[a]	Q1a3-M346
9	UID_1989.030	XY[a]	A[a]	m.d.
10	UID_1990.033.01	XY[a]	Other[a]	Q1a3-M346
11	UID_1991.038	XY[a]	C[a]	Q1a3-M346
12	UID_1993.043	XY[a]	G2a[a]	K-M9
13	UID_1993.044.03	XY[a]	A[a]	Q1a3-M346
14	UID_1994.048	XY[a]	C[a]	Q1a3-M346
15	UID_1994.053.02	XY[a]	A[a]	m.d.
16	UID_1994.055.02	XY[a]	G2a[a]	Q-M242
Kurma XI (EBA)				
1	KUR_2002.001	XY	D	Not assigned
2	KUR_2002.007.02	XY	D	Q1a3-M346
3	KUR_2002.010	NA	Z	Q1a3-M346
4	KUR_2002.015	XY	Z	Q1a3-M346
5	KUR_2003.017	XY	D	Q-M242
6	KUR_2003.018	XY	D	Q-M242
7	KUR_2003.019	XY	F	Other (L914 SNP)

m.d. = missing data, no results were obtained from these samples. Not assigned: the data obtained were not adequate enough to assign a Y-chromosomal haplogroup.
[a] Molecular sexing and mtDNA haplogroup determined by Mooder et al. (2005, 2006).
[b] Molecular sexing and for one sample mtDNA determined by Thomson (2005).

Table 3), as some of the key SNPs did not amplify either by SNaPshot I or II reactions. Three individuals from the LN-EBA (Ust'-Ida and Kurma XI) cemeteries show a previously undescribed polymorphism, L914 SNP (T>G transversion) (Table 3).

Despite the low analytical success rate, Lokomotiv demonstrated the highest degree of heterogeneity in Y-chromosomal haplogroup distribution with four individuals belonging to haplogroup K-M9, two to haplogroup R1a1-M17 and one to haplogroup C3-M217.

All individuals from the other EN cemetery, Shamanka II, belonged to one haplogroup, K-M9. The Y-chromosomal haplogroups from the LN-EBA cemetery Ust'-Ida, showed one individual with haplogroup K-M9, two individuals (Other- L914 SNP) and the remaining eleven individuals belonging to haplogroup Q-M242 and its sub-haplogroup Q1a3-M346. In the other LN-EBA cemetery, Kurma XI, one individual carried the L914 SNP and all remaining individuals belonged to Q-M242 and sub-haplogroup Q1a3-M346 (Table 3). None of the analyzed individuals specifically from Ust'-Ida or Kurma XI belonged to sub-haplogroup Q1a2-M25.

3.2. Statistical results

Fisher's exact test results obtained from the two EN cemeteries, Lokomotiv and Shamanka II, showed a value of (Fisher's exact test; $p = 0.0625$) close to the statistical significance level 0.05. This would indicate a similar paternal background. However, the number of individuals analyzed may not be representative of the entire male population from the two cemeteries, and analyzing more male samples would be valuable.

The Fisher's exact test obtained from the two LN-EBA cemeteries, Ust'-Ida and Kurma XI, showed a strong statistical similarity ($p = 1.00$), which is indicative of sharing the paternal origin between these two LN-EBA cemeteries.

Fisher's exact test obtained between Kurma XI vs. Lokomotiv and Kurma XI vs. Shamanka II showed significant statistical differences between them ($p = 0.0023$ and $p = 0.0002$; respectively). Similarly, Fisher's exact test between Ust'-Ida and Lokomotiv ($p = 0.0002$), and Ust'-Ida and Shamanka II ($p < 0.0001$) also demonstrated a significant statistical difference.

4. Discussion

4.1. EN cemeteries populations' archaeological context

Individuals from the two EN cemeteries of Cis-Baikal showed a Y-chromosomal haplogroup distribution (Fisher's exact test; $p = 0.0625$), which approached a statistical significance level p value of 0.05. The lack of Y-chromosomal haplogroup (only K-M9) variation in Shamanka II is striking. By contrast, there were three different Y-chromosomal haplogroups (K-M9, R1a1-M17, C3-M217) among the seven individuals represented at Lokomotiv, which indicates a high level of paternal heterogeneity at this cemetery. The statistics suggest a relatively close paternal biological relationship between the two EN communities. The two EN cemeteries are located on two different micro-regions in Cis-Baikal area, Lokomotiv on the Angara River Valley and Shamanka II on the South Baikal region. Stable isotope ratios, reflecting diet; from the two micro-regions show different signatures depending on the different types of fish and aquatic foods available in each area. Individuals

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

from Lokomotiv and Shamanka II would be expected to show different stable isotope signatures because they are far apart from each other and located on two different fisheries, Lokomotiv on the riverine Angara and Shamanka II on lacustrine Lake Baikal. However, Lokomotiv and Shamanka II have similar stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) as indicated by Weber and Bettinger (2010). These two large EN populations probably had similar adaptive strategies and access to both fisheries, the Angara and Baikal. Also, this can be an indication for an interaction between the populations of these two EN cemeteries (Weber and Bettinger, 2010).

Further investigation of other male individuals from Lokomotiv and Shamanka II, to detect if the variation in haplogroup distribution would increase or decrease with increasing the sample size and if other Y-chromosomal haplogroups would be detected in Shamanka II, is necessary because the Y-chromosomal results from both EN cemeteries (Lokomotiv and Shamanka II) might be biased by the small sample size (Lokomotiv, n = 7, and Shamanka II, n = 9).

Lokomotiv show a high degree of variability in strontium isotope ratios within and between individuals proposing differences in mobility during the individual's life (Haverkort et al., 2010). Bone and teeth strontium isotope ratios from Lokomotiv showed a degree of inconsistency suggesting differences in mobility between the individuals. Linking this variability to the Y-chromosomal haplogroup results at the individual level we observe that the only three males belonging to haplogroup K-M9 (LOK_1980.010, age 20–25 years, LOK_1980.016, age 45–55 years and LOK_1990.042, age 40–50 years) did not show a high degree of variability in $^{87}Sr/^{86}Sr$ between bones and teeth suggesting lower mobility during their lifespan. By contrast, the only male in the EN population belonging to haplogroup C3-M217 (LOK_1985.031.02, age 25–30 years) demonstrated a strikingly different $^{87}Sr/^{86}Sr$ ratio, between bones and teeth, from the other three individuals at Lokomotiv from whom both strontium isotope and Y-haplogroup analysis is available. This difference suggests that he was born in one location, and although buried in the Lake Baikal area, spent his adulthood outside the region as his $^{87}Sr/^{86}Sr$ from femur is quite different from the other Lokomotiv individuals. No further information could be gleaned about this individual. He was buried in a double grave, and therefore unlikely to be 'elite' because Mooder et al. (2006) suggested that in the EN culture (Kitoi culture) 'elite' people were buried in a single grave (Mooder et al., 2006). The other individual (LOK_1985.031.01, age 35–50 years) buried with the C3-M217 male (LOK_1985.031.02), in a double grave, had an undetermined morphological sex (Lieverse, 2010).

For Shamanka II, no strontium isotope data from Shamanka II has been published yet.

4.2. LN-EBA cemeteries populations' archaeological context

Individuals from the two LN-EBA cemeteries (Ust'-Ida and Kurma XI) showed no significant differences in their Y-chromosomal haplogroup distribution (Fisher's exact test; p = 1.00) with haplogroup Q-M242 being the predominant in both populations (n = 11/14 individuals in Ust'-Ida, and n = 5/6 individuals in Kurma XI). Ust'-Ida and Kurma XI belong to two different micro-regions in the Lake Baikal area (Fig. 1). Y-chromosomal haplogroups from Ust'-Ida were obtained from 12 LN and two EBA individuals. All Kurma XI individuals, from whom Y-chromosomal haplogroups were obtained, belonged to the EBA period.

Individuals of all ages, including subadults and adults, are buried at Ust'-Ida. However, at Kurma XI there were no individuals under the age of 15 or older than 50 years, which could indicate that this is an 'exclusive' cemetery rather than a community burial ground (McKenzie, 2010). The two males (UID_1987.005 and UID_1989.026.01), who carry the Y-chromosomal L914 SNP in Ust'-Ida, are both juvenile (Tables 1 and 3). One male (UID_1987.005) was buried in a single grave, but the other male (UID_1989.026.01) was buried in a multiple grave. The only individual in Kurma XI carrying the Y-chromosomal L914 SNP (KUR_2003.019) was a young adult and was in one of the three sitting position graves at Kurma XI. There was no obvious relation between the two individuals at Ust'-Ida carrying the Y-chromosomal L914 SNP as both had different mtDNA haplogroups (Table 3). One was buried in a single grave (UID_1987.005) while the other was buried in a multiple grave (UID_1989.026.01). The Kurma XI individual (KUR_2003.019) carrying Y-chromosomal polymorphism L914 belonged to mtDNA haplogroup F (Table 3). However, no Y-chromosomal haplogroup data were obtained from the other two sitting burials in Kurma XI to compare them genetically to each other.

The Strontium isotope ratios of individuals from Ust'-Ida show a low variability when compared to Lokomotiv individuals, which might suggest a lower mobility level during lifetime (Haverkort et al., 2010). No Strontium isotope data have been published for Kurma XI individuals yet.

Despite the many differences in burial practices and mtDNA haplogroup distribution between LN-EBA Ust'-Ida and Kurma XI cemeteries, the Y-chromosomal haplogroup frequency and distribution is very similar, implying that both Ust'-Ida and Kurma XI had a similar paternal origin.

4.3. Correlation between maternal and paternal background in Cis-Baikal EN and LN-EBA populations

Comparing the Y-chromosomal haplogroup distribution findings to the mtDNA haplogroup distribution reflecting the paternal and maternal background might provide insight into the genetic relationship between the individuals in the various Cis-Baikal cemeteries. In Lokomotiv, the two individuals (LOK_1980.006 and LOK_1981.024.01) carrying R1a1-M17 Y-chromosomal haplogoup, both belong to mtDNA haplogroup F (Mooder et al., 2005). Insufficient evidence at present to imply that these two males are related, as there is a lack in other DNA sequence data (e.g. autosomal DNA analysis). The only individual at Lokomotiv (LOK_1985.031.02) with the Y-chromosomal haplogroup C3-M217, had the mtDNA haplogroup A, presented at a low frequency (13%, n = 4/31) in Lokomotiv (Mooder et al., 2005). This individual also showed different strontium isotope ratios, indicating a higher degree of mobility than other Lokomotiv individuals (Haverkort et al., 2010). The evidence may suggest that this male's uncommon genetic background and unique strontium isotope ratio imply that he was from outside the Lokomotiv locality.

All three individuals at Lokomotiv carrying the K-M9 Y-chromosomal haplogroup carried different mtDNA haplogroups (Mooder et al., 2005) (Table 3), so no connection between maternal and paternal genetic background can be concluded.

All males in Shamanka II cemetery belong to one Y-chromosomal haplogroup (K-M9), although there are five different mtDNA haplogroups represented (Table 3). The lack of variation in Y-chromosomal haplogroups in this population makes it difficult to draw conclusions about the genetic relationship between the individuals, but further Y-chromosomal analysis would be required to analyze subhaplogroups of K-M9.

In the Ust'-Ida burials, one major Y-chromosomal haplogroup, Q-M242 and its sub-haplogroup Q1a3-M346, is represented. The exceptions were two individuals (UID_1987.005 and UID_1989.026.01) with the novel Y-chromosomal SNP, L914 and one male (UID_1993.043) with the K-M9 haplogroup.

The two males in Ust'-Ida (UID_1987.005 and UID_1989.026.01) carrying the L914 SNP belong to different mtDNA haplogroups (Other and A, respectively) (Table 3). This finding indicates that there is no maternal relation between these two Ust'-Ida individuals carrying Y-chromosomal L914 SNP. Also, 83.3% (n = 5/6) of males in Kurma XI belong to Y-chromosomal haplogroup Q-M242 or its sub-haplogroup Q1a3-M346. These males belong to either mtDNA haplogroup D (three individuals) or Z (two individuals) (Table 3), and the only male carrying Y-

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

chromosomal polymorphism L914 has a different mtDNA haplogroup (haplogroup F) than the other males (Table 3). Mitochondrial DNA haplogroup F is present in 25% (n = 3/12) of individuals in the EBA Kurma XI cemetery (Moussa, 2015). The other two individuals carrying the mtDNA haplogroup F are females, and there are no other males in Kurma XI cemetery carrying Y-chromosomal polymorphism L914. Therefore, no relation can be drawn between mtDNA and Y-chromosomal haplogroups' distributions at this time.

4.4. Genetic context of the Cis-Baikal prehistoric population and the connection with the modern populations

The modern Cis-Baikal populations carry a heterogeneous distribution of Y-chromosomal haplogroups, each with a different origin and composition.

Haplogroup K-M9 is represented in both EN cemeteries (57.1%, n = 4/7) in Lokomotiv and 100%, n = 9/9 in Shamanka II), but with low frequency in the LN-EBA cemetery Ust'-Ida (7.1%, n = 1/14) and absent in EBA Kurma XI. It is believed that the origin of haplogroup K-M9 is from Southwest Asia (Nasidze et al., 2005). K-M9 is found with high frequency in the south Siberian populations, for example, in the Tuvans and Mongolians and is also found in one central Asian population (Uigur) (Chen et al., 2011). K-M9 is an ancient haplogroup with an estimated age of 40,000–53,900 years. Haplogroup K with its designated M9 mutation (Fig. 3) is considered the ancestral haplogroup that defines each of L, M, NO, P, S and T haplogroups (Karafet et al., 2008).

The second Y-chromosomal haplogroup found in high frequency in the LN-EBA populations is Q-M242 or its sub-haplogroup Q1a3-M346. Haplogroup Q-M242 or its sub-haplogroup Q1a3-M346 is found in 78.6% (n = 11/14) of Ust'-Ida individuals and in 83.3% (n = 5/6) of Kurma XI individuals. The approximate age of this haplogroup is 17,700 ± 4800 years. Haplogroup Q-M242 is widely distributed across Siberia (Karafet et al., 2002), and it is also widely distributed in Central to South Asia, West Eurasia and northern East Asia, which might be indicative of Q-M242 expansion through northern Eurasia as a migratory route (Zhong et al., 2011). Individuals carrying haplogroup Q-M242 might have migrated from Siberia via the Altai/Baikal area to the Americas (Bortolini et al., 2003; Seielstad et al., 2003; Zegura et al., 2004), which suggest the presence of this haplogroup in the area just after the Last Glacial Maximum (Malyarchuk et al., 2011). A study done by Malyarchuk et al. (2011) examining several northern East Asian sub-haplogroups of Q-M242, showed that Q1a3-M346 is more frequent than other sub-haplogroups (Q1a-MEH2, Q1a2-M25 and Q1a3a-M3) in the Siberian populations. Q1a3-M346 is found in the Altaians, Todjins, Tuvinians, Khakassians and Sojots, and rarely found in the Kalmyks population (Malyarchuk et al., 2011) (Table 4).

Haplogroup R1a1-M17 was identified in 28.6% (n = 2/7) of males in the Lokomotiv population. This haplogroup is not only defined by the M17 marker, but also by M173 marker (Fig. 3) (Y Chromosome Consortium, 2002; Karafet et al., 2008). R1a1 is widely distributed in Eurasia, including western Eurasia, southern Asia, central Asia and the Siberian population, particularly southern Siberia. It is believed that the R1a1 haplogroup is associated with the early migration of the Indo-Europeans eastward (Semino et al., 2000). The estimated age of R1a1-M17 is about *13,800 cal BP* as estimated by the SNP evolution rate (Underhill et al., 2000). It is also widely distributed in the southern and northern modern Altaian populations (Khar'kov et al., 2007). R1a1-M17 is found in the Tuvinian population (Kharkov et al., 2013), Khakassian population (Khar'kov et al., 2011), and in the native Yakuts of the Sakha Republic (Khar'kov et al., 2008). In prehistoric populations, the presence of the R1a1-M17 haplogroup was evident in the ancient Kurgan culture, a Late Bronze Age culture (5000) found in southern Siberia. Twelve males analyzed from the ancient Kurgan people belong to R1a1-M17 haplogroup except for one belonging to C3 (Keyser et al., 2009).

Haplogroup C3 is defined by the M217 SNP on the Y-chromosome (Fig. 3). Only one C3 individual was found in the Cis-Baikal population. However, it is detected in several parts of Asia including the Central, South, Southeast and East of Asia, and also in Siberia and the Americas. There are many sub-haplogroups of C3 (Zhong et al., 2010), but these were not investigated in this study. The C3 haplogroup dated back in Siberia to 11,900 ± 4800 years ago. A possible source of this haplogroup in Siberia is Mongolia and/or Lake Baikal (Karafet et al., 2002).

The L914 SNP, a previously undescribed (T>G) transversion, was found in the LN-EBA populations. L914 SNP does not define a specific Y-chromosomal haplogroup and is not cited in the modern world population, which might indicate that this is a rare mutation site that is not defined yet.

5. Conclusion

The EN and the LN-EBA populations at Lake Baikal might be paternally genetically distinct; however, this insight might change with analyzing more males from all the mentioned cemeteries, as the number of males obtained in this study may not be representative of the entire population. The earlier conception of the biological discontinuity between the EN and the LN-EBA groups in the Cis-Baikal area suggested by Weber et al. (2002) (Weber, 1995) could be supported, so far, from the analysis of the paternal background of the area. The differences in the Y-chromosomal signatures of the EN and LN-EBA suggest displacement of males in the Cis-Baikal population during the EN period and replacement by a genetically different population during the LN-EBA

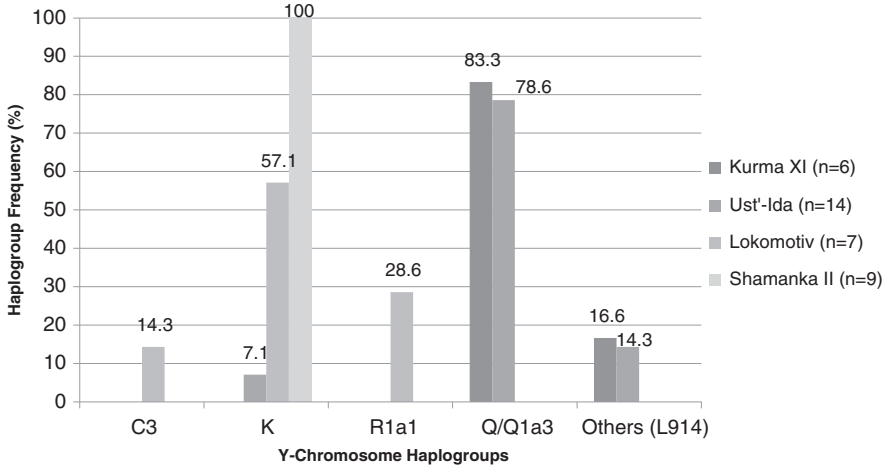

Fig. 3. A column chart representing Y-chromosomal haplogroup frequency distribution in percentage for the four studied cemeteries (Lokomotiv, Shamanka II, Ust'-Ida, and Kurma XI).

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

Table 4
Explanation to Fig. 3. (Number of male individuals from each of the four studied cemeteries and the Y-chromosomal haplogroups they are assigned to.)

Cemetery	Y-chr. haplogroups	C3-M217	K-M9	R1a1-M17	Q-M242/Q1a3-M346	Others (L914 SNP)
Kurma XI (n = 6)		0	0	0	5/6 (83.3%)	1/6 (16.6%)
Ust'-Ida (n = 14)		0	1/14 (7.1%)	0	11/14 (78.6%)	2/14 (14.3%)
Lokomotiv (n = 7)		1/7 (14.3%)	4/7 (57.1%)	2/7 (28.6%)	0	0
Shamanka II (n = 9)		0	9/9 (100%)	0	0	0

periods. As shown in the results, there is little resemblance in the Y-chromosomal haplogroups shared between the EN and LN-EBA. There could be a similarity in Y-chromosomal haplogroup distribution between the two EN (Kitoi culture) cemeteries (Lokomotiv and Shamanka II), which could indicate a common paternal ancestor and possible social interaction, even if the new mtDNA data from Shamanka II showed that Lokomotiv and Shamanka II are maternally different from each other. Sharing a common paternal ancestor between Lokomotiv and Shamanka II needs to be further investigated through analyzing more male samples from both cemeteries (Lokomotiv and Shamanka II) as the results could be biased due to the small sample sizes from both cemeteries. The Y-chromosomal haplogroup distribution of the two LN-EBA cemeteries is similar, indicating a unified paternal origin for the area during the LN-EBA period. However, interestingly the mtDNA haplogroup distribution in EBA Kurma XI is different from LN Ust'-Ida and also from EBA Ust'-Ida (Moussa, 2015).

Based on Y-chromosomal haplogroup analysis of both the EN and LN-EBA cemeteries, it is clear that the Y-chromosomal haplogroups of the prehistoric Cis-Baikal population are represented in the contemporary Siberian populations. The haplogroups found in the prehistoric study groups can mark migration events from Lake Baikal area to the south towards Mongolia, and the Altai Republic as for haplogroup C3-M217 and R1a1-M17, respectively. Furthermore, the ancient hunter-gatherer groups that inhabited Lake Baikal played a major role in the formation of the Native American tribes evident in the existence of sub-haplogroup Q1a3 in the LN-EBA people. This is evident in the high frequency of sub-haplogroup Q1a3a-M3 in the Native American population (Schurr and Sherry, 2004). The sister sub-haplogroup of Q1a3a-M3 is sub-haplogroup Q1a3-M346 (Malyarchuk et al., 2011), which is identified in the LN-EBA populations of Cis-Baikal.

Analyzing more male samples would be beneficial to observe possibly other males carrying Y-chromosomal haplogroup C3-M217 and compare their strontium isotope ratios to examine if these males share similar mobility levels.

Acknowledgements

We owe our sincere acknowledgements to the Baikal Archaeology Project team for their support, encouragement and their involvement in the incredible fieldwork in Russia. Also, we would like to extend a special thank you to the Social Sciences and Humanities Research Council of Canada for its continuous financial support (Major Collaborative Research Initiative grants nos. 410-2000-1000, 412-2005-1004 and 412-2011-1001). We would like to express our deepest gratitude to Dr. Rong-Cai Yang (ARD Professor of Statistical Genomics, Department of Agricultural, Food and Nutritional Science) for his help with the statistical analysis in this paper. As for Susan Kenny and Simona Veniamin from The Applied Genomic Centre, we would like to deeply thank them for their help and efforts in the SNaPshot and Sequencing experiments in this paper.

References

Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijn, M.H., Coulson, A.R., Drouin, J., et al., 1981. Sequence and organization of the human mitochondrial genome. Nature 290 (5806), 457–465 Apr 9.

Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., Howell, N., 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. 23 (2), 147 Oct.

Bazaliiskii, V.I., 2000a. In: Bondarchuk, L. (Ed.), Shamanka II Field Report, Graves 5, 8-11, Trans. University of Alberta, Edmonton Baikal Archaeology Project.

Bazaliiskii, V.I., 2000b. In: Bondarchuk, L. (Ed.), Shamanka II Field Report, Graves 11-19, Trans 2. University of Alberta, Edmonton Baikal Archaeology Project.

Bazaliiskii, V.I., 2003. The Neolithic of the Baikal region on the basis of mortuary material. In: Weber, A.W., Mckenzie, H.G. (Eds.), Prehistoric Foragers of the Cis-Baikal. Canadian Circumpolar Press, Siberia Edmonton, pp. 37–50.

Bazaliiskii, V.I., 2010. Mesolithic and Neolithic mortuary complexes in the Baikal region. In: Weber, A.W., Katzenberg, M.A., Schurr, T.G. (Eds.), Prehistoric Hunter-Gatherers of the Baikal Region, Siberia: Bioarchaeological Studies of Past Life Ways Philadelphia. University of Pennsylvania Press, USA, pp. 51–86.

Bazaliiskiy, V.I., Savelyev, N.A., 2003. The wolf of Baikal: the "Lokomotiv" early Neolithic cemetery in Siberia (Russia). Antiquity 77, 20–30.

Bortolini, M.C., Salzano, F.M., Thomas, M.G., Stuart, S., Nasanen, S.P., Bau, C.H., et al., 2003 Sep. Y-chromosome evidence for differing ancient demographic histories in the Americas. Am. J. Hum. Genet. 73 (3), 524–539.

Bouakaze, C., Keyser, C., Amory, S., Crubezy, E., Ludes, B., 2007 Nov. First successful assay of Y-SNP typing by SNaPshot minisequencing on ancient DNA. Int. J. Legal Med. 121 (6), 493–499.

Chen, Z., Zhang, Y., Fan, A., Zhang, Y., Wu, Y., Zhao, Q., et al., 2011. Brief communication: Y-chromosome haplogroup analysis indicates that Chinese Tuvans share distinctive affinity with Siberian Tuvans. Am. J. Phys. Anthropol. 144, 492–497.

Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289 (5482), 1139 Aug 18.

Gerasimova, M.M., 1992. Cherepa iz Fofanovskogo mogil'nika (r. Oka, Selenga). In: Masson, V.M. (Ed.), Drevnosti Baikala. Irkutskii gosudarstvennyi unversitet, Irkutsk, pp. 97–111.

Goebel, T., 1999. Pleistocene human colonization of Siberia ana peopling of the Americas: an ecological approach. Evol. Anthropol. 8 (6), 208–227.

Goldstein, L.G., 1981. One-dimensional archaeology and multi-dimensional people: spatial organization and mortuary analysis. In: Chapman, R., Kinnes, I., Randsborg, K. (Eds.), The Archaeology of Death Cambridge. Cambridge University Press, England, pp. 53–70.

Goriunova, O.I., Novik, A.G., Mamonova, N.N., 2004. Drevnie pogrebeniia mogil'nika Uliarba na Baikale: Neolit-paleometall (Ancient Graves at the Uliarba Cemetery on Lake Baikal: Neolithic and Paleometal). Novosibirsk, Izdatel'stvo Instituta Arkheologii i Etnografii .

Gould, J.L., Gould, G.F., 2002. Biostats Basics: A Student Handbook. W.H. Freeman and Company, New York, USA .

Gusmao, L., Carraccedo, A., 2003. Y chromosome-specific STRs. Profiles DNA 3–6.

Gustafson, E., 2007. Teeth and DNA: The Mining of Mesolithic and Neolithic Teeth in a Non-destructive Manner. University of Alberta, Edmonton .

Hammer, M.F., 1994. A recent insertion of an alu element on the Y chromosome is a useful marker for human population studies. Mol. Biol. Evol. 11 (5), 749–761 Sep.

Hammer, M.F., Zegura, S.L., 1996. The role of the Y chromosome in human evolutionary studies. Evol. Anthropol. 5, 116–134.

Hammer, M.F., Spurdle, A.B., Karafet, T., Bonner, M.R., Wood, E.T., Novelletto, A., et al., 1997. The geographic distribution of human Y chromosome variation. Genetics 145 (3), 787–805 Mar.

Hammer, M.F., Karafet, T., Rasanayagam, A., Wood, E.T., Altheide, T.K., Jenkins, T., et al., 1998. Out of Africa and back again: nested cladistic analysis of human Y chromosome variation. Mol. Biol. Evol. 15 (4), 427–441 Apr.

Haverkort, C.M., Bazaliiski, V.I., Savel'ev, N.A., 2010. Identifying hunter-gatherer mobility patterns using strontium isotopes. In: Weber, A.W., Katzenberg, M.A., Schurr, T.G. (Eds.), Prehistoric Hunter-Gatherers of the Baikal Region. University of Pennsylvania Museum of Archaeology and Anthropology Philadelphia, Siberia Philadelphia, PA, pp. 217–238.

Jobling, M.A., Tyler-Smith, C., 1995. Fathers and sons: the Y chromosome and human evolution. Elsevier Sci. 11 (11), 449–456.

Karafet, T.M., Osipova, L.P., Gubina, M.A., Posukh, O.L., Zegura, S.L., Hammer, M.F., 2002. High levels of Y-chromosome differentiation among native Siberian populations and the genetic signature of a boreal hunter-gatherer way of life. Hum. Biol. 74 (6), 761–789 Dec.

Karafet, T.M., Mendez, F.L., Meilerman, M.B., Underhill, P.A., Zegura, S.L., Hammer, M.F., 2008. New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. Genome Res. 18 (5), 830–838 May.

Katzenberg, M.A., Saunders, S.R., 2008. Stable isotope analysis, a tool for studying past diet, demography and life history. In: Katzenberg, M.A. (Ed.), Biological Anthropology of the Human Skeleton Hoboken. Wiley-Liss, pp. 413–442.

Katzenberg, M.A., Weber, A.W., 1999. Stable isotope ecology and palaeodiet in the Lake Baikal region of Siberia. J. Archaeol. Sci. 26, 651–659.

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003

Katzenberg, M.A., Bazaliiskii, V.I., Goriunova, O.I., Savel'ev, N.A., Weber, A.W., 2010. Diet reconstruction of prehistoric hunter-gatherers in the Lake Baikal region. In: Weber, A.W., Katzenberg, M.A., Schurr, T.G. (Eds.), Prehistoric Hunter-Gatherers of the Baikal Region, Siberia: Bioarchaeological Studies of Past Life Ways Pennsylvania. University of Pennsylvania Museum of Archaeology and Anthropology, Philadelphia, pp. 175–192.

Kemp, B.M., Smith, D.G., 2005. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci. Int. 154 (1), 53–61 Nov 10.

Keyser, C., Bouakaze, C., Crubezy, E., Nikolaev, V.G., Montagnon, D., Reis, T., et al., 2009. Ancient DNA provides new insights into the history of south Siberian Kurgan people. Hum. Genet. 126 (3), 395–410 Sep.

Kharkov, V.N., Khamina, K.V., Medvedeva, O.F., Simonova, K.V., Khitrinskaya, I.Y., Stepanov, V.A., 2013. Gene-pool structure of Tuvinians inferred from Y-chromosome marker data. Genetika 49 (12), 1416–1425 Dec.

Khar'kov, V.N., Stepanov, V.A., Medvedeva, O.F., Spiridonova, M.G., Voevoda, M.I., Tadinova, V.N., et al., 2007. Gene pool differences between northern and southern Altaians inferred from the data on Y-chromosomal haplogroups. Genetika 43 (5), 675–687 May.

Khar'kov, V.N., Stepanov, V.A., Medvedev, O.F., Spiridonova, M.G., Maksimova, N.R., Nogovitsyna, A.N., et al., 2008. The origin of Yakuts: analysis of Y-chromosome haplotypes. Mol. Biol. (Mosk) 42 (2), 226–237 Mar–Apr.

Khar'kov, V.N., Khamina, K.V., Medvedeva, O.F., Shtygasheva, O.V., Stepanov, V.A., 2011. Genetic diversity of Khakassian gene pool: subethnic differentiation and the structure of Y-chromosome haplogroups. Mol. Biol. (Mosk) 45 (3), 446–458 May–Jun.

Kivisild, T., Tolk, H.V., Parik, J., Wang, Y., Papiha, S.S., Bandelt, H.J., et al., 2002. The emerging limbs and twigs of the East Asian mtDNA tree. Mol. Biol. Evol. 19 (10), 1737–1751 Oct.

Lawenstam, H.A., Weiner, S., 1989. On Biomineralization. Oxford University Press, New York .

Lee, H.C., Pagliaro, M., Berka, K.M., Folk, N.L., Anderson, D.T., Ruano, G., et al., 1991. Genetic markers in human bone: 1. Deoxyribonucleic acid (DNA) analysis. J. Forensic Sci. 36, 320–330.

Lieverse, A.R., 2010. Health and behavior in mid-Holocene Cis-Baikal: biological indicators of adaptation and culture change. In: Weber, A.W., Katzenberg, M.A., Schurr, T.G. (Eds.), Prehistoric Hunter-Gatherers of the Baikal Region, Siberia: Bioarchaeological Studies of Past Life Ways Philadelphia. University of Pennsylvania Museum of Archaeology and Anthropology, PA, pp. 135–173.

Lieverse, A.R., Pratt, V., Schulting, R.J., Cooper, M.L., Bazaliiskii, I., Weber, A.W., 2014. Point taken: an unusual case of incisor agenesis and mandibular trauma in Early Bronze Age Siberia. Int. J. Paleopathology 6, 53–59.

Malyarchuk, B., Derenko, M., Denisova, G., Maksimov, A., Wozniak, M., Grzybowski, T., et al., 2011. Ancient links between Siberians and Native Americans revealed by subtyping the Y chromosome haplogroup Q1a. J. Hum. Genet. 56 (8), 583–588 Aug.

Mamonova, N.N., 1973. K voprosu o drevnem naselenii Priangar'ia po paleoantropologicheskim dannym. In: Smirnov, A.P. (Ed.), Problemy arkheologii Urala i Sibiri Moskva, pp. 18–28 Nauka.

Mamonova, N.N., 1980. Drevnee naselenie Angary i Leny v serovskoe vremia po dannym antropologii. In: Okladnikov, A.P., Alekseev, V.P. (Eds.), Paleontropologiia Sibiri Moskva. Nauka, pp. 64–88.

Mamonova, N.N., 1983. K voprosu o mezhgrupovykh razlichiiakh v neolite Pribaikal'ia. Voprosy Antropologii 1, 88–103.

McKenzie, H.G., 2010. Variability in Bronze Age mortuary practices in the little sea microregion of Cis-Baikal. In: Weber, A.W., Katzenberg, M.A., Schurr, T.G. (Eds.), Prehistoric Hunter-Gatherers of the Baikal Region, Siberia: Bioarchaeological Studies of Past Life Ways 3260 South Street, Philadelphia, PA. University of Pennsylvania Museum of Archaeology and Anthropology, pp. 87–106.

Mooder, K.P., 2004. Mitochondrial DNA and Prehistoric Siberians from Baikal University of Alberta .

Mooder, K.P., Weber, A.W., Bamforth, F.J., Lieverse, A.R., Schurr, T.G., Bazaliiski, V.I., et al., 2005. Matrilineal affinities and prehistoric Siberian mortuary practices: a case study from Neolithic Lake Baikal. J. Archaeol. Sci. 32, 619–634.

Mooder, K.P., Schurr, T.G., Bamforth, F.J., Bazaliiski, V.I., Savel'ev, N.A., 2006. Population affinities of Neolithic Siberians: a snapshot from prehistoric Lake Baikal. Am. J. Phys. Anthropol. 129 (3), 349–361 Mar.

Mooder, K.P., Thomson, T., Weber, A.W., Bazaliiskii, V., Bamforth, F., 2010. Uncovering the genetic landscape of the prehistoric Cis-Baikal. In: Weber, A.W., Katzenberg, M.A., Schurr, T.G. (Eds.), Prehistoric Hunter-Gatherers of the Baikal Region, Siberia: Bioarchaeological Studies of Past Lifeways Philadelphia. University of Pennsylvania Museum of Archaeology and Anthropology Press, pp. 107–119.

Moussa, N.M., 2015. Maternal and Paternal Polymorphisms in Prehistoric Siberian Populations of Lake Baikal. University of Alberta, Edmonton .

Nakahori, Y., Takenaka, O., Nakagome, Y., 1991. A human X-Y homologous region encodes "amelogenin". Genomics 9 (2), 264–269 Feb.

Nasidze, I., Quinque, D., Dupanloup, I., Cordaux, R., Kokshunova, L., Stoneking, M., 2005. Genetic evidence for the Mongolian ancestry of Kalmyks. Am. J. Phys. Anthropol. 128 (4), 846–854 Dec.

Naumova, O.Y., Rychkov, S.Y., 1998. Siberian population of the New Stone Age: mtDNA haplotype diversity in the ancient population from the Ust'-Ida I burial ground, dated 4020–3210 BC by 14C. Anthropol. Anz. 56, 1–6.

Naumova, O.Y., Rychkov, S.Y., Bazaliiskii, V.I., Mamonova, N.N., Sulerzhitskii, L.D., Rychkov, Y.G., 1997. Molecular genetic characteristics of the Neolithic population of the Baikal region: RFLP of the ancient mtDNA from skeletal remains found in the Ust-Ida I burial ground. Russ. J. Genet. 33, 1215–1221.

Perez-Lezaun, A., Calafell, F., Seielstad, M., Mateu, E., Comas, D., Bosch, E., et al., 1997. Population genetics of Y-chromosome short tandem repeats in humans. J. Mol. Evol. 45 (3), 265–270 Sep.

Perez-Lezaun, A., Calafell, F., Comas, D., Mateu, E., Bosch, E., Martinez-Arias, R., et al., 1999. Sex-specific migration patterns in Central Asian populations, revealed by analysis of Y-chromosome short tandem repeats and mtDNA. Am. J. Hum. Genet. 65 (1), 208–219 Jul.

Rohland, N., Hofreiter, M., 2007. Ancient DNA extraction from bones and teeth. Nat. Protoc. 2 (7), 1756–1762.

Schurr, T.G., Sherry, S.T., 2004. Mitochondrial DNA and Y chromosome diversity and the peopling of the Americas: evolutionary and demographic evidence. Am. J. Hum. Biol. 16 (4), 420–439 Jul–Aug.

Schurr, T.G., Sukernik, R.I., Starikovskaya, Y.B., Wallace, D.C., 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), 1–39 Jan.

Seielstad, M., Yuldasheva, N., Singh, N., Underhill, P., Oefner, P., Shen, P., et al., 2003. A novel Y-chromosome variant puts an upper limit on the timing of first entry into the Americas. Am. J. Hum. Genet. 73 (3), 700–705 Sep.

Semino, O., Passarino, G., Oefner, P.J., Lin, A.A., Arbuzova, S., Beckman, L.E., et al., 2000. The genetic legacy of Paleolithic *Homo sapiens* sapiens in extant Europeans: a Y chromosome perspective. Science 290 (5494), 1155–1159 Nov 10.

Sosnovskaia, N.S., 1996. Issledovanii mogil'nika Kurma XI (poberezh'e Malogo moria ozera Baikal). Arkheologiia, paleoekologiia i etnologiia Sibiri Dal'nego Vostoka 2, 47–49.

Su, B., Xiao, J., Underhill, P., Deka, R., Zhang, W., Akey, J., et al., 1999. Y-chromosome evidence for a northward migration of modern humans into Eastern Asia during the last Ice Age. Am. J. Hum. Genet. 65 (6), 1718–1724 Dec.

Sullivan, K.M., Mannucci, A., Kimpton, C.P., Gill, P., 1993. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. BioTechniques 15 (4), 636–638 Oct. (640-1).

Thomas, M.G., Skorecki, K., Ben-Ami, H., Parfitt, T., Bradman, N., Goldstein, D.B., 1998. Origins of Old Testament priests. Nature 394 (6689), 138–140 Jul 9.

Thomson, T., 2006. Examination of mtDNA Polymorphisms at the Early Neolithic Shamanka II Cemetery, Lake Baikal, Siberia. University of Alberta, Edmonton .

Tiutrin, A.A., Bazaliiskii, V.I., 1996. Mogil'nik v ust'e reki Idy v doline Angary. Akheologiia i etnologiia Sibiri in Dal'nego Vostoka 1, 85–90.

Underhill, P.A., Shen, P., Lin, A.A., Jin, L., Passarino, G., Yang, W.H., et al., 2000. Y chromosome sequence variation and the history of human populations. Nat. Genet. 26 (3), 358–361 Nov.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., et al., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40 (15), e115 Aug 1.

Weber, A., 1995. Neolithic and early Bronze Age of the Lake Baikal region: a review of recent research. J. World Prehistory 9 (1), 99–165.

Weber, A.W., Goriunova, O.I., 2005. Khronologiia mogil'nika Kurma XI (oz. Baikal) po arkheologicheskim i radiouglerodnym dannym. Izvestiia Laboratorii drevnikh tekhnologii IGTU 3, 186–190.

Weber, A.W., Bettinger, R., 2010. Middle Holocene hunter-gatherers of Cis-Baikal. Siberia: an overview for the new century. J. Anthropol. Archaeol. 29, 491–506.

Weber, A.W., Goriunova, O.I., 2011. Fieldwork methods at Kurma XI. In: Weber, A.W., Goriunova, O.I., Mckenzie, H.G., Lieverse, A.R. (Eds.), Kurma XI, A Middle Holocene Hunter-Gatherer Cemetery on Lake Baikal, Siberia: Archaeological and Osteological Materials, sixth ed. Canadian Cirumpolar Institute Press, Edmonton, Alberta, Canada, pp. 13–22.

Weber, A.W., Link, D.W., Katzenberg, M.A., 2002. Hunter-gatherer culture change and continuity in the middle Holocene of the Cis-Baikal, Siberia. J. Anthrop. Archaeol. 21 (2), 230–299.

Weber, A.W., Goriunova, O.I., McKenzie, H.G., Lieverse, A.R., 2011a. Kurma XI, a Middle Holocene Hunter-Gatherer Cemetery on Lake Baikal, Siberia: Archaeological and Osteological Materials. sixth ed. Northern Hunter-Gatherers Research SeriesCanadian Circumpolar Institute (CCI Press), Edmonton, Alberta, Canada Volume.

Weber, A.W., Metcalf, M., Goriunova, O.I., Sekerin, A.P., Ovodov, N.D., 2011b. Grave descriptions: archaeology. In: Weber, A.W., Goriunova, O.I., McKenzie, H.G., Lieverse, A.R. (Eds.), Kurma XI, a Middle Holocene Hunter-Gatherer Cemetery on Lake Baikal, Siberia: Archaeological and Osteological Materials, sixth ed. Canadian Circumpolar Institute Press, Edmonton, Alberta, Canada, pp. 23–79.

Weber, A.W., White, D., Bazaliiskii, V.I., Goriunova, O.I., Savel'ev, N.A., Katzenberg, M.A., 2011c. Hunter–gatherer foraging ranges, migrations, and travel in the middle Holocene Baikal region of Siberia: insights from carbon and nitrogen stable isotope signatures. J. Anthropol. Archaeol. 30, 523–548.

Weber, A.W., Schulting, R.J., Bronk Ramsey, C., Bazaliiskii, V.I., 2016. Biogeochemical data from the Shamanka II Early Neolithic cemetery on southwest Baikal: chronological and dietary patterns. Quaternary Int. 405 (Part B), 233–254 2016.

Wolf, U., Schempp, W., Scherer, G., 1992. Molecular biology of the human Y chromosome. Rev. Physiol. Biochem. Pharmacol. 121, 147–213.

Y Chromosome Consortium, 2002. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. Genome Res. 12 (2), 339–348 Feb.

Zegura, S.L., Karafet, T.M., Zhivotovsky, L.A., Hammer, M.F., 2004. High-resolution SNPs and microsatellite haplotypes point to a single, recent entry of Native American Y chromosomes into the Americas. Mol. Biol. Evol. 21 (1), 164–175 Jan.

Zhong, H., Shi, H., Qi, X., Xiao, C., Jin, L., Ma, R.Z., et al., 2010. Global distribution of Y-chromosome haplogroup C reveals the prehistoric migration routes of African exodus and early settlement in East Asia. J. Hum. Genet. 55, 428–435.

Zhong, H., Shi, H., Qi, X.B., Duan, Z.Y., Tan, P.P., Jin, L., et al., 2011. Extended Y chromosome investigation suggests postglacial migrations of modern humans into East Asia via the northern route. Mol. Biol. Evol. 28 (1), 717–727 Jan.

Please cite this article as: Moussa, N.M., et al., Y-chromosomal DNA analyzed for four prehistoric cemeteries from Cis-Baikal, Siberia, Journal of Archaeological Science: Reports (2016), http://dx.doi.org/10.1016/j.jasrep.2016.11.003