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

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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. Buekers, 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 \(-70^\circ\text{C}\) 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 MgCl₂ (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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{AluI} 10397 site gain were further defined using primers flanking SNPs characteristic of haplogroups A, B, F and, in certain cases, the \textit{DdeI} 10394 site gain, as well as the \textit{DdeI} 1715 site loss that defines haplogroup X.

\textbf{mtDNA haplogroup assignment}

Amplified PCR products were digested with restriction enzymes targeting SNPs defining different mtDNA haplogroups. These haplogroups included M, defined by an \textit{DdeI}/\textit{AluI} site gain at nucleotide pair (np) 10394/10397; A, by the \textit{HaeIII} site gain at np 663; C by the \textit{HincII} site loss at np 13259; D by an \textit{AluI} site loss at np 5176; F by a \textit{HincII} site loss at np 12406, G2a by a \textit{CfoI} 7598 site loss; and X by the \textit{DdeI} 1715 site loss. Restriction digests were prepared by mixing 5 U of the relevant restriction enzyme and 1 \(\mu\)L of 10X buffer (NEB or Invitrogen), diluting the mixture to a final volume of 10 \(\mu\)L 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, Sojets, 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
$CjoI$ 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.
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 Ketitic 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 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**

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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

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<th>Prehistoric Groups</th>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>G2a/E</th>
<th>F</th>
<th>OTHER</th>
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*Haplogroup assignment derived solely from HVI sequence data
Table 2: Reportable HVI sequences found in the Kitoi and Serovo-Glazkovo

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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<sub>ST</sub>. 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
Figure 2
Figure 3:

- 1st PC explains 69% of variance
- 2nd PC explains 18% of variance

Variables: LOK, KT, SH, UID, EG, SJ, BT, TV, EVTD, TF
Figure 4:
### Appendix 1: Primers for Asian-specific coding region and HVI substitutions analysed in this study

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<th>mtDNA Haplogroup</th>
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