Population affinities of Neolithic Siberians: A snapshot from prehistoric Lake Baikal

ARTICLE *in* AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY · MARCH 2006

Impact Factor: 2.51 · DOI: 10.1002/ajpa.20247 · Source: PubMed

5 AUTHORS, INCLUDING:

Karen Mooder
British Columbia Institute of Technology
3 PUBLICATIONS **49** CITATIONS
SEE PROFILE

Theodore Schurr
University of Pennsylvania
87 PUBLICATIONS **5,430** CITATIONS
SEE PROFILE

Nikolai Aleksandrovich Savel'ev
Irkutsk State University
64 PUBLICATIONS **310** CITATIONS
SEE PROFILE

Population Affinities of Neolithic Siberians: A Snapshot from Prehistoric Lake Baikal

Mooder KP,[1*] Schurr TG,[2] Bamforth FJ,[1] Bazaliiski VI,[3] and Savel'ev NA[3].

[1]Human Identification Laboratory for Archaeology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada T6G 2G3

[2] Department of Anthropology, University of Pennsylvania, Philadelphia, PA, USA

[3] Department of Archaeology and Ethnography, Irkutsk State University, Irkutsk, Russia

Key Words: Ancient DNA, mtDNA, biological discontinuity, Siberia

*To whom correspondence should be addressed: kmooder@ualberta.ca

ABSTRACT

Archaeological evidence supports the inhabitation of the Lake Baikal region since the Palaeolithic. Both metric and non-metric osteological studies suggest that Neolithic Cis-Baikal populations are the ancestors of the contemporary inhabitants of the region. To date, ancient DNA data have not been used to corroborate this biological continuity hypothesis. This study presents a temporal snapshot of the Cis-Baikal Neolithic by examining mtDNA diversity in two cemetery populations situated on the Angara River downstream of Lake Baikal. The 800 years separating the use of the two cemeteries is thought to represent a biocultural hiatus in the Cis-Baikal region, one that ended when a new group migrated into the area. To assess the likelihood that genetic continuity exists between these two Neolithic groups, we have examined both mtDNA coding region and hypervariable region I (HVI) polymorphisms from skeletal remains excavated from both cemeteries (Lokomotiv and Ust'-Ida). The mtDNA haplogroup distributions of the two cemetery populations differ significantly, suggesting that they were biologically distinct groups. When the biological distance between these Neolithic groups is compared with modern Siberian and other East Eurasian groups, the post-hiatus group (Serovo-Glazkovo) generally aligns with contemporary Siberians, while the pre-hiatus (Kitoi) individuals are significantly different from all but modern Kets and Shorians living in the Yenisey and Ob River basins to the west of Lake Baikal. These results suggest that the Lake Baikal region experienced a significant depopulation event during the sixth millennium BP, and was re-occupied by a new immigrant population some 800 years later.

Archaeological evidence definitively supports the inhabitation of the Lake Baikal region in the southwest of eastern Siberia since the Upper Palaeolithic (Okladnikov, 1959, 1964; Alekseev, 1998; Derev'anko 1998). However, little is known about the population history of the region and whether biological continuity can be demonstrated from around 40,000 years ago to the present day. Lithic evidence from excavations at the Upper Palaeolithic sites of Mal'ta and Buret' in the Angara River basin suggests that the earliest inhabitants of the Cis-Baikal region (i.e., north and west of Lake Baikal) had a material culture similar to those of contemporaneous eastern European groups (Okladnikov, 1959, 1964). It is well established that the Altai region of western Siberia has been the site of notable admixture between East and West Eurasian populations since the Upper Palaeolithic (Derenko et al., 2001a, 2002a, 2003). However, limited osteological evidence (Turner, 1987; Ishida and Dodo, 1996) and Palaeolithic artistic representations (Debets, 1951; cited in Okladnikov, 1959, 1964) suggest that the original inhabitants of eastern Siberia including the Cis-Baikal, were East Eurasian in origin.

The abundance of both habitation and cemetery sites along the major river basins in the southwestern part of East Siberia speak to its rich population history. Many of these sites were excavated by Russian archaeologists (i.e., Gerasimov, 1958; Okladnikov, 1959, 1964), although very few skeletal collections found within them have been extensively characterised. For this reason, clarifying the biological character of these past Siberian groups will help to piece together the population history of the region, and to better understand how these past peoples contributed to the population structure of Siberia as it is today.

In this study, we attempt to elucidate the population history of prehistoric Siberia by examining two Neolithic Cis-Baikal cemetery populations located on the Angara River, downstream from Lake Baikal. The use of each cemetery, known as Lokomotiv and Ust'-Ida, flanks an 800-year gap in the archaeological record that is thought to represent a biocultural hiatus

in the Cis-Baikal region (Weber, 1995; Weber et al., 2002). Differences in the material culture, burial practices and subsistence strategies as observed in the mortuary record suggest that the two populations were dissimilar. In addition, Russian anthropologists believe the groups using Lokomotiv and Ust'-Ida were biologically discrete based on observed variation in their cranial traits (Gerasimova, 1991; cited in Weber, 1995).

The Lokomotiv cemetery is located in the current-day city of Irkutsk at the confluence of the Irkut and Angara Rivers, and was used by a pre-hiatus population known as the Kitoi (**Fig. 1**). The Kitoi are characterised by a subsistence strategy heavily reliant on fishing and unique mortuary rituals, including the widespread use of red ochre and decapitation in burials (Bazaliiskii and Savelyev, 2003). Weber et al. (2002) proposed that the Kitoi were a population in decline towards the latter half of their existence due to stresses imposed by, among other things, a selective subsistence strategy and social isolation. The Kitoi culture disappeared from the archaeological record after approximately 6000 years before present (BP) and, for most of the next 800 years, no evidence of cemeteries can be found in the Cis-Baikal region. Around 5100 B.P., a different culture emerged in Cis-Baikal, as evidenced by the mortuary record at Ust'-Ida, a cemetery located approximately 100 km downstream of Lokomotiv at the mouth of the Ida River.

The graves at Ust'-Ida represent two groups known as the Serovo and Glazkovo (**Fig. 1**). Although Serovo and Glazkovo mortuary rituals vary slightly, scholars believe that their other cultural behaviour, such as mobility patterns and subsistence strategies, are similar enough to warrant their treatment as a single group (Weber, 1995; Weber et al., 2002). The Serovo-Glazkovo differ from the Kitoi in both the associated material culture found within the graves and their mortuary behaviour, mobility patterns and subsistence strategies. They used fire as a part of mortuary ritual, although traces of ochre were also found in the occasional grave (Tiutrin and Bazaliiskii, 1996; Weber et al., 2002). Furthermore, tool kits and stable isotope data retrieved from

Serovo-Glazkovo skeletal material suggest that they primarily hunted for food (Weber et al., 2002). Investigators have proposed that the Serovo-Glazkovo community using Ust'-Ida was larger and healthier than the Kitoi, due to the increased incidence of osteoarthritis and enamel hypoplasia in the Kitoi and the abundance of Serovo-Glazkovo subadults in the mortuary record (Link, 1996; Weber et al., 2002).

Although the health, demography, and subsistence strategies of the Kitoi and Serovo-Glazkovo have been investigated (Link, 1996, 1999; Lieverse, 1999; Katzenberg and Weber, 1999; Weber et al., 2002), a comprehensive biological affinities analysis of these skeletal collections has never been published. However, there would be substantial value in estimating the biological (i.e., genetic) distance between the Kitoi and the Serovo-Glazkovo, as such an approach would allow a more direct testing of the biological discontinuity hypothesis for Neolithic Cis-Baikal. Biodistance studies of skeletal populations have traditionally focused on variation in dental, cranial and post-cranial metric and non-metric traits (e.g., Johnson and Lovell, 1994; Prowse and Lovell, 1995, 1996; Hemphill, 1999). However, continued advances in ancient DNA (aDNA) analysis have made it possible to catalogue variation in prehistoric cemetery populations using genotypic rather than phenotypic data (e.g., Stone and Stoneking, 1998; Carlyle et al., 2000; Kaestle and Smith, 2001; Keyser-Tracqui et al., 2003). It is generally thought that biological distances between groups are best estimated using genotypic traits, as phenotypes are often expressed through the action of multiple genes or other epigenetic influences (Larsen, 1997; Gelehrter et al., 1998).

The principal objective of this study was to assess the biological distance between the Neolithic Kitoi and Serovo-Glazkovo using mtDNA data. The high copy number of mtDNA in hard tissue facilitates its retrieval from human skeletal remains (Wallace et al., 1987; Holland et al., 1993; Stone and Stoneking, 1998; Carlyle et al., 2000; Kaestle and Smith, 2001). With the retrieval of sufficient, authentic mtDNA data to characterise both the Kitoi and Serovo-Glazkovo, it may

be possible to provide evidence to support the occurrence of a significant depopulation event in the Cis-Baikal region during the Middle Neolithic, followed by the emergence of a new group in the same area almost a millennium later.

For years, knowledge of the mtDNA structure of living, indigenous Siberian groups was limited to the distributions of haplogroups A, B, C and D and their implications for hypotheses concerning the peopling of the Americas (Schurr et al., 1990; Torroni et al., 1992, 1993a, b). When scholars began to explore variation in the mtDNA hypervariable (HV) regions, it became evident that the mtDNA composition of indigenous Siberian populations was highly diverse (Shields et al., 1993; Starikovskaya et al., 1998; Schurr et al., 1999). Since then, both coding region and HVI data have been comprehensively described for many modern Siberian groups (Derenko et al., 2000, 2001a, b, 2002a, 2003; Derbeneva et al. 2002; Pakendorf et al. 2003; Schurr and Wallace 2003).

The recent expansion of the Siberian mtDNA data set has allowed relationships between indigenous Siberian groups to be characterised with greater sensitivity. It has recently been established that the matrilineal structure of South Siberian populations are highly differentiated by geography (Derenko et al., 2003). This finding is consistent with the tenet that groups exchange genes with their neighbors (Cavalli-Sforza et al., 1996; Hedrick, 2000). Thus, groups living in the regions proximate to Cis-Baikal are expected to share a greater degree of biological affinity with each other than with populations outside the region. Furthermore, several aDNA studies have demonstrated the continuity of mtDNA population structure of North America through thousands of years (e.g., Carlyle et al., 2000; O'Rourke et al., 2000; Kaestle and Smith, 2001; Malhi et al. 2002). These data suggest that analogous concerns about the settlement of and population continuity in South-Central Siberia may be elucidated through the analysis of aDNA diversity in the Cis-Baikal region. Moreover, it is unclear whether groups inhabiting the Cis-Baikal region today are descendents of those who inhabited it during the Neolithic (Lopatin, 1940; Okladnikov,

1964; Naumova and Rychkov, 1998). Thus, by comparing mtDNA diversity in modern and Neolithic Siberians, it will be possible to determine whether the Kitoi or Serovo-Glazkovo contributed to the matrilineal population structure of the Cis-Baikal region today.

MATERIALS AND METHODS

Neolithic population samples

The skeletal remains representing the Kitoi and Serovo-Glazkovo analysed in this study were excavated from the Lokomotiv and Ust'-Ida cemeteries by Russian archaeologists from Irkutsk State University (ISU) during the last two decades of the 20th century (Tiutrin and Bazaliiskii, 1986; Bazaliiski, 2003; Bazaliiskiy and Savelyev, 2003). Lokomotiv is considered the largest Neolithic cemetery in North Asia, and was first discovered during construction of the Trans-Siberian Railway in the late 1800s (Bazaliiskii and Savelyev, 2003). Non-calibrated radiocarbon dates (Isotrace, University of Toronto) from Lokomotiv suggest that this cemetery was used from approximately 7250 to 6040 BP. These dates correspond to the period between 6125 and 4885 B.C. when calibrated with the methodology of Stuiver et al. (1998) (R. Buekens, personal communication). In total, 70 graves containing 124 burials were excavated from Lokomotiv, and the retrieved skeletal remains were curated in the Department of Archaeology and Ethnography at ISU.

The Ust'-Ida cemetery was excavated after a dam on the Angara River downstream of the cemetery flooded in 1986, causing 11 graves to erode out of the bank (Tiutrin and Bazaliiskii, 1996). Uncalibrated radiocarbon dates obtained from skeletal remains at Ust'-Ida place the use of this cemetery between approximately 4960 and 3590 BP. These dates correspond to a period between 3710 and 2020 B.C. when calibrated using the methodologies of Stuiver et al. (1998) (R.

Buekens, personal communication). From 1987 to 1996, a total of 59 graves containing 64 individuals were excavated.

Based on differences in body orientation, two grave typologies have been identified at Ust'-Ida (Tiutrin and Bazaliiski, 1996). The first is the Serovo type (n= 37), in which burials are found oriented in an extended supine position with the heads pointing to the south. The second is the Glazkovo type (n=12), in which burials are either found in an extended supine or flexed body position with the heads oriented to the north. While there is little overlap between the ^{14}C dates for the Serovo and Glazkovo graves at Ust'-Ida (A. Weber, personal communication), these groups are treated as a single community for a number of reasons. Firstly, based on similarities in their osteological traits and material culture, Russian anthropologists (*i.e.*, Gerasimov, 1955; Maumanova, 1983; Gerasimova, 1991; cited in Weber, 1995) believe that the Serovo and Glazkovo are biologically and culturally continuous. Secondly, many Serovo-Glazkovo cemeteries are known to exist throughout the Cis-Baikal region, and the dates corresponding to the use of both grave types overlap (Weber, 1995; Weber et al., 2002). Finally, the Glazkovo sample at Ust'-Ida is, at present, too small to make any meaningful inferences about how they differ from the Serovo.

Overall, judging from macroscopic examination, the skeletal remains from both cemeteries appear to be well preserved. The grave pits at Lokomotiv were generally dug to depths exceeding 50 cm into the lower levels of reddish brown loam corresponding to the Holocene climatic optimum i.e., the Atlantic period (Bazaliiski, 2003). Many of the grave pits at Ust'-Ida were dug to the upper levels of the reddish brown loam and backfilled with limestone slabs and cobbles (Tiutrin and Bazaliiski, 1996). The climate of the Lake Baikal region is continental with warm summers, cold winters and limited precipitation. Average temperatures for Cis-Baikal region range from a high of 20 °C during July to a low of -26 °C in January (Atlas SSSR 1984; cited in Weber et al., 2002). Regions of discontinuous permafrost have also been noted in the area around Lake

Baikal but not in the Angara valley (Weber et al., 2002). The combination of neutral soil pH, good soil drainage and cool temperatures favors the preservation of DNA within the osteological material (Rogan and Salvo, 1990, Eglington and Logan, 1991; Geigl, 2002).

Both thoracic and cervical vertebrae were sampled from 40 Lokomotiv and 42 Ust'-Ida skeletal remains for DNA analysis to be carried out at the University of Alberta. These samples were well preserved with negligible erosion of the vertebral bodies. Vertebrae were selected for analysis for two reasons. First, vertebrae were readily available across a majority of individuals recovered from these sites. Furthermore, vertebrae are expected to contain a greater quantity of DNA per unit mass of tissue than long bones due to their increased ratio of cancellous to cortical bone (e.g., Lee et al., 1991). The vertebrae used in this study were subject to osteological examination prior to DNA sampling (i.e., Link, 1996, 1999).

Specimen preparation

To remove any exogenous DNA contaminating the vertebral material, several steps were undertaken. All samples were initially prepared for analysis by removing several millimeters of the cortical bone surface with a sterile scalpel, followed by immersion in a 10% bleach solution[1] (with time dependent on the observed porosity of the sample). The samples were then briefly rinsed in sterile, distilled water and exposed to UV irradiation at 254 nm for a minimum of an hour within closed, sterile containers. The samples (within their sterile containers) were then immersed in liquid nitrogen for 20 to 60 minutes (depending on the sample size) and crushed with a sterile mortar and pestle. The pulverized samples were then transferred to sterile polypropylene tubes and stored at –70 °C until extraction.

Contamination controls

All pre- and post-PCR manipulations of the samples analysed in this study were undertaken in physically separated rooms. The laminar flow cabinet in which all pre-PCR sample manipulations were carried out was decontaminated with undiluted industrial strength bleach prior to each use. All supplies and reagents used in this study were rendered DNA-free using methods suitable for the material being treated. All racks, pipettors and containers were treated with undiluted bleach prior to use. Pipette tips and tubes were autoclaved in small batches. Reagents were exposed to UV light for a minimum of 20 minutes to denature any decontaminating DNA (e.g., Ou et al., 1991). Those reagents known to degrade when exposed to UV irradiation were instead autoclaved. Both extraction and negative (no template) PCR controls were used to detect the presence of systematic contamination. Positive control material was added to PCR reaction vessels only in the post-PCR area. To detect spurious contamination, all samples were extracted in duplicate, and PCR amplifications from each extract were executed multiple, independent times.

DNA extraction

All specimens were extracted using a guanidium thiocyanate protocol first proposed by Boom and others (1990) with several modifications. The modified protocol used 0.5 grams of bone per extract, incubated in extraction buffer overnight at 65 °C for a minimum of 16 hours. This was followed by a silica-binding step where the bone supernatant was incubated with 500 μL of extraction buffer and 40 μL of silica, and placed on a rotator at room temperature for two hours. The silica pellets were washed twice, first with wash buffer, and then with 70% ethanol, followed by a single wash with acetone. After the silica pellets were dry, the DNA was eluted from the silica with 100 μL of water in a 56 °C water bath for one hour. The resulting DNA extracts were transferred to sterile microfuge tubes, and stored at –20 °C until analysis.

PCR amplification

PCR amplifications were performed using either a Perkin-Elmer 2400 thermocycler (Foster City, CA) or a MJ TC Minicycler (MJ Research, Boston, MA). Each 50 μL reaction mix consisted of 5 μL 10X PCR Buffer (Invitrogen), 0.2mM of each dNTP (PE Biosystems), 1.5 mM $MgCl_2$ (Invitrogen), 200 pmol of each relevant primer, 15 μg of BSA (NEB), 1.25 U of Platinum Taq DNA Polymerase (Invitrogen). DNA extracts were not quantitated; instead, a standard 8 μL of template was added to each reaction mixture.

All primers used in this study and their respective annealing temperatures are listed in **Appendix 1.** These represent primers derived from other aDNA studies in addition to those designed in this study. The primers created to flank the *CfoI* site loss at np 7598 were originally designed to identify haplogroup E individuals. However, recent modifications to the East Eurasian mtDNA tree suggest that Siberian individuals harboring the *CfoI* 7598 site loss actually belong to haplogroup G2a (Yao et al., 2002), as haplogroup E has only been characterised in Tibetan (Torroni et al., 1994) and southern Chinese (Kivisild et al., 2002) groups at negligible frequencies (Derenko et al., 2003). Haplogroup G2a status has been assigned in this study based on the *CfoI* 7598 site loss.

Amplifications using the PE 2400 thermocycler consisted of an initial denaturation step at 94 °C for 2 minutes, followed by 40 cycles of 30 s at 94 °C, 1 min at relevant annealing temperature and 30 s at 72 °C. Reaction conditions using the MJ PTC Minicycler were modified slightly in that denaturation took place at 95 °C for 60s, and the annealing and extension steps were increased to 90s and 60s, respectively. Regardless of the thermocycler used, amplifications were completed with a final extension step of 5 minutes at 72 °C.

The limited amount of sample available for analysis in this study necessitated a low-resolution approach to the characterization of mtDNA sequence variation. The aDNA samples were initially analysed for a single nucleotide polymorphism (SNP) defining an *AluI* 10397 site gain defining mtDNA macrohaplogroup M. Those individuals belonging to M were further characterised using PCR primers flanking SNPs defining haplogroups C, D and G2a. Those individuals lacking the *AluI* 10397 site gain were further defined using primers flanking SNPs characteristic of haplogroups A, B, F and, in certain cases, the *DdeI* 10394 site gain, as well as the *DdeI* 1715 site loss that defines haplogroup X.

mtDNA haplogroup assignment

Amplified PCR products were digested with restriction enzymes targeting SNPs defining different mtDNA haplogroups. These haplogroups included M, defined by an *DdeI*/*AluI* site gain at nucleotide pair (np) 10394/10397; A, by the *HaeIII* site gain at np 663; C by the *HincII* site loss at np 13259; D by an *AluI* site loss at np 5176; F by a *HincII* site loss at np 12406, G2a by a *CfoI* 7598 site loss; and X by the *DdeI* 1715 site loss. Restriction digests were prepared by mixing 5 U of the relevant restriction enzyme and 1 μL of 10X buffer (NEB or Invitrogen), diluting the mixture to a final volume of 10 μL with sterile water, and adding it to the entire volume of amplified product. All restriction digests were incubated overnight at 37 °C. The digested products were visualized using a Fluor-S Multimager with the Quantity One software package (BioRad) after electrophoresis on 10% polyacrylamide gels and staining with ethidium bromide (10 mg/mL). Only data from completely digested PCR products (as deduced through comparison with a modern DNA digestion control) were considered interpretable results (e.g., Kolman and Tuross, 2000).

Sequencing of the mtDNA HVI was performed using primers flanking a 176 bp region of HVI from np 16191 to 16367. The sequences for these primers are also listed in **Appendix 1**. This fragment was targeted because it contains a majority of the informative polymorphisms characterising East Eurasian HVI variation. The reagent concentrations and conditions for the primary sequencing PCRs were the same as those used for SNP amplifications except that 50, rather than 40, cycles of PCR were performed. The primary PCR products for cycle sequencing were purified using the QuickStep 2 PCR Purification Kit (Edge Biosystems). Sequencing of 75 ng of template was undertaken for both the H and L strands using an ABI 377 sequencer and the Big Dye Terminator v. 3.1 Kit (Applied Biosystems), following standard manufacturer specifications. The resulting sequence data were read manually, and deviations from the Cambridge reference sequence (Anderson et al., 1981) were scored.

Evaluation of authenticity

A series of criteria were used to evaluate the authenticity of the aDNA data produced in this study. First, mtDNA haplogroups were only assigned to individuals with SNP data reproduced from a minimum of two independent and temporally separated extraction events. Likewise, HVI data were only reported if reproduced from independent and temporally discrete extractions. Secondly, since a series of coding-region SNPs beget a well-defined HVI motif (i.e., Kivisild et al., 2002; Yao et al., 2003), individuals possessing discordant SNP and HVI markers were rejected from the analysis. Thirdly, all data reported in this study made phylogenetic sense and reflected polymorphisms congruent with the geographic location under study (i.e., mtDNA substitutions found in populations inhabiting East Eurasia today). Finally, all sequence data reflecting the HVI motif of the analyst or the osteologist who worked with this skeletal material were rejected from the analysis.

Molecular sexing of the Lokomotiv and Ust'-Ida skeletal material also provided support for the authenticity of the mtDNA coding-region and HVI data. Using PCR primers for the amelogenin locus first targeted by Mannucci et al. (1994), the molecular sex of 16 individuals from both Lokomotiv and Ust'-Ida were determined, and found to agree with the morphological sex assignment (i.e., Link, 1996; A. Lieverse, personal communication) in all but two cases, for a concordance rate of 93% (Mooder, 2004).

Statistical analysis

Modern East Eurasian population data were incorporated into this study to provide a comparative framework in which to evaluate the Kitoi and Serovo-Glazkovo contribution to the matrilineal gene pool of Siberia. Only modern populations with comprehensive mtDNA coding-region data were used in this study, as the HVI dataset for Lokomotiv and Ust'-Ida is, to date, incomplete. **Figure 2** shows approximate geographic locations of these groups. The populations used for comparative analysis span most of Siberia as well as parts of Central and East Eurasia. The Siberian groups inhabit areas extending from the Sayan Plateau to the west of Lake Baikal, and include the Tofalars, Sojots, Tuvinians and Todjins (Derenko et al., 2001a, 2002a, 2003). The Buryats sampled by Derenko et al. (2002a, 2003) were drawn from the entire Buryat Republic, a region bounded by Lake Baikal on the west and Mongolia and China to the south. Groups inhabiting the Yenisey and Ob River basins to the northwest of Cis-Baikal include the Shorians (Derenko et al., 2002a) and the Kets (Derbeneva et al., 2002a). The Evenk data compiled by Derenko et al. (2002a) were sampled from various communities to the north and east of Lake Baikal in the Evenk Autonomous Okrug.

Haplogroup assignments were also compared against HVI data obtained from a northern Mongolian cemetery population known as Egyin Gol, which dates to the 3rd century B.C. (Keyser-

Tracqui et al., 2003). Egyin Gol is located on a tributary of the Selenga River, which drains into the east side of Lake Baikal. Because of the relative geographic proximity of Egyin Gol to Lokomotiv and Ust'-Ida, the inclusion of this cemetery in the analysis allowed us to better characterize the population structure of prehistoric Siberia.

The mtDNA haplogroup distributions of prehistoric and modern populations were compared using a number of statistical algorithms. An exact test of population differentiation (i.e., Raymond and Rousset, 1995), using *Arlequin* 2.000 (Schneider *et. al.*, 2000), was used to test the null hypothesis that the groups at Lokomotiv and Ust'-Ida represent a homogenous population. This test is considered to be analogous to a Fisher's exact test with a two-by-two contingency table expanded to a table of a size defined by the number of populations by the number of haplogroups examined in this study. An exact test approach is preferable when dealing with small sample sizes, as the chi-square test for homogeneity assumes that any given cell has a minimum frequency of five (Gould and Gould, 2002).

Biological distances between prehistoric and modern populations were estimated from mtDNA haplogroup frequencies using various algorithms; these included pairwise F_{ST} in *Arlequin 2.00* (Schneider et al., 2000), CONTML in PHYLIP ver 3.5c (Felsenstein, 1998) and the PROXCAL multidimensional scaling (MDS) program in SPSS 12.0. As all three approaches produced similar relationships among populations, only the F_{ST} results are presented here. These are shown in the form of a two-dimensional map using principal components (PC) analysis (MINITAB 13.3).

RESULTS

Analytical success

Reproducible mtDNA coding region data were retrieved for 31 of 40 (78%) Lokomotiv and 39 of 42 (93%) Ust'-Ida individuals. The HVI sequences produced from the 22 Lokomotiv and 27 Ust'-Ida individuals selected for sequencing fall into one of three categories. The first includes 10 Lokomotiv (45%) and 17 Ust'-Ida (68%) individuals from whom reproducible HVI motifs concordant with the SNP haplogroup assignments were retrieved. The second subset consists of eight individuals from each of Lokomotiv and Ust'-Ida who were noted to have concordant SNP and HVI motifs but due to sample constraints, have not generated reproducible HVI motifs. The remaining category consists of equivocal HVI data from Lokomotiv (n= 4) and Ust'-Ida (n=2). This category included contaminating sequences derived from handling by the osteologist who analysed these collections and ambiguous sequence variants resulting from postmortem DNA modification that were not consistent with published haplogroup motifs. The reduced retrieval rate of authentic coding region and HVI data from Lokomotiv specimens compared with those from Ust'-Ida can be attributed to the increased handling of Lokomotiv skeletal material, which occurred through osteological examination of commingled communal burials (Link, 1996). As only the samples in the first category meet our laboratory's authenticity criteria, these are the only HVI sequences reported in this study.

Neolithic mtDNA haplogroup assignments

Through analysis of coding-region SNPs, five characteristic East Eurasian mtDNA haplogroups were identified for 28 of 31 (90%) Lokomotiv and 30 of 39 (77%) Ust'-Ida individuals. The haplogroup frequencies for Lokomotiv, Ust'-Ida and all other populations included for comparative analysis in this study are presented in **Table 1**. Although all five East Eurasian haplogroups are shared between individuals at Lokomotiv and Ust'-Ida, their frequency distributions are remarkably different. Overall, Lokomotiv has higher frequencies of haplogroups

D and F, while Ust'-Ida has higher frequencies of haplogroups A and C. Neither population has haplogroup B nor X mtDNAs. In addition, HVI sequencing was used to further resolve haplogroup assignments of two individuals from Lokomotiv and nine from Ust Ida who lacked coding-region SNPs defining haplogroups A to G2a and, thus, were initially classified as haplogroup 'Other'. The summarized HVI sequence data from Lokomotiv and Ust'-Ida is presented in **Table 2.**

The entire set of haplogroup 'Other' individuals from Lokomotiv lacked the *DdeI*/*AluI* 10394/10397 site gains, as did three from Ust'-Ida. As such, these individuals were expected to have polymorphisms representative of macrohaplogroup N haplotypes, which encompass both West and East Eurasian mtDNAs (e.g., Kivisild et al., 2002). Two N individuals from Lokomotiv and one from Ust'-Ida possessed HVI sequence variants characteristic of haplogroup U5a (16256-16270), a mtDNA cluster which is thought to have West Eurasian origins and an estimated coalescence time of 50 000 years (Richards et al., 1998; Sykes, 1999).

Two other N individuals from Ust'-Ida were characterised by a single T to C transition at 16311. This polymorphism is found in both West and East Eurasian mtDNAs, either alone as seen in haplogroup H, or in association with other sequence variants from haplogroups A, D5, K, R, U4, U5a and U5b within macrohaplogroup N (Kivisild et al., 2002; Kong et al., 2003). The presence of the 16311 variant in the absence of any other substitutions within the region sequenced effectively ruled out all but haplogroups H, R, or U4, with the latter two having additional sequence variants that occur outside the portion of the HV1 sequenced in this study.

The remaining four haplogroup 'Other' individuals from Ust'-Ida were defined by the *AluI* 10397 site gain characteristic of macrohaplogroup M. Two of these had a HVI motif with substitutions at 16223-16227-16262-16278. While this sequence is identical to that seen in a G2a mtDNA from one Ust'-Ida individual, both of these 'Other' individuals lacked the diagnostic G2a

CfoI 7598 site loss for this haplogroup. The remaining Ust'-Ida M individual for whom an HVI motif was determined had only the C to T transition at 16223. When observed alone, this substitution may define either haplogroup G4 or an undifferentiated M lineage (Kivisild et al., 2002; Yao et al., 2003). The HVI status of the three remaining 'Other' individuals awaits confirmatory sequencing. By evaluating these haplogroup 'Other' individuals for additional coding-region and HV markers, their haplogroup status may be further resolved.

Population affinities of the Kitoi and Serovo-Glazkovo

Population genetic models reflect the assumption that populations with similar mtDNA haplogroup distributions are more likely to share a common maternal ancestry than those groups whose distributions are disparate (e.g., Kaestle and Horburgh, 2002). Through analysis of molecular variance (i.e., AMOVA) testing of mtDNA coding-region and HVI data, Derenko et al. (2003) have suggested that modern Siberian populations do not cluster by language or anthropological (i.e., phenotypic) variation; instead, only geography significantly influences the biological distance between groups. Consequently, shared matrilineal population affinities between the Kitoi and modern Cis-Baikal groups would suggest that matrilineal ancestors of the Kitoi remained in the Cis-Baikal region. If the Kitoi were instead observed to have similar mtDNA haplogroup distributions to those of modern groups outside the Cis-Baikal region, then this would suggest that the Kitoi left Cis-Baikal and settled elsewhere. Likewise, population continuity in the Cis-Baikal region from the Neolithic through to modern times would be supported if biological distances between the Serovo-Glazkovo and modern Cis-Baikal populations were small.

An important caveat to note here is that mtDNA genome is in essence, a single locus. Thus, our interpretations about the population structure of these Neolithic Cis-Baikal groups reflect only population variation determined from the transmission of mtDNA from generation to

generation. To fully understand the genetic structure of Neolithic Siberia, it will be necessary to characterize other loci such as the Y-chromosome which will take place in the future.

Kitoi population affinities

A PC map constructed from the F_{ST} matrix representing mtDNA haplogroup distributions in prehistoric and modern East Eurasian populations is shown in **Figure 3**. The first two principal components explained 87% of the total variance arising from the mtDNA haplogroup distributions. At first glance, most modern populations appeared to cluster as a function of physical distance from each other, but two general groupings also appeared in this plot. The first cluster occupying the left portion of the PC map was comprised of modern groups inhabiting the Ob and Yenisey River basins to the west and north of Cis-Baikal, while the other cluster was composed of groups living east of the Yenisey and those living to the northeast of Cis-Baikal.

With 72% of their mtDNAs belonging to haplogroups D (22%) and F (49%), the Kitoi did not cluster with modern groups such as the Sojots, Buryats and Tuvinians, who occupy regions close to Lake Baikal. Instead, they were located in a somewhat isolated position in the PC plot relatively close to the Shorians and Kets. Interestingly, the biological distances between the Kitoi and Shorians, and the Kitoi and Kets, are not statistically significant, with respective P values of 0.21 and 0.06. The Shorians have a combined frequency of haplogroups D and F of 50% (Derenko et al., 2001a, 2002a) and also the highest frequencies of West Eurasian haplogroups (36%) seen in Western Siberian populations, the most prevalent of which is haplogroup is H. In addition, the Kets have the second highest frequency of haplogroup F (24%) seen in Siberian populations, but also low frequencies of D (3%) (Sukernik et al. 1996; Derbeneva et al., 2002a; Schurr and Wallace 2003). However, Kets were also similar to Lokomotiv individuals in having moderate frequencies of haplogroup A (8%) and also lacking haplogroup B mtDNAs.

When HVI data were evaluated, the modern Kets and the Neolithic Kitoi were found to share a single U5a sequence (16256-16270) at frequencies of 5% and 6%, respectively. Similarly, these groups also shared common haplogroup A (16223-16290-16319) C (16223-16298-16327) and F (16232-16249-16304-16311) sequences. Therefore, the Kets share a minimum of 50% of their HVI sequences with the Kitoi. The further characterization of Lokomotiv HVI sequences might increase the proportion of shared haplotypes. Unfortunately, an analogous comparison between the Kitoi and Shorians could not be made because Shorian HVI datasets have yet to be published.

Serovo-Glazkovo population affinities

The population affinities of the Serovo-Glazkovo are strikingly different than those of the Kitoi. In the PC plot of the F_{ST} distance matrix, the Serovo-Glazkovo fell into the milieu of modern Siberian populations who live east of the Yenisey River and those proximate to Cis-Baikal (**Fig. 3**). The association between these groups was largely due to the similar distributions of haplogroups C and F. However, the Serovo-Glazkovo and the modern groups differed in the frequencies of haplogroups D and G2a. While the Serovo-Glazkovo had a low frequency of haplogroup D (5%) and a moderate frequency of G2a (10%), the opposite trend was observed for most other Siberian groups living between the Yenisey River and Lake Baikal. The only exceptions were the Sojots and Buryats, who had high frequencies of haplogroup D (50% and 33%, respectively) and moderate frequencies of haplogroup G2a (9% and 14%, respectively).

Intriguingly, the Serovo-Glazkovo also had 26% haplogroup A mtDNAs. This frequency was higher than almost all other Asian populations discussed in this study. The only other Siberian populations known to have comparable frequencies of haplogroup A are the Chukchi (67%) and Siberian Yupik (80%), who inhabit northeastern Siberia (Starikovskaya et al. 1998; Schurr et al.

1999). In contrast, the average haplogroup A frequency in modern Siberian populations living proximate to Lake Baikal is around 4% (Derenko et al., 2002a; Schurr and Wallace 2003).

DISCUSSION

Biological distance between the Kitoi and Serovo-Glazkovo

The principal goal of this study was to test the hypothesis that a depopulation event occurred in Cis-Baikal during the Middle Neolithic, causing an approximate 800-year hiatus in the population history of the region. This biological discontinuity hypothesis evolved with the discovery that no cemetery sites whose dates fall between the use of Lokomotiv and Ust'-Ida have been found in the Cis-Baikal region (Weber, 1995; Weber et al., 2002). Thus, by estimating the biological distance between the Kitoi and Serovo-Glazkovo by examining the differences in their respective mtDNA haplogroup distributions, we were able to generate an additional line of evidence to test this hypothesis.

Although the Kitoi and Serovo-Glazkovo share all six mtDNA haplogroups identified in this study, their distributions are significantly different (P=0.0001). These differences are largely seen in the moderate proportions of haplogroups A (26%), C (28%) and G2a (10%) in Ust'-Ida individuals, and the predominance of haplogroup D (23%) and F (48%) mtDNAs in the Lokomotiv sample. Thus, it is likely that the Kitoi and Serovo-Glazkovo do not share a common matrilineal origin. Coupled with the information from the archaeological record, the disparate mtDNA haplogroup distributions of the Kitoi and Serovo-Glazkovo strongly suggest that a population shift occurred after a biological hiatus in the Cis-Baikal region during the seventh millennium BP.

However, explaining the process by which two biologically distinct groups emerged in the Cis-Baikal Neolithic is a complex task, as both cultural and environmental factors must be taken

into consideration. Archaeological evidence suggests that the Kitoi social relations were shaped by power and sex imbalances (Weber et al., 2002). If such social complexities created intra-community tension, this may have precipitated the dispersal of the group. Alternatively, if the climate in Cis-Baikal became inhospitable during the Middle Neolithic or if resources became scarce, then the Kitoi may have left the region to settle elsewhere. In both scenarios, the Serovo-Glazkovo would represent a different population who migrated into the area after the disappearance of the Kitoi. If the relocation of the Kitoi and subsequent immigration of the Serovo-Glazkovo caused the population shift observed in Cis-Baikal, then we might observe population affinities and ethnographic similarities between the Kitoi and groups in other regions of Siberia.

Alternatively, the Kitoi may have been afflicted by population stresses that eventually contributed to their demise (Link, 1996, 1999; Weber et al., 2002). The Kitoi are believed to have had lower reproduction rates than the Serovo-Glazkovo, based largely on the lower frequency of subadults in the mortuary record at Lokomotiv compared with Ust'-Ida. A greater degree of enamel hypoplasia was also observed in the Kitoi compared with the Serovo-Glazkovo. As enamel hypoplasia is thought to be a marker of nutritional stress during childhood (Larsen, 1997), its presence suggests that Kitoi food procurement may have been inconsistent.

The Kitoi also appear to have had a lower life expectancy than the Serovo-Glazkovo. This difference is suggested by the increased frequency of individuals in the 35 to 50 year age class buried at Lokomotiv compared to that at Ust'-Ida, whose largest age class consisted of individuals greater than 50 years old (Link, 1996). However, other than osteoarthritis, which is found in both cemeteries, there is little osteological evidence to suggest that either the Kitoi or Serovo-Glazkovo were affected by disease (Lieverse, 1999; Weber et al., 2002).

In an effort to clarify these issues, Weber et al. (2002) evaluated the subsistence strategies and mobility patterns of the Kitoi and Serovo-Glazkovo, as well as the distribution and size of cemetery sites throughout the Cis-Baikal region. Based on this evidence, they proposed that, while at least a few of the Kitoi communities (*i.e.*, Lokomotiv and Kitoi on the Angara River, Shamanka II on Lake Baikal) were likely to have been larger than those of the Serovo-Glazkovo, they were socially and reproductively isolated units. Unlike the Serovo-Glazkovo, whose high mobility would have promoted population growth through the actions of migration and gene flow, the Kitoi may have suffered from the combined effects of genetic isolation, diminished health and fertility.

If the Kitoi decreased in number over time, an increased amount of mtDNA homogeneity among individuals (i.e., a genetic bottleneck) might be observed towards the latter stages of cemetery use at Lokomotiv. **Figure 4** shows the distribution of Kitoi mtDNA haplogroups across three arbitrary chronological periods. When these distributions are compared using an exact test in *Arlequin*, they are almost identical (P = 0.987), suggesting that the matrilineal population structure of the Kitoi remained relatively stable throughout the time Lokomotiv was used. Thus, it seems unlikely that the cessation of Lokomotiv use coincided with the extinction of the Kitoi.

Although the similar matrilineal structure of the prehistoric Kitoi and modern Shorians and Kets speaks to population affinities between these three groups, the limited osteological evidence for the Kitoi is not concordant with this interpretation. Gerasimova (1991; cited in Weber, 1995) believed Kitoi crania to have overt "Mongoloid" characteristics, based on remains from the Fofanovo cemetery in the Selenga River basin. This phenotype contrasts with the more "Europeoid" features defining the Uralic type that characterizes both the Shorians and the Kets (Potapov, 1964; Popov and Dolgikh, 1964). The Shorians also share ethnographic affinities with the Kets, who inhabit the Middle to Lower Yenisey River basin, and speak a unique language (i.e., Ket) that is neither Samoyedic nor Turkic in origin (Popov and Dolgikh, 1964). However, given

that the anthropological type defining the Kitoi is based on just one cemetery sample, a more extensive examination of Kitoi crania from both Lokomotiv and Shamanka II sites will be needed to better define Kitoi biological affinities.

Interestingly, there are a few notable geographic and ethnographic affinities between the Kets and the Kitoi. The Kets and the Kitoi are connected by the Angara/Yenisey watershed that could easily have served as a natural migratory route for groups living on the upper Angara River. The first Russians to reach the Yenisey noted the presence of a Ket group known as the Asans on the lower reaches of the Angara River, near the confluence of the Yenisey. The Asans were later thought to merge with Evenks, who today occupy territory immediately to the east of where modern Kets live (Popov and Dolgikh, 1964). However, it should be noted that due to their dissimilar mtDNA distributions, the Evenks cluster far away from both the Kitoi and Kets on the PC map (Fig 3). While many Kets are hunters, there is a strong fishing tradition among northern Kets, just as is seen in the Kitoi. Popov and Dolgikh (1964) also remarked on the Kettic mortuary ritual of sacrificing and burying of dogs with the dead. A similar ritual was practiced by the Kitoi, as evidenced by a burial at Lokomotiv that contained the remains of a wolf in association with a human skull (Bazaliiskiy and Savelyev, 2003).

In contrast, no such cultural affinities are observed between the Shorians and the Kitoi. The Shorians use hunting as a primary subsistence strategy and only recently have begun burying their dead in the ground. Before the 20th century, the Shorians wrapped their dead in birch bark and deposited them in logs (Potapov, 1964). Therefore, although the Kitoi, Kets and Shorians share similar mtDNA haplogroup distributions, it seems that that there are stronger affinities between the Neolithic Kitoi and modern Kets.

Given that large biological distances exist between the Kitoi and modern Siberian populations who live proximate to Cis-Baikal, it is reasonable to conclude that the Kitoi left the

upper reaches of the Angara River to settle along the Yenisey River basin. The interaction of the Kitoi with regional groups along the Yenisey may have resulted in the creation of a population from which the founders of the Kets, Shorians and other similar groups evolved. In the future, we will attempt to corroborate this hypothesis with Y chromosome data, the analysis of which is underway (Schurr et al., in progress). The Kitoi mtDNA data set will also be enhanced with the analysis of mtDNA and Y-chromosome variation in human remains from the Shamanka II site, which is currently being excavated (Bazaliiski, 2003). Similarly, the excavation and analysis of additional cemetery populations in the Angara and Yenisey River basins may further illuminate the prehistoric population structure of Siberia.

Ancient links between the Serovo-Glazkovo and the Huns?

Although no overt associations are observed between the Serovo-Glazkovo and modern groups occupying the Cis-Baikal region today, there is an intriguing link between the Ust'-Ida group and the cemetery population from Egyin Gol (Keyser-Tracqui et al., 2003), which is located in the Selenga River basin that drains into Lake Baikal on its east side. Egyin Gol was used for approximately five centuries by the Xiongnu, or Huns (Keyser-Tracqui et al., 2003). The Huns were nomadic pastoralists who inhabited and controlled a large part of Mongolia and Trans-Baikal (i.e., south and east of Lake Baikal) from the third century B.C. to the third century A.D., at which time their realm of influence was diminished by the emergence of the Chinese Han dynasty (Marx, 2000). Few inferences about the mortuary behaviour of the Huns can be made from the Keyser-Tracqui et al. (2003) study except for the notable patterning of a single affluent burial surrounded by double interments. The same burial pattern was reportedly practiced by the Sakha (i.e., Yakut), who inhabit the Lena River basin north of Lake Baikal (Francfort et al., 2000; cited in Keyser-Tracqui et al., 2003). In contrast, this kind of mortuary ritual has not been observed at Ust'-Ida;

this finding is not surprising given that the Serovo-Glazkovo are thought to have had a relatively egalitarian social structure (Weber, 1995; Weber et al., 2002).

Interestingly, both of these cemetery populations share the same East Eurasian mtDNA haplogroups with the exception of one haplogroup B individual excavated from Egyin Gol. Although the Egyin Gol group has a higher frequency of haplogroup D and a lower frequency of haplogroup G2a than the Serovo-Glazkovo, the two groups have similar frequencies of haplogroups A, C and F (**Table 1**). In addition, the Serovo-Glazkovo and Egyin Gol groups share a number of HVI sequences. The most frequent haplogroup A sequence at Ust'-Ida (16223-16290-16319) is also found in 75% of Egyin Gol samples. Likewise, 66% of the C, 50% of the F, and 100% of the U5a sequences are shared between the two cemetery groups. However, the G2a sequence in the Egyin Gol sample (16223-16227-16278-16362) differs from both G2a sequences found in the Serovo-Glazkovo (16223-16227-16262-16278). Interestingly, the G2a sequence in the Serovo-Glazkovo also differs from the single Kitoi G2a sequence (16223-16227-16278) and has not yet been characterised in any other modern Siberian group but has been identified in a sample of Han Chinese recently analysed by Yao et al., (2002).

Russian scholars generally believe that the Xiongnu were immigrants who did not interact with the indigenous groups in the region (Okladnikov, 1964). However, the similarity of Serovo-Glazkovo and Xiongnu mtDNA haplogroup distributions suggest that gene flow may have occurred between matrilineal descendents of the Serovo-Glazkovo and groups representing the northern extent of the Huns. This association is compelling, as it suggests that temporal stability was maintained in the regional matrilineal gene pool of Lake Baikal for over four millennia (i.e., from 4200 B.C. to 200 A.D.). Furthermore, the insignificant biological distance between the Serovo-Glazkovo and Xiongnu also reinforces the notion of a population shift in the Cis-Baikal region during the fifth millennia B.C. The Kitoi, who are biologically distinct from the Serovo-

Glazkovo, do not share obvious population affinities with the Xiongnu, making it even more unlikely that the Kitoi contributed to the subsequent population structure of the Cis-Baikal region.

While this study demonstrates the likelihood that a relatively stable matrilineal population structure was maintained in the Lake Baikal region from the time of the Serovo-Glazkovo through to the Xiongnu, the degree to which the Serovo-Glazkovo contributed to the genetic make-up of modern Siberian groups is not clear. Further characterisation of both mtDNA and Y chromosome haplotypes in the Serovo-Glazkovo from Ust'-Ida, as well as from other Serovo-Glazkovo cemeteries in the Cis-Baikal region, will help to illuminate the biological structure of this group and its relationship to modern Siberians.

Ancient peopling of Siberia

A principal tenet of Siberian population history holds that the western regions of Siberia were simultaneously inhabited by both West and East Eurasian groups from as early as the Palaeolithic (i.e., Okladnikov, 1964). This region has long been seen as a junction between East and West, and recent mtDNA evidence has reinforced this view in revealing the presence of many West Eurasian mtDNA lineages in western Siberian groups (e.g., Derenko et al., 2001a, b, 2002a, b, 2003; Derbeneva et al., 2002; Schurr and Wallace 2003; Schurr et al. 2004). Whether this heterogeneity extended as far east as Lake Baikal is not yet clear. On the basis of similarities in material culture between the Upper Palaeolithic Lake Baikal occupations of Mal'ta and Buret' with sites from Eastern Europe (i.e., West Eurasia), Okladnikov (1964) proposed that a West Eurasian group inhabited the Lake Baikal region during the Upper Palaeolithic. However, Okladnikov (1964) predicted that the growth of East Eurasian groups in the region eventually resulted in the replacement of these Upper Palaeolithic West Eurasians. Therefore, modern populations in the

region of Lake Baikal who have not exchanged genes with Russian groups are expected to have a higher proportion of East Eurasian mtDNA polymorphisms than their western neighbors.

The mtDNA lineages characterising the two Neolithic cemetery populations examined in this study generally support this assessment. Although the mtDNA data sets of the Kitoi and the Serovo-Glazkovo are not yet complete, our limited coding region and HVI data sets suggest that 90% of the Kitoi and 85% of the Serovo-Glazkovo mtDNAs are of East Eurasian origin. Comparatively, the proportions of East Eurasian haplogroups found in modern Siberian populations range from a minimum of 60% in the Altaians to a maximum of 92% in the Evenks (**Table 1) (**Derenko et al., 2001, 2002a; Schurr and Wallace 2003).

The only definitive non-East Eurasian haplogroup identified in either population is haplogroup U5a. The geographic origins of haplogroup U5a are ambiguous, as it has been observed in both West and East Eurasian populations (Richards et al., 1998; Sykes, 1999; Derenko et al., 2002b, 2003), but it has great temporal depth. This haplogroup has been detected both in many modern Siberian groups (e.g., Derbeneva et al., 2002a, b; Derenko et al., 2003; Pakendorf et al., 2003; Schurr et al. 2004), as well as other prehistoric Asian cemetery populations (e.g., Oota et al., 1999; Keyser-Tracqui et al., 2003). By exploring the geographic origins of haplogroup U5a, it may be possible to reveal another facet of Siberian population history.

CONCLUSIONS

Analysis of mtDNA coding-region and HVI polymorphisms from two temporally distinct Siberian cemetery groups provides compelling evidence to support the occurrence of a biological hiatus during the Cis-Baikal Neolithic. The Kitoi and Serovo-Glazkovo had unique matrilineal population structures, suggesting that these two groups did not share an ancestor-descendent relationship. The significant biological distances observed between the Kitoi and modern Cis-

Baikal groups, together with the intriguing affinities seen between the Kitoi and groups from the Yenisey and Ob river basins, suggests that the Kitoi left the region. The Serovo-Glazkovo likely migrated into Cis-Baikal about a millennia later where, as evidenced from an intriguing affinity with an Upper Mongolian Hun cemetery, their matrilineal descendents remained for at least the next four thousand years. This study demonstrates how archaeological studies investigating the population history of a particular region can benefit by integrating aDNA data into their interpretations. Future work of the Baikal Archaeology Project will follow this approach with other Cis-Baikal cemeteries, ultimately creating a comprehensive snapshot regarding the peopling of this region.

ACKNOWLEDGEMENTS

This research was supported with funding from the Social Sciences and Humanities Research Council of Canada, the Canadian Circumpolar Institute, and the May and Stanley Smith Charitable Trust. We thank Martin Somerville and Mark Hicks at the Stollery Children's Hospital Molecular Diagnostics Laboratory for their generous assistance in conducting the HVI sequencing. We are grateful for Henry Harpending's advice concerning the population genetic analysis. We thank Ann O'Neill and Vicki Listoe for their technical assistance with this project. Finally, we extend our appreciation to the two anonymous reviewers whose valuable comments enhanced the quality of this paper.

LITERATURE CITED

Alekseev V. 1998. The physical specificities of Paleolithic hominids in Siberia. In: Derev'anko A., editor. The Paleolithic of Siberia. Urbana: University of Illinois Press. p. 122–136.

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, and Young IG. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

Atlas SSSR [Atlas of the USSR]. Glavnoe upravlenie geodezii I kartografi pri Sovete ministrov SSSR, Moscow. [In Russian]

Bazaliiskii VI. 2003. The Neolithic of the Baikal region on the basis of mortuary materials. In: McKenzie H and Weber A, editors. Prehistoric Foragers of the Cis-Baikal, Siberia. Edmonton: Canadian Circumpolar Press. p 37–50.

Bazaliiskiy VI, and NA Savelyev. 2003. The wolf of Baikal: the "Lokomotiv" Early Neolithic cemetery in Siberia (Russia). Antiquity 77:20–30.

Bentley RA, Chikhi L, and Price TD. 2003. The Neolithic transition in Europe: comparing broad scale genetic and local scale isotopic evidence. Antiquity 77:63-66.

Bentley RA, Price TD, and Stephan E. 2004. Determining the 'local' Sr-87/Sr-86 range for archaeological skeletons: a case study from Neolithic Europe. J Archaeol Sci 31:365-375.

Boom R, Sol CJ, Salimans MM, Jansen CL , Wertheim–van Dillen PM , and van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503.

Brown MD, Hosseini SH, Torroni A, Bandelt HJ, Allen JC, Schurr TG, Scozzari R, Cruciani F, and Wallace DC. 1998. mtDNA haplogroup X: an ancient link between Europe/Western Asia and North America? Am J Hum Genet 63:1852–61.

Carlyle SW, Parr RL, Hayes MG, and O'Rourke DH. 2000. Context of maternal lineages in the Greater Southwest. Am J Phys Anthropol 113:85–101.

Cavalli–Sforza LL, Menozzi P, and Piazza A. 1996. The history and geography of human genes . Princeton University Press. Princeton, N.J.

Crawford MH, McComb J, Schanfield MS, and Mitchell RJ. 2002. Genetic structure of pastoral populations of Siberia: the Evenki of central Siberia and the Kizhi of Gorno Altai. In: Human Biology of Pastoral Populations. Cambridge: Cambridge University Press. p 10–49.

Debets GF. 1951. Antropologicheski issledovaniia v Kamchatskoi oblasti. Trudy Severo–vostochnoi Ekspeditsii I. SSSR: Trudy Instituta Etnografii. [In Russian]

Derbeneva OA, Starikovskaya EB, Volodko NV, Wallace DC, and Sukernik RI. 2002a. Mitochondrial DNA variation in the Kets and Nganasans and its implications for the initial peopling of northern Eurasia. Russ J Genet 11:1554–1560.

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

Derenko MV, Malyarchuk BA, Dambueva IK, Shaikhaev GO, Dorzhu CM, Nimaev DD, and Zakharov IA. 2000. Mitochondrial DNA variation in two South Siberian aboriginal populations: implications for the genetic history of North Asia. Hum Biol 72:945-973.

Derenko MV, Denisova GA, Malayarchuk BA, Dambueva IK, Luzina FA, Lotosh EA, Dorzhu CM, Karamchakova ON, Solovenchuk LL, and Zakharov IA. 2001a. The structure of gene pools of the ethnic populations of Altai–Sayan region based on mitochondrial DNA polymorphism data. Russ J Genet 37:1402–1410.

Derenko MV, Grzybowski T, Malyarchuk BA, Czarny J, Miscicka–Sliwka D, and Zakharov IA. 2001b. The presence of mitochondrial haplogroup X in Altaians from South Siberia. Am J Hum Genet 69:237–241.

Derenko MV, Malayarchuk BA, Denisova GA, Dambueva IK, Kakpakov VT, Dorzhu Ch. M, Luzina FA, Lotosh EA, Ondar UN, Aplina MI, and Zakharov IA. 2002a. Molecular genetic differentiation of the ethnic populations and South and East Siberia based on mitochondrial DNA polymorphism. Russ J Genet 38:1409–1416.

Derenko MV, Malyarchuk BA, and Zakharov IA. 2002b. Origin of Caucasoid-specific mitochondrial DNA lineages in the ethnic groups of the Altai-Sayan Region. Russ J Genet 38:1098-1103.

Derenko MV, Grzybowski T, Malayarchuk BA, Dambueva IK, Denisova GA, Czarny J, Dorzhu CM , Kakpakov VT, Miscicka–Sliwka D, Wozniak M, and Zakharov IA. 2003. Diversity of mitochondrial DNA lineages in South Siberia. Ann Hum Genet 67:391–411.

Eglington G, and Logan GA. 1991. Molecular preservation. Phil Trans R Soc Lond B 333:315–328.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. *Distributed by the author*. Department of Genetics, University of Washington, Seattle.

Forster P, Cali F, Rohl A, Metspalu E, D'Anna R, Mirisola M, De Leo G, Flugy A, Salerno A, Ayala G, Kouvatsi A, Villems R, and Romano V. 2002. Continental and subcontinental distributions of mtDNA control region types. Int J Legal Med 116:99–108.

Francfort HP, Ligabue G, and Samashev Z. 2000. La fouille d'un kourgane Scythe gele du IVe siecle av. notre ere a Berel dans l'Altai (Kazakhstan). In: Academie des Inscriptions et Belles Lettres . Paris: p 775-806. [In French]

Geigel EM. 2002. On the Circumstances surrounding the preservation and analysis of very old DNA. Archaeometry 44:337–342.

Gelehrter TD, Collins FS, and Ginsburg D. 1998. Principles of medical genetics. Baltimore: Williams and Williams.

Gerasimov MM. 1955. Osnovy vosstanovleniia litsa po cherepu. Trudy Instituta Etnografii. Novaia seriia 27. [In Russian]

Gerasimov MM 1958. Paleoliticheskaya stoyanka Ma'lta (raskopki 1956–1957 godov) [The Paleolithic site of Ma'lta [excavations of 1956–1957]. Sovetskaya Etnografiya 3. [In Russian]

Gerasimova MM. Cherepa iz Fofanovskogo mogil'nika (r. Oka, Selenga) [Skulls from the Fofanovo cemetery (Oka, Selenga rivers)]. 97-111. 1991. Irkutsk, IGU. Masson, V. M. [In Russian]

Gould JL, and Gould GF. 2002. Biostats basics: a student handbook. New York: W.H. Freeman and Company.

Handt O, Krings M, Ward RH, and Paabo S. 1996. The retrieval of ancient human DNA sequences. Am J Hum Genet 59:368–376.

Hedrick PW. 2000. Genetics of populations. Boston: Jones and Bartlett Publishers.

Hemphill BE. 1999. Foreign elites from the Oxus civilization? A craniometric study of anomalous burials from Bronze Age Tepe Hissar. Am J Phys Anthropol 110:421–434.

Holland MM, Fisher DL, Mitchell LG, Rodriquez WC, Canik JJ, Merril CR, and Weedn VW. 1993. Mitochondrial DNA sequence analysis of human skeletal remains: identification of remains from the Vietnam War. J Forensic Sci 38:542-53.

Ishida H, and Dodo Y. 1996. cranial morphology of the Siberians and East Asians. In: Akazawa T. and Szathmary E.J.E. editors. Prehistoric Mongoloid Dispersals. Oxford : Oxford University Press. p 113–124.

Johnson AL, and Lovell NC. 1994. Biological differentiation at Predynastic Naqada, Egypt: An analysis of dental morphological traits. Am J Phys Anthropol 93:427–433.

Kaestle FA and Smith DG. 2001. Ancient mitochondrial DNA evidence for prehistoric population movement: the Numic expansion. Am J Phys Anthropol 115:1–12.

Kaestle FA, and Horsburgh KA. 2002. Ancient DNA in anthropology: methods, applications, and ethics. Am J Phys Anthropol Suppl 35:92–130.

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

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

Kivisild T, Tolk HV, Parik J, Wang Y, Papiha SS, Bandelt HJ, and Villems R. 2002. The emerging limbs and twigs of the East Asian mtDNA tree. Mol Biol Evol 19:1737–51.

Kolman CJ and Tuross N. 2000. Ancient DNA analysis of human populations. Am J Phys Anthropol 111:5-23.

Kong QP, Yao YG, Sun C, Bandelt HJ, Zhu CL, and Zhang YP. 2003. Phylogeny of East Asian mitochondrial DNA lineages inferred from complete sequences. Am J Hum Genet 73:671–676.

Larsen CS. 1997. Bioarchaeology: interpreting behavior from the human skeleton. New York: Cambridge University Press.

Lee HC, Pagliaro EM, Berka KM, Folk NL, Anderson DT, Ruano G, Keith TP, Phipps P, Herrin GL, Garner DD, and Gaensslen RE. Genetic markers in human bone: I. deoxyribonucleic acid (DNA) analysis. J For Sci 36:320-330.

Lieverse AR. 1999. Diet and the aetiology of dental calculus. Int. J. Osteoarch 9:219–232.

Link DW. 1996. Hunter–gatherer demography and health in the Cis-Baikal Neolithic. Unpublished PhD dissertation. University of Chicago, Chicago, Illinois.

Link DW 1999. Boreal forest hunter–gatherer demography and health during the Middle Holocene of the Cis-Baikal, Siberia. Arct Anthropol 36:51–72.

Lopatin IA 1940. The extinct and near–extinct tribes of Northeastern Asia as compared with the American Indian. Am Antiquity 5:202–208.

Malhi RS, and Smith DG. 2002. Brief communication: haplogroup x confirmed in prehistoric North America. Am J Phys Anthropol 119:84-86.

Mamonova NN. 1983. K voprosu o mezhgrupovykh razlichiiakh v neolite Pribaikal'ia [On interpopulational differences during the Neolithic of Lake Baikal region]. Voprosy antropologii 1:88-103. [In Russian]

Mannucci A, Sullivan KM, Ivanov PL, and Gill P. 1994. Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin . Int J Leg Med 106:190-193.

Marx I. The Xiongnu culture–third century BCE. http://www.silk-road.com/artl/xiongnu1.shtml accessed November 21, 2003.

McComb J, Crawford MH, Osipova L, Karaphet T, Posukh O, and Schanfield MS. 1996. DNA interpopulational variation in Siberian Indigenous populations: The mountain Altai. Am J Hum Biol 8:599–607.

Medvedev G. 1998. Upper Paleolithic sites in South–Central Siberia. In: The Paleolithic of Siberia: new discoveries and interpretations. Urbana: University of Illinois Press. p. 122–136.

Merriwether DA, Hall WW, Vahlne A, and Ferrell RE. 1996. mtDNA variation indicates Mongolia may have been the source for the founding population of the new world. Am J Hum Genet 59:204–212.

Mooder K, Schurr TG, Bamforth F, and Bazaliiskii V. 2003. Mitochondrial DNA and archaeology: the genetic characterisation of prehistoric Siberian hunter–gatherers. In: Prehistoric Foragers of the Cis-Baikal, Siberia. CCI Press. p 193–202.

Mooder KP. 2004. mtDNA and prehistoric Siberian hunter-gatherers: characterising matrilineal population affinities in Neolithic and Bronze Age Cis-Baikal. Unpublished PhD dissertation, University of Alberta, Edmonton, Alberta. 268 pp.

Naumova OY, Rychkov SY, Bazaliiskii VI, Mamonova NN, Sulerzhitskii LD, and Rychkov YG. 1997. molecular genetic characteristics of the Neolithic population of the Baikal Region: RFLP of the ancient mtDNA from osseous remains found in the Ust'-Ida I burial ground. Genetika 33:1418–1425. [In Russian]

Okladnikov AP. 1955. The history of the Yakut ASSR. Volume 1: Yakutia before its incorporation into the Russian state Moscow: izdatel'stvo Akademii Nauk SSSR. [In Russian]

Okladnikov AP. 1959. Ancient population of Siberia and its cultures. Cambridge, U.S.A: Peabody Museum.

Okladnikov AP. 1964. Ancient population of Siberia and its cultures. In: Levin MG and Potapov LP, editors. The Peoples of Siberia. Chicago: University of Chicago Press. p. 13–98.

Oota H, Saitou N, Matsushita T, and Ueda S. 1999. Molecular genetic analysis of remains of a 2,000–year–old human population in China–and its relevance for the origin of the modern Japanese population. Am J Hum Genet 64:250–258.

O'Rourke DH, Hayes MG, and Carlyle SW. 2000. Spatial and temporal stability of mtDNA haplogroup frequencies in Native North America. Hum Biol 72:15-34.

Ou CY, Moore JL, and Schochetman G. 1991. Use of UV irradiation to reduce false positivity in polymerase chain reaction. Biotechniques 10:442–445.

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.

Popov AA and Dolgikh BO. 1964. The Kets. In: Levin MG and Potapov LP, editors. The Peoples of Siberia. Chicago: University of Chicago Press. p. 607–619.

Potapov LP. 1964. The Shors. In: Levin MG and Potapov LP, editors. The Peoples of Siberia. Chicago: University of Chicago Press. p. 440–473.

Price TD, Burton JH, and Bentley RA. 2002. The characterization of biologically available strontium isotope ratios for the study of prehistoric migration. Archaeometry 44:117-135.

Prince AM, and Andrus L. 1992. PCR: how to kill unwanted DNA. Biotechniques 12:358–360.

Prowse TL, and Lovell NC. 1996. Concordance of cranial and dental morphological traits and evidence for endogamy in Ancient Egypt. Am J Phys Anthropol 101:237–246.

Raymond M, and Rousset F. 1995. An exact test for population differentiation. Evolution 49:1280–1283.

Richards MB, Macaulay VA, Bandelt HJ, and Sykes BC. 1998. Phylogeography of mitochondrial DNA in Western Europe. Ann Hum Genet 62:241-260.

Rogan PK, and Salvo JJ. 1990. Study of nucleic acids isolated from ancient remains. Yearbook Phys Anthropol 33:195–214.

Rubicz R, Melvin KL, and Crawford MH. 2002. Genetic evidence for the phylogenetic relationship between Na–Dene and Yeniseyan Speakers. Hum Biol 74:743–760.

Schneider S, Roessli D, and Excoffier L. 2000. ARLEQUIN ver 2.000. A software package for population genetics data analysis. Distributed by the authors.

Schurr TG, Ballinger SW, Gan YY, Hodge JA, Merriwether DA, Lawrence DA, Knowler WC, Weiss KM, and Wallace DC. 1992. Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. Am J Hum Genet 46:613–623.

Schurr TG, Sukernik RI, Starikovskaya YB, and 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.

Schurr TG, and Wallace DC. 2003. Genetic prehistory of Paleoasiatic-speaking peoples of northeastern Siberia and their links to Native American populations. In: Kendall L, Krupnik I editors. Constructing Cultures Then and Now: Celebrating Franz Boas and the Jesup North Pacific Expedition. Baltimore: Smithsonian Institution Press. p. 239-58.

Schurr TG, Zhadanov SI, and Osipova LP. 2004. mtDNA variation in indigenous Altaians, and their genetic relationships with Siberian and Mongolian populations. Am J Phys Anthropol Suppl 123(38):176 [Abstr.].

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

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

Starikovskaya YB, Sukernik RI, Schurr TG, Kogelnik AM, and 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.

Stone AC, and Stoneking M. 1998. mtDNA analysis of a prehistoric Oneota population: implications for the peopling of the New World. Am J Hum Genet 62:1153–1170.

Stuiver M, Reimer PJ, Bard E, Beck JW, Burr GS, Hughen KA, Kromer B, Mccormac G, Van Der Plicht J, and Spurk M. 1998. INTCAL98 radiocarbon age calibration, 24,000-0 Cal BP. Radiocarbon 40:1041-1083.

Sukernik RI, Schurr TG, Starikovskaya EB, and Wallace DC. 1996. Mitochondrial DNA variation in native Siberians, with special reference to the evolutionary history of Americans Indians. studies on restriction polymorphism. Genetika 32(3):432-39 [In Russian].

Sykes B. 1999. The molecular genetics of European ancestry. Phil Trans R Soc Lond B 354:131–139.

Tiutrin AA, and Bazaliiskii V. 1996. Mogil'nik v ust'e reki Idy v doline Angary [A cemetery at the mouth of the Ida river in the Angara River Valley]. Berdnikova NE et al. Arkheologiia, paleoekologiia i ehnologiia Sibiri i Dal'nego Vostoka. IGU. [In Russian]

Tokarev SA, and Gurvich IS. 1964. The Yakuts. In: Levin MG and Potapov LP, editors. The Peoples of Siberia. Chicago: University of Chicago Press. p. 243–304.

Torroni A, Miller JA, Moore LG, Zamudio S, Zhuang J, Droma T, and Wallace DC. 1994. Mitochondrial DNA analysis in Tibet: implications for the origin of the Tibetan population and its adaptation to high altitude. Am J Phys Anthropol 93:189–199.

Torroni A, Schurr TG, Yang CC, Szathmary EJ, Williams RC, Schanfield MS, Troup GA, Knowler WC, Lawrence DN, and Weiss KM et al. 1992. Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130:153–162.

Torroni A, Sukernik RI, Schurr TG, Starikorskaya YB, Cabell MF, Crawford MH, Comuzzie AG, and Wallace DC. 1993a. mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with Native Americans. Am J Hum Genet 53:591–608.

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, et al. 1993b. Asian Affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563-590.

Turner CG II. 1987. Late Pleistocene and Holocene population history of East Asia based on dental variation. Am J Phys Anthropol 73:305–321.

Vasilevich GM, and Smolyak AV. 1964. The Evenks. In: Levin MG and Potapov LP, editors. The Peoples of Siberia. Chicago: University of Chicago Press. p. 620–654.

Wallace DC, Ye J, Neckelmann SN, Singh G, Webster KA, and Greenberg BD. 1987. Sequence analysis of cDNAs for the human and bovine ATP synthase B subunit: Mitochondrial genes sustain seventeen times more mutations. Curr Genet 12:81-90.

Wallace DC. 1995. Mitochondrial DNA variation in human evolution, degenerative disease, and aging. Am J Hum Genet 57:201–23.

Wallace DC, Brown MD, and Lott MT. 1999. Mitochondrial DNA variation in human evolution and disease. Gene 238:211–230.

Weber AW. 1995. The Neolithic and Early Bronze Age of the Lake Baikal Region, Siberia: A review of recent research. J World Prehist 9:99–165.

Weber AW, Link DW, and Katzenberg MA. 2002. Hunter–Gatherer culture change and continuity in the Middle–Holocene of the Cis-Baikal, Siberia. J Anthropol Archaeol 21:230–299.

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

Yao YG, Kong QP, Man XY, Bandelt HJ, and Zhang YP. 2003. Reconstructing the evolutionary history of China: a caveat about inferences drawn from ancient DNA. Mol Biol Evol 20:214-219.

Footnote (p. 9)

[1] 10 % v/v solution composed of industrial strength bleach containing 12% sodium hypochlorite.

Table 1: mtDNA Haplogroup Frequencies of Prehistoric and Modern East Eurasian Populations

	n	A	B	C	D	G2a/E	F	OTHER
Prehistoric Groups								
Lokomotiv[1]	31	0.13	0.00	0.03	0.23	0.03	0.48	0.10
Ust'-Ida[1]	39	0.26	0.00	0.28	0.05	0.10	0.08	0.23
Eygin-Gol[5]*	46	0.17	0.02	0.13	0.41	0.02	0.09	0.15
Modern Groups								
Shorians[2]	42	0.00	0.02	0.07	0.10	0.00	0.43	0.38
Sojots[2]	34	0.09	0.03	0.18	0.50	0.09	0.00	0.12
Kets[4]	38	0.08	0.00	0.16	0.03	0.00	0.24	0.50
Tofalars[4]	58	0.05	0.03	0.62	0.00	0.02	0.00	0.28
Todjins[4]	48	0.04	0.08	0.48	0.04	0.00	0.02	0.33
Evenks[4]	79	0.04	0.05	0.48	0.27	0.03	0.01	0.13
Yakuts[4]	62	0.00	0.05	0.42	0.27	0.02	0.02	0.23
Buryat[4]	91	0.02	0.07	0.29	0.33	0.14	0.01	0.14
Tuvnians[4]	90	0.01	0.08	0.48	0.18	0.02	0.02	0.21

[1]This Study, [2] Derenko et al., (2002a), [3]Derbeneva et al., (2002a), [4]Derenko et al., (2001b),
[5] Keyser-Tracqui et al., (2003)

*Haplogroup assignment derived solely from HVI sequence data

Table 2: Reportable HVI sequences found in the Kitoi and Serovo-Glazkovo

Hap	16223	16227	16232	16249	16256	16262	16270	16278	16288	16290	16298	16304	16311	16319	16327	Lokomotiv (n = 10)	Ust'-Ida (n=17)
CRS	**C**	**A**	**C**	**T**	**C**	**C**	**C**	**C**	**T**	**C**	**T**	**T**	**T**	**G**	**C**		
A	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	2	3
	T	G								T				A			2
C	T	.	.	.	.	.	.	.	.	.	C	.	.	.	T	1	2
	T										C						1
D	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.		2
	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	
F	.	.	A	C	.	.	.	.	.	.	.	C	C	.	.	2	
G2a	T	G	.	.	.	.	.	T	.	.	.	.	.	.	.	1	
	T	G	.	.	.	T	.	T	.	.	.	.	.	.	.		1
U5a	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	2	1
Other	T	G				T		T					C				2
	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.		2
	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1

Figure Legends

Figure 1: Location of Neolithic Cis-Baikal cemeteries analysed in this study

Figure 2: : Map of modern Siberian populations used for comparison in this study

Figure 3: PC map of mtDNA haplogroup distributions for prehistoric and modern populations as estimated by F_{ST}. Key for populations: LOK = Lokomotiv, UID= Ust'-Ida, SH=Shorians, KT=Kets, TF=Tofalars, TD= Todjins, TV=Tuvinians, EV=Evenki, BT=Buryats, SJ=Sojots, EG=Egyin Gol

Figure 4: Lokomotiv mtDNA haplogroup distribution through duration of cemetery use. E= Early Neolithic (7140–6750 BP) M = Middle Neolithic (i.e., 7140–6750 BP) and L = Late Neolithic (6620–6200 BP)

Figure 1

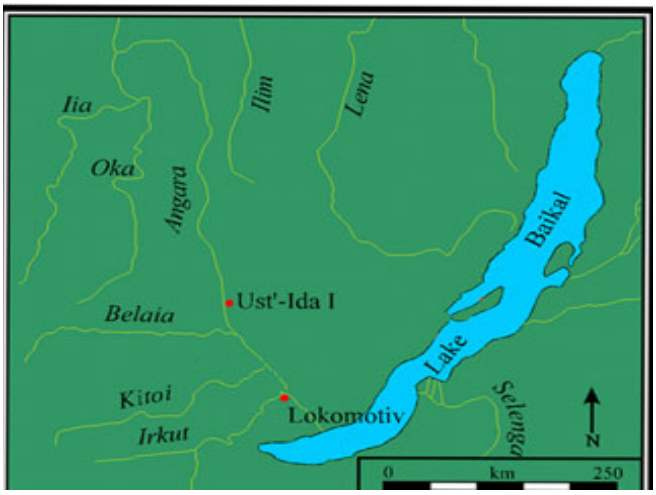

Iia
Oka
Angara
Ilim
Lena
Baikal
Lake
Belaia
Ust'-Ida I
Kitoi
Irkut
Lokomotiv
Selenga
N
0
km
250

Figure 2


Laptev Sea
Kara Sea
Tiksi
KHREBET CHERSKOGO
VERKHOYANSK KHREBET
Magadan
Petropavlov
Kamchatsk
Arctic Circle
SIBERIA
RUSSIA
KHREBET BYZHUGO
Sea of Okhotsk
Kets
Evenks
Shorians
Tofalars
Sojots
Lake Baikal
KHREBET
Khabarovsk
Sakhali
Todjins
Irkutsk
Buryats
Tuvinians
Egyin Gol
Ulaanbaatar
ALTAY MTS.
MONGOLIA
DESERT
DA HINGGAN LING
MANCHURIAN PLAIN
Harbin
Changchun
Vladivostok
Shenyang
NORTH KOREA
Sea of Japan
SIKHOTE-ALIN

Figure 3:

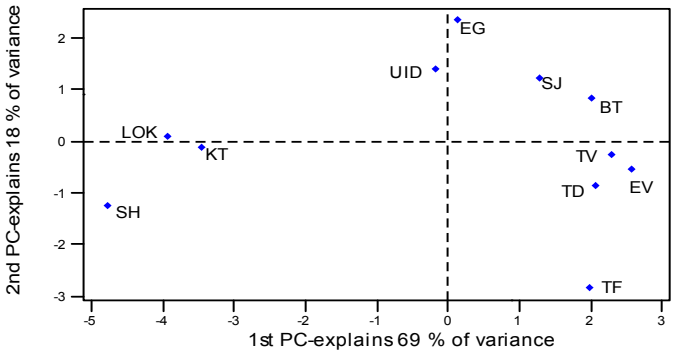
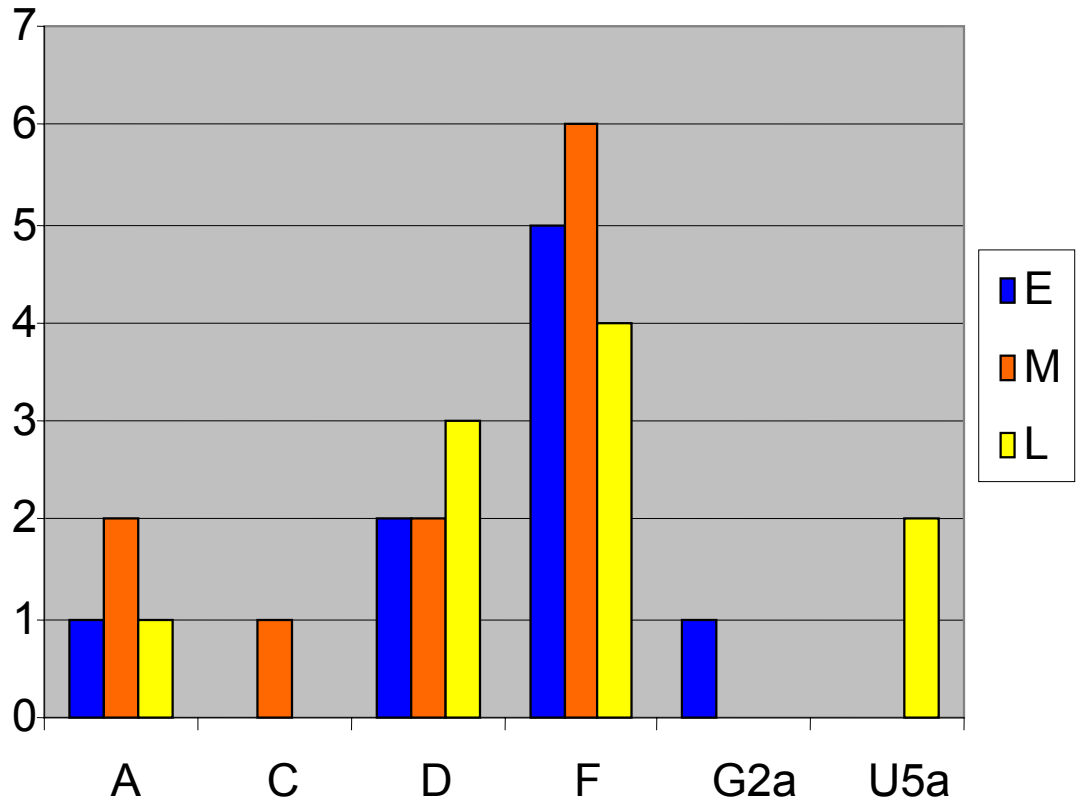

EG
UID
SJ
BT
LOK
KT
TV
EV
TD
SH
TF
2nd PC-explains 18 % of variance
1st PC-explains 69 % of variance
2
1
0
-1
-2
-3
-5
-4
-3
-2
-1
0
1
2
3

Figure 4:



Appendix 1: Primers for Asian-specific coding region and HVI substitutions analysed in this study

mtDNA Haplogroup	RFLP	Primer	Sequence	Length (bp)	Anneal Temperature	Reference
A	+HaeIII 663	L635 H708	5'-TGAAAATGTTTAGACGGCCTCACATG 5'-TAGAGGGTGAACTCACTGGAAC	108	58 °C	Handt et al., 1996
B	COII/tRNA^{-Lys} 9 bp deletion	L8196 H8297	5'-ACAGTTTCATGCCCATCGTC 5'-ATGCTAAGTTAGCTTTACAG	112/121	58 °C	Handt et al.,1996
C	-HincII 13259	L13257 H13393	5'-AATCGTAGCCTTCTCCACTTCA 5'-TCCTATTTTTCGAATATCTTGTTC	180	58 °C	Handt et al.,1996
D	-AluI 5176	L5127 H5189	5'-ACTACCGCATTCTACTACTCA 5'-GGGTGGATGGAATTAAGGGTGT	106	54 °C	Handt et al.,1996
F	-HincII 12406	L12368 H12473	5'-CCCTGACTTCCCTAATTCCC 5'-TGTTGTGGGGAAGAGACTGA	125	56 °C	this study
G2a	-CfoI 7598	L7495 H7615	5′-TGATAGGGGAAGTAGCGTCTT 5′-ATGGCCTCCATGACTTTTTC	140	54°C	this study
M	+AluI 10397/ +DdeI 10394	L10361 H10458	5'-TCTGGCCTATGAGTGACTACAA 5'-TGAGGGGCATTTGGTAAATATG	120	56 °C	this study
X	-DdeI 1715	L1688 H1752	5′-AACTTAACTTGACCGCTCT 5′-TGCGCCAGGTTTCAATTTCTA	107	54°C	this study
HVI	n/a	H16346 L16211	5′-CCCATGCTTACAAGCAAGTA 5′-CAGTTTAGGGAAGAGCAGGG	176	54 °C	this study