

## Middle Holocene Siberian genomes reveal highly connected gene pools throughout North Asia

### Highlights

- A distinctive Middle Holocene Siberian ancestry is found in Altai hunter-gatherers
- It results from a mixture of paleo-Siberian and ancient North Eurasian ancestries
- A contemporaneous Altai individual carries ancient Northeast Asian ancestry
- Northeastern Siberians experienced a prolonged Native American-related geneflow

### Authors

Ke Wang, He Yu, Rita Radzevičiūtė, ..., Choongwon Jeong, Johannes Krause, Cosimo Posth

### Correspondence

ke\_wang@fudan.edu.cn (K.W.),  
krause@eva.mpg.de (J.K.),  
cosimo.posth@uni-tuebingen.de (C.P.)

### In brief

Wang et al. describe a distinctive genetic profile in Altai hunter-gatherers that is derived from a mixture between paleo-Siberian and ancient North Eurasian ancestries. This and ancient genomic data from the Russian Far East and Kamchatka reveal a connected gene pool across vast areas of North Asia and North America by at least the early Holocene.

Article

# Middle Holocene Siberian genomes reveal highly connected gene pools throughout North Asia

Ke Wang,<sup>1,2,\*</sup> He Yu,<sup>1,3</sup> Rita Radzevičiūtė,<sup>1</sup> Yuriy F. Kiryushin,<sup>4</sup> Alexey A. Tishkin,<sup>4</sup> Yaroslav V. Frolov,<sup>4</sup> Nadezhda F. Stepanova,<sup>5</sup> Kirill Yu. Kiryushin,<sup>6</sup> Artur L. Kungurov,<sup>7</sup> Svetlana V. Shnaider,<sup>8</sup> Svetlana S. Tur,<sup>4</sup> Mikhail P. Tiunov,<sup>9</sup> Alisa V. Zubova,<sup>10,11</sup> Maria Pevzner,<sup>12</sup> Timur Karimov,<sup>13</sup> Alexandra Buzhilova,<sup>14</sup> Viviane Slon,<sup>15,16,17</sup> Choongwon Jeong,<sup>18</sup> Johannes Krause,<sup>1,\*</sup> and Cosimo Posth<sup>1,19,20,21,\*</sup>

<sup>1</sup>Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, Leipzig 04103, Germany

<sup>2</sup>Department of Anthropology and Human Genetics, School of Life Sciences, Fudan University, Songhu Rd. 2005, Shanghai 200433, China

<sup>3</sup>State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Yiheyuan Rd. 5, Beijing 100871, China

<sup>4</sup>Department of Archaeology, Ethnography and Museology, Altai State University, pr. Lenina 61, Barnaul 656049, Russia

<sup>5</sup>Laboratory of Archaeology and Ethnography of South Siberia, Institute of Archaeology and Ethnography, Siberian Branch, Russian Academy of Sciences, Acad. Lavrentiev Avenue 17, Novosibirsk 630090, Russia

<sup>6</sup>Department of Recreational Geography, Service, Tourism and Hospitality, Institute of Geography, Altai State University, pr. Lenina 61, Barnaul 656049, Russia

<sup>7</sup>Altai State Museum of Regional Studies, ul. Polzunova 46, Barnaul 656049, Russia

<sup>8</sup>Archaeozoology in Siberia and Central Asia – ZooSCAn, CNRS – IAET SB RAS International Research Laboratory, IRL 2013, Institute of Archaeology and Ethnography, Siberian Branch, Russian Academy of Sciences, Acad. Lavrentiev Avenue 17, Novosibirsk 630090, Russia

<sup>9</sup>Federal Science Center of East Asian Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences, Pr-t 100-let Vladivostoka 159, Vladivostok 690022, Russia

<sup>10</sup>Department of Anthropology, Peter the Great Museum of Anthropology and Ethnography (the Kunstkamera), Russian Academy of Sciences, Universitetskaya Naberezhnaya 3, St. Petersburg 199034, Russia

<sup>11</sup>North-East Interdisciplinary Scientific Research Institute of Far East Branch of the Russian Academy of Sciences, Portovaya Street 16, Magadan 685000, Russia

<sup>12</sup>Laboratory for Isotope Geochemistry and Geochronology, Geological Institute of RAS, Pyzhevsky lane 7, Moscow 119017, Russia

<sup>13</sup>D-REAMS Laboratory, Weizmann Institute of Science, Herzl Street 234, Rehovot 7610001, Israel

<sup>14</sup>Research Institute and Museum of Anthropology, Lomonosov Moscow State University, Mokhovaya 11, Moscow 125009, Russia

<sup>15</sup>Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, Leipzig 04103, Germany

<sup>16</sup>Department of Anatomy and Anthropology and Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, P.O.B 39040 Ramat Aviv, Tel Aviv 6997801, Israel

<sup>17</sup>The Dan David Center for Human Evolution and Biohistory Research, Tel Aviv University, 12 Klausner St., Tel Aviv 6997801, Israel

<sup>18</sup>School of Biological Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea

<sup>19</sup>Archaeo- and Palaeogenetics, Institute for Archaeological Sciences, Department of Geosciences, University of Tübingen, Hölderlinstr. 12, Tübingen 72074, Germany

<sup>20</sup>Senckenberg Centre for Human Evolution and Palaeoenvironment at the University of Tübingen, Hölderlinstr. 12, Tübingen 72074, Germany

<sup>21</sup>Lead contact

\*Correspondence: [ke\\_wang@fudan.edu.cn](mailto:ke_wang@fudan.edu.cn) (K.W.), [krause@eva.mpg.de](mailto:krause@eva.mpg.de) (J.K.), [cosimo.posth@uni-tuebingen.de](mailto:cosimo.posth@uni-tuebingen.de) (C.P.)

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## SUMMARY

The peopling history of North Asia remains largely unexplored due to the limited number of ancient genomes analyzed from this region. Here, we report genome-wide data of ten individuals dated to as early as 7,500 years before present from three regions in North Asia, namely Altai-Sayan, Russian Far East, and the Kamchatka Peninsula. Our analysis reveals a previously undescribed Middle Holocene Siberian gene pool in Neolithic Altai-Sayan hunter-gatherers as a genetic mixture between paleo-Siberian and ancient North Eurasian (ANE) ancestries. This distinctive gene pool represents an optimal source for the inferred ANE-related population that contributed to Bronze Age groups from North and Inner Asia, such as Lake Baikal hunter-gatherers, Okunevo-associated pastoralists, and possibly Tarim Basin populations. We find the presence of ancient Northeast Asian (ANA) ancestry—initially described in Neolithic groups from the Russian Far East—in another Neolithic Altai-Sayan individual associated with different cultural features, revealing the spread of ANA ancestry ~1,500 km further to the west than previously observed. In the Russian Far East, we identify 7,000-year-old individuals that carry Jomon-associated ancestry indicating genetic links with hunter-gatherers in the Japanese archipelago. We also report multiple phases of Native American-related gene flow into north-eastern Asia over the past 5,000 years, reaching the Kamchatka Peninsula and central Siberia. Our findings highlight largely interconnected population dynamics throughout North Asia from the Early Holocene onward.

## INTRODUCTION

North Asia covers an extensive portion of the Eurasian continent stretching from western to northeastern Siberia and connects to North America through Beringia (Figure 1A). Despite representing a corridor for past population movements, the peopling history of North Asia remains poorly studied. Starting from the Upper Paleolithic, multiple distinct human genetic lineages have been present in this vast area. In the Altai-Sayan region (hereafter Altai), the oldest modern humans with genomic data published thus far were found at the Afontova Gora site (AG2 and AG3) and dated to ~17,000 years before present (BP; dates are calibrated if not specified otherwise).<sup>1,2</sup> These individuals carry the typical ancient North Eurasian (ANE) ancestry, a widely distributed gene pool that was first identified in a 24,000-year-old individual (MA1) from the Mal'ta site near the Lake Baikal in south-central Siberia.<sup>2</sup> Thereafter, there is a large gap of ~12,000 years, during which the genomic profile of human populations in the Altai region is unknown. Across this timespan, hunter-gatherer groups in neighboring regions were shown to harbor different ancestries, which raise questions regarding their movements and connections. Specifically, populations in the northwest of the Altai (i.e., in western Siberia) exhibit high levels of ANE ancestry,<sup>3</sup> whereas populations to the east of the Altai (i.e., in eastern Siberia) exhibit high levels of ancient Northeast Asian (ANA) ancestry.<sup>4–7</sup> The latter gene pool was first identified in the Russian Far East in Neolithic hunter-gatherers from Devil's Gate Cave (DevilsCave\_N),<sup>5,6</sup> and, to date, the westernmost presence of ANA ancestry was reported in the Lake Baikal region and in Central Mongolia by ~7,500 and ~5,600 BP, respectively.<sup>4,7</sup> Finally, a third distinct gene pool, named here “paleo-Siberian” ancestry, was identified in the 14,000-year-old Ust-Kyakhta-3 genome (UKY) close to Lake Baikal<sup>7</sup> and the 9,000-year-old Kolyma genome from northeastern Siberia (Kolyma\_M)<sup>6</sup> and shows deep genetic links with Native American-related ancestry.<sup>6,7</sup> At present, it remains unclear how these diverse human lineages—present in North Asia from the Upper Paleolithic onward—have interacted with each other and how they relate to Neolithic and Bronze Age populations in the region, as well as to Native American groups. Additional genome-wide data of hunter-gatherer individuals from North Asia has thus the potential to uncover past genetic transformations that contributed to the formation of present-day Siberian populations.

## RESULTS

### Genome-wide ancient DNA data

In this study, we generate genome wide data from ten newly reported individuals from Inner Asia and northeastern Siberia, dated to as early as 7,500 BP. Genomic data from Inner Asia includes six ancient hunter-gatherer individuals from four archaeological sites in the Altai region, spanning from 7,500 to 5,500 BP (Table 1; Data S1A). Among them, one individual from the Nizhnytykesken Cave-I was dated to ~6,500 BP and was found in a site containing rich burial goods with a religious costume and objects interpreted as possible representation of shamanism (see STAR Methods). The genomic data from Northeast Asia encompass a 7,000-year-old hunter-gatherer from the Letuchaya Mysh Cave in the Russian Far East and three ~500 uncalibrated BP

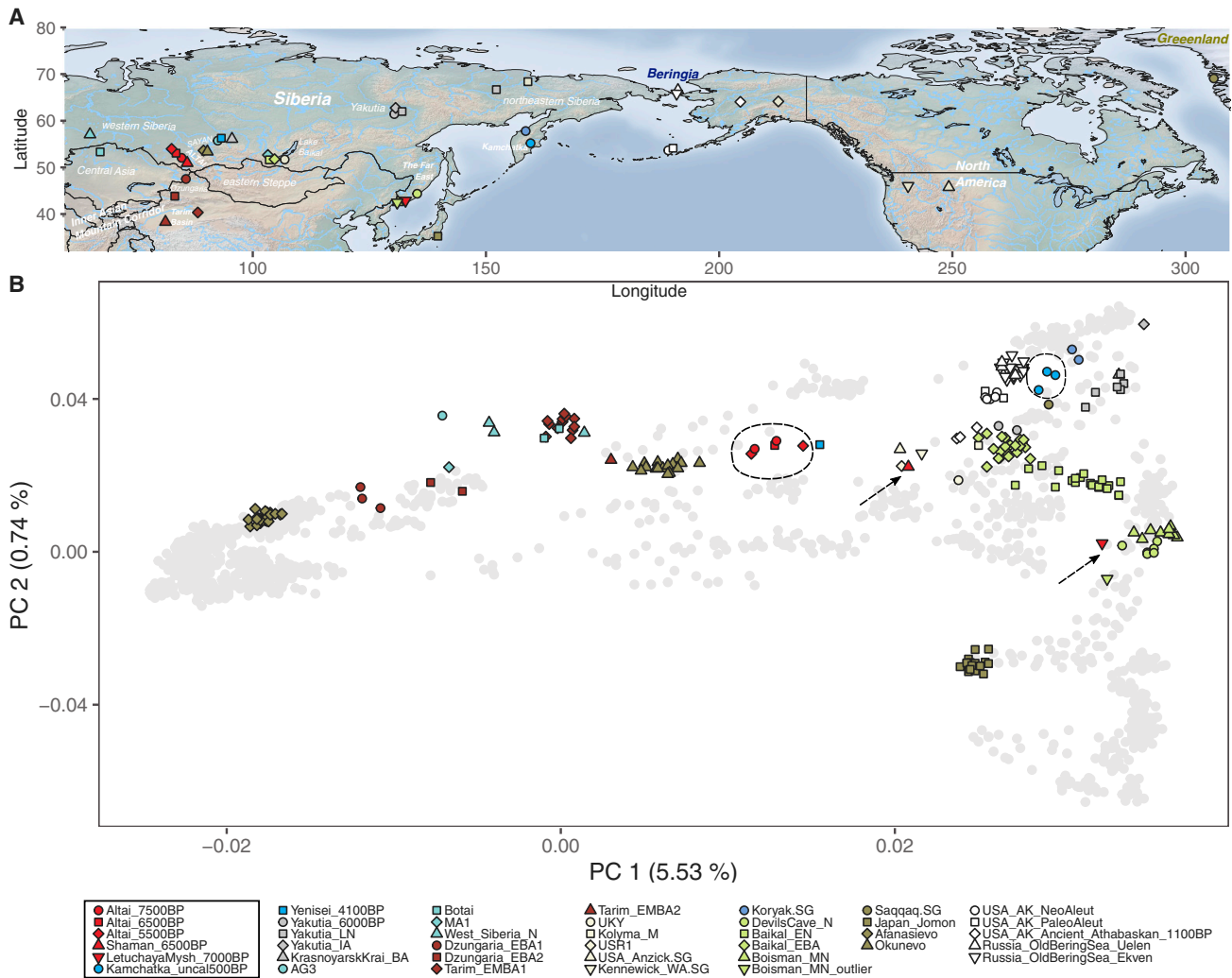
individuals from the Kamchatka Peninsula (Figure 1). We examined contamination levels in mitochondrial DNA (mtDNA) for all individuals and on the X chromosomes for nine males. The Letuchaya Mysh individual was found to carry 14% ± 1% and 40% ± 4% contamination on the mtDNA and X chromosome, respectively, and was therefore filtered to only retain DNA fragments with the typical signature of ancient DNA.<sup>8</sup> For all other data, contamination levels were found to be negligible (Table 1). We assigned mtDNA haplogroups for the newly reported individuals to various haplogroups known to be prevalent in Eurasia and in the Americas, such as mtDNA haplogroups C and G1b, which are dominant in Arctic Siberia and Kamchatka, respectively (Table 1). The Y chromosome haplogroups are instead assigned either to Q1a1 or C2b (Table 1), which are highly common in present-day northeastern Eurasians.

### Distinctive gene pool of Neolithic Altai hunter-gatherers

We carried out principal component analysis (PCA) based on modern-day Eurasian and Native American populations (Figures 1 and S1), which maximizes the west-to-east Eurasian genetic variation on a left-to-right cline of the first principal component (PC1). The eastern (right) part of this cline is composed of populations with high ANA ancestry, such as Neolithic hunter-gatherers from Devil's Gate Cave in the Russian Far East.<sup>5,6</sup> The western (left) part of this cline is composed of populations with western Eurasian ancestry, such as eastern European hunter-gatherers (EHG),<sup>9</sup> who are contemporaneous to our newly reported Neolithic Altai hunter-gatherers. Another gene pool that was largely distributed in northern Eurasia since the Upper Paleolithic, the ANE ancestry,<sup>1,2</sup> deviates from this west-to-east cline, shifting slightly upward on PC2.

In PCA space (Figure 1B), we find that five newly reported ancient Altai hunter-gatherers (labeled as “Altai\_7500BP,” “Altai\_6500BP,” and “Altai\_5500BP” based on their radiocarbon dates and genetic profile) (Table 1) fall in the middle of the west-to-east Eurasia cline, close to the slightly younger BZK002 individual (“Yenisei\_4700BP”)<sup>7</sup> from the upper Yenisei River region to the east of the Altai. In particular, the Altai hunter-gatherers neither fall close to populations with high ANA ancestry on the right nor to populations with high ANE and western Eurasian steppe ancestries on the left. In fact, the earliest record of genetic ancestry related to nomadic pastoralists from the western Eurasian steppe appears in the Altai at ~5,000 BP and is associated with the spread of the Afanasievo culture.<sup>10</sup> Conversely, contemporaneous hunter-gatherers from the Baikal region (“Baikal\_EN”)<sup>7,11</sup> and another newly reported individual from the Altai (“Nizhnytykesken\_6500BP”) are much closer to the eastern side of this cline (Figure 1B). The distinct PCA placement of the main cluster containing Altai hunter-gatherer individuals hints at a previously undescribed genetic composition for this group.

We carried out outgroup  $f_3$  statistics in the form  $f_3(\text{Altai}_7500\text{BP}/6500\text{BP}/5500\text{BP}, \text{test population}; \text{Mbuti})$  to examine which populations show the highest genetic affinity to the Altai hunter-gatherer groups throughout time. All groups present the highest genetic similarity among each other, and to Yenisei\_4700BP, as suggested from their PCA positioning (Figure 1B).<sup>7</sup> The outgroup  $f_3$  statistics also indicate high allele sharing with populations carrying elevated levels of ANE ancestry (Figure S2), such as AG3 and MA1,<sup>1,2</sup> Neolithic



**Figure 1. Geographic location and genetic profiles of newly studied individuals**

(A) Map with sampling locations of the newly reported individuals and related ancient genetic groups.

(B) Principal component analysis. Ancient individuals are shown in symbols with filled colors. This panel reports ancient individuals projected onto the PC space calculated with modern-day Eurasian individuals, shown in gray dots. Newly reported samples are highlighted by dashed circles or arrows and with a black box in the legend.

See also [Figure S1](#) and [Data S1A](#) and [S1B](#).

hunter-gatherers from western Siberia (West\_Siberia\_N, ~8,000–6,000 BP),<sup>3</sup> horse herders associated with the Botai culture from Kazakhstan (Botai, ~5,500–5,300 BP),<sup>11</sup> and Bronze Age agropastoralists from the Tarim Basin (Tarim\_EMBA, ~4,000 BP).<sup>12</sup> Hence, we use these ANE-related populations to model the western Eurasian ancestry component in the Neolithic Altai hunter-gatherers, as well as in the Bronze Age Yenisei\_4700BP individual. To complement the ANE ancestry, we first included Neolithic ANA-related populations from eastern Siberia, such as from the Lake Baikal region and the Russian Far East, as possible second ancestry components (see [STAR Methods](#)). Two-source admixture modeling performed with *qpAdm*<sup>13</sup> reveals that the proposed models using ANE- and ANA-related populations are not feasible for Altai hunter-gatherers ([Data S2A](#)). Notably, the present-day Native American

population Mixe in the *qpAdm* outgroup list ([STAR Methods](#)) shows extra genetic affinity to the Altai hunter-gatherer groups when compared to the proposed 2-source admixture models.

Hence, we investigated other available ancient individuals/populations to the east of the Altai as the possible second sources, in addition to ANE. We focused in particular on genomes that are known to be related to the ancestry present in Native American populations, such as the UKY genome from the Lake Baikal (14,000 BP),<sup>7</sup> the Kolyma\_M genome from northeastern Siberia (9,000 BP),<sup>6</sup> and the 6,000-year-old Yakutia population from central Siberia (Yakutia\_6000BP),<sup>14</sup> as well as the 13,000-year-old Anzick 1 and the 8,000-year-old Kennewick genomes from North America<sup>15,16</sup> ([STAR Methods](#); [Data S2A](#)). We find that the “paleo-Siberian” ancestry present in UKY, Kolyma\_M, and Yakutia\_6000BP outperforms the other tested ancestral

**Table 1. Summary information of the ancient individuals with newly reported genome-wide data**

Genetic label	Individual	Date (95% CI) direct date	Sex	1240k SNPs	Y chr haplogroup	mtDNA haplogroup	mtDNA contam. (%)	X chr contam. (%)
Altai_7500BP	FRS001	5,478–5,390 cal BCE	M	73,258	CT	U2e1b	1 ± 1	2.8 ± 6.1
	FRS002	5,479–5,390 cal BCE	M	653,871	Q1a1	C	2 ± 1	1.1 ± 0.3
Altai_6500BP	NVR001	4,325–4,180 cal BCE	M	428,365	Q1a1	D4j	2 ± 1	2.8 ± 0.8
Altai_5500BP	TZB001	3,487–3,359 cal BCE	M	659,140	C2b1	C4+152	2 ± 1	1.9 ± 0.4
	TZB002	3,973–3,818 cal BCE	M	281,236	C2b	R1b	3 ± 1	5.8 ± 1.5
Nizhnetytkesken_6500BP	NIZ001	4,445–4,337 cal BCE	M	732,200	C2b1a1	A	1 ± 1	0.4 ± 0.1
LetuchayaMysh_7000BP	Letuchaya Mysh.pmd	4,935–4,729 cal BCE	M	242,242	C2b	D4b1a2a	–	4.7 ± 1.8
Kamchatka_500uncalBP	KMT001	1,646 ± 68 <sup>14</sup> C years	F	258,456	–	G1b	1 ± 1	–
	KMT002	1,578 ± 52 <sup>14</sup> C years	M	372,840	Q1a1	G1b	1 ± 1	1.1 ± 0.4
	KMT003	1,118 ± 31 <sup>14</sup> C years	M	320,342	Q1a1	G1b	1 ± 1	1.1 ± 0.7

See also [Data S1A](#).

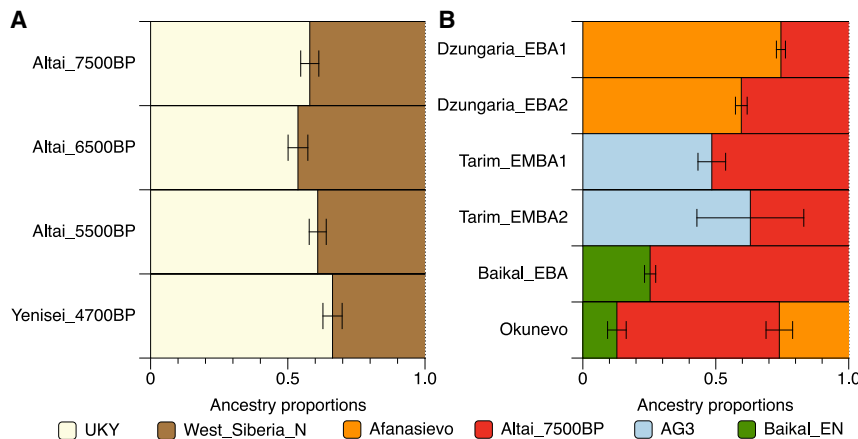
source populations ([Data S2A](#)). This is further confirmed with a 3-source competing model (i.e., using both ANA and paleo-Siberian ancestry proxies, in addition to ANE), which suggests that the Altai hunter-gatherer groups are better modeled with the paleo-Siberian ancestry carried by UKY and Kolyma\_M than with a contribution from an ANA-related population, such as DevilsCave\_N. This improved model could be explained by the previously described distinct genetic affinity of UKY and Kolyma\_M with Native American ancestry.<sup>6,7</sup> Furthermore, to enhance the resolution in ancestry modeling, we pooled together the three Altai hunter-gatherer groups (Altai\_HG, composed of Altai\_7500BP, Altai\_6500BP, and Altai\_5500BP) and found that Botai and West\_Siberia\_N provide a better fit than AG3 for the ANE component ([Data S2A](#)). The result is confirmed when adding EHG to the outgroup set ([Data S2H](#)). This suggests that Botai and West\_Siberia\_N groups from central Asia—both carrying EHG-related ancestry<sup>12</sup>—work as a more proximal source than the local Upper Palaeolithic AG3 genome for the ANE component present in the Middle Holocene Altai\_HG gene pool. The admixture modeling of Altai hunter-gatherers thus confirms their close affinity to Botai and West\_Siberia\_N shown in outgroup  $f_3$  statistics ([Figure S2](#)). Therefore, we were able to successfully model Altai\_7500BP, Altai\_6500BP, Altai\_5500BP, and the closely related Yenisei\_4700BP as a mixture of UKY to represent the paleo-Siberian ancestry component (ranging from 53.7% to 66.3%) and West\_Siberia\_N to represent the ANE ancestry component (ranging from 33.7% to 46.3%) ([Figure 2A](#); [Data S2](#)).

Such marginal but detectable differences in the amount of paleo-Siberian/ANE ancestries among the newly reported Altai hunter-gatherer time transect are consistent with pairwise  $f_4$  statistics in the form  $f_4(\text{ancient Altai group 1, ancient Altai group 2, test, Mbuti.DG})$  ([Figure S2](#)). These statistics suggest that Altai\_7500BP shares extra genetic affinity to the Anzick 1 genome compared to Altai\_6500BP ( $Z = 3.2$ ), and Altai\_5500BP shares extra genetic affinity to the Kennewick genome compared to Altai\_6500BP ( $Z = 3.239$ ). Both Anzick 1 and Kennewick are among the oldest ancient Native American individuals sequenced from North America<sup>15,16</sup> and fall in PCA space to the right of the Altai hunter-gatherers along the PC1 cline ([Figure 1B](#)). As neither Anzick 1 nor Kennewick represents a good proxy for one of the

two ancestry sources in the Altai groups ([Data S2A](#)), this extra genetic affinity to ancient Native Americans in Altai\_7500BP and Altai\_5500BP is better explained by varying proportions of the paleo-Siberian ancestry, which shares distant ties with Native Americans ([Data S2A](#)).<sup>7</sup> Moreover, we estimated the admixture time between the paleo-Siberian ancestry (UKY genome) and the ANE ancestry (ANE-related groups pooled together) in Altai\_7500BP, Altai\_6500BP, and Altai\_5500BP. This spans between ~11,000 and 7,400 BP ([Data S2E](#); [Figure S3](#)), possibly reflecting multiple and/or continuous admixture pulses in the formation process of the Altai hunter-gatherer gene pool. However, due to wide confidence intervals in the admixture dates, more data are required to assess the modes and tempos of this admixture pattern. Overall, we show that ancient Altai hunter-gatherers carry a previously unknown Siberian genetic profile that started forming in the Early Holocene as the result of a distinctive admixture between paleo-Siberian and ANE ancestries.

### Genetic legacy of Altai hunter-gatherers in neighboring and later populations

To the east of the Altai region, Early Neolithic (EN) and Early Bronze Age (EBA) Baikal hunter-gatherers also share extra genetic affinity with ANE-related groups compared to ANA-related groups located further to the east ([Figure 1](#)). Hence, we examined if the newly described ancestry of Altai hunter-gatherers might have contributed to the genetic profile of previously reported Baikal hunter-gatherers. We find that all three Altai groups can be modeled as ancestral sources for Baikal\_EN and Baikal\_EBA in 2-source admixture models using *qpAdm* ([Data S2D](#)). For Baikal\_EN, Altai\_HG contributes ~20% genetic ancestry when DevilsCave\_N is used as the second source ([Data S2D](#)). Instead, for Baikal\_EBA, the genetic contribution of Altai\_HG is ~25% when Baikal\_EN is used as the second source ([Data S2D](#)). Notably, using the software<sup>3</sup> DATES, the admixture time for Baikal\_EBA is estimated to 123 ± 30 generations before the group's age, corresponding to 8,202 ± 995 BP. This date is around 1,000 years earlier than the admixture date obtained using ANE populations as source, replacing Altai\_HG ([Figure S3](#); [Data S2D](#)). Such an admixture time for Baikal\_EBA is instead consistent with the obtained estimate for Baikal\_EN when



**Figure 2. The distinctive genetic profile of the newly reported Altai hunter-gatherer groups and related ancient populations**

(A) Ancestry modeling of Neolithic Altai hunter-gatherers and Yenisei\_4700BP.

(B) Genetic modeling of Altai\_7500BP contribution into Bronze Age individuals from the Tarim Basin, Lake Baikal, and associated with the Okunevo culture.

Horizontal bars represent  $\pm 1$  standard error.

See also [Figures S2](#) and [S3](#), [Data S1](#) and [S2](#).

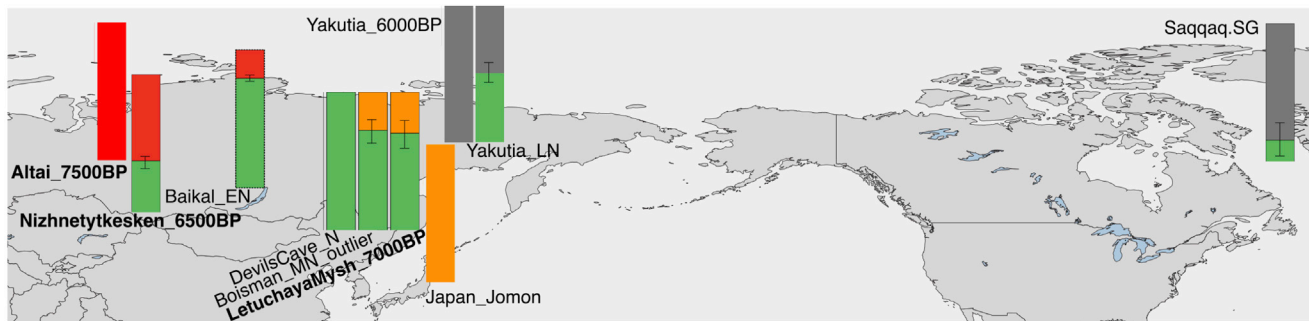
Altai\_HG and ANA ancestries are used as admixture sources ( $8,296 \pm 624$  BP) ([Figure S3](#); [Data S2D](#)). In a competitive 3-source admixture model with Altai\_HG, ANE, and Baikal\_EN as ancestry sources, the estimated proportion of ANE ancestry in Baikal\_EBA is either negative or with large standard errors overlapping with zero. Moreover, adding Altai\_HG to the *qpAdm* outgroup populations violates the previously successful models with ANA and ANE as ancestry sources. Instead, the combination of the Altai hunter-gatherer groups and ANA ancestry to model both Baikal\_EN and Baikal\_EBA fits even when Tarim\_EMBA1 is added to the outgroup list ([Data S2D](#)). Taken together, these results suggest that Altai hunter-gatherers represent a viable proxy for the ANE-related ancestry that is found in the Lake Baikal region from the early Middle Holocene and shaped the genetic profile of Baikal hunter-gatherer populations.

The newly generated genomic data from the Altai hunter-gatherers sheds new light also on the genetic origins of later populations in the central Eurasian Steppe. The Bronze Age Okunevo culture is an archaeological phenomenon developed in the Minusinsk Basin following the Afanasievo culture and dated to  $\sim 4,600$ – $3,900$  BP.<sup>17</sup> Previous studies reported Y chromosome haplogroup Q in Okunevo-associated individuals,<sup>18</sup> which is also observed in the Altai hunter-gatherers. Furthermore, their genome-wide data are modeled here as a 3-source mixture between Baikal hunter-gatherer, Botai, and Afanasievo ancestries or in a simpler 2-source mixture between Yenisei\_4700BP and Afanasievo ancestry.<sup>7</sup> Since Yenisei\_4700BP is well modeled as the mixture between Altai\_HG and Baikal\_EBA ([Data S2A](#)), which outperforms the previously reported Botai plus Baikal\_EBA model in a 3-source competing model test, we refine the genetic composition of the Okunevo-associated group using Altai\_HG as the main ancestry source, with Afanasievo and Baikal hunter-gatherers as the two additional sources ([Data S2D](#)). We find that the ancestry carried by Altai\_HG, geographically located to the south of the Minusinsk Basin, contributed to more than half ( $56.4\% \pm 6\%$ ) of the Okunevo-related gene pool ([Figure 2B](#); [Data S2D](#)). We estimate the admixture time in Okunevo between Altai\_HG and Baikal\_EBA to be  $59 \pm 22$  generations before the date of the analyzed group—i.e.,  $\sim 6,000 \pm 700$  BP ([Figure S3](#); [Data S2D](#)). The incoming Afanasievo ancestry admixed with Altai\_HG and Baikal\_EBA ancestries much more recently ( $18 \pm 3$  and  $16 \pm 4$  generations before the Okunevo's

date, respectively), overlapping in time with the presence of the Afanasievo culture in the region  $\sim 5,300$ – $4,500$  BP ([Figure S3](#); [Data S2D](#)). Therefore, we were able to trace in the Altai hunter-gatherers the main ancestry component of Okunevo-related groups and to propose that their gene pool was shaped by an admixture with ancestry related to Baikal hunter-gatherers during the 7<sup>th</sup> to 6<sup>th</sup> millennium BP, followed by a gene flow from Afanasievo-associated pastoralists during the EBA. This corroborates the archaeological record, according to which the beginning of the Okunevo culture immediately succeeded the end of the Afanasievo culture.<sup>17</sup>

Within the Altai region itself, we investigated if the Neolithic hunter-gatherer ancestry was preserved in the previously reported Middle-Late Bronze Age groups ("Altai\_MLBA"). The gene pool of Altai\_MLBA has been described as deriving from two temporally proximal sources: Baikal\_EBA and a Middle-Late Bronze Age population from the western Eurasian steppe (Sintashta\_MLBA).<sup>4</sup> We cannot find any 2-source admixture model that explains Altai\_MLBA using Altai\_HG as a source. However, using a 3-source admixture, we were able to successfully model Altai\_MLBA as Baikal\_EN plus Altai\_HG (estimated here to  $27.2\% \pm 4.1\%$ ) and Sintashta\_MLBA ([Data S2D](#)). This suggests that at least two additional gene flows are needed to shape the genetic profile of Bronze Age populations in the Altai, besides the local hunter-gatherer ancestry contribution. We could also use the same 3-source admixture model for Khövsgol\_LBA, a Late Bronze Age group from the eastern Eurasian steppe<sup>19</sup> ([Data S2D](#)), which was previously described as the combination of western Eurasian steppe and EBA Baikal ancestries.<sup>4,19</sup>

The Altai region also represents a geographic corridor connecting the Minusinsk Basin to the northeast with the Dzungarian and Tarim Basins in Inner Asia to the south. A recent study reported an isolated ANE-related genetic profile in Tarim\_EMBA individuals ( $\sim 4,100$ – $3,700$  BP) and modeled the EBA Dzungarian individuals ( $\sim 5,000$ – $4,800$  BP) as a mixture of this local genetic profile, in addition to Baikal\_EBA and Afanasievo-related ancestries.<sup>12</sup> We investigated if the newly described Altai hunter-gatherer gene pool could improve the previously proposed admixture model. In contrast to that 3-source proximal model with Tarim\_EMBA1 as the local source,<sup>12</sup> we find that our newly produced Altai hunter-gatherer genome-wide data allow for a simpler 2-source distal model where Dzungarian individuals are a mixture between Altai\_HG and Afanasievo-related ancestries ( $25\%$  Altai\_HG ancestry for Dzungaria\_EBA1 and  $40\%$  for



**Figure 3. Distribution of ANA ancestry across North Asia**

ANA ancestry (in green) is modeled as contributing ancestry to ancient groups spanning from the Altai to Greenland throughout North Asia and northern America. Here, we show the admixture modeling for Nizhnetytkesken\_6500BP, Baikal\_EN, Boisman\_MN\_outlier, LetuchayaMysh\_7000BP, Yakutia\_LN, and Saqqaq along the longitude in bar charts, using Japan\_Jomon (orange), Altai\_7500BP (red), Yakutia\_6000BP (gray), and DevilsCave\_N (green) as sources. The labels of newly reported individuals are written in bold. Horizontal bars represent  $\pm 1$  standard error.

See also [Figure S4](#), [Data S1](#) and [S2](#).

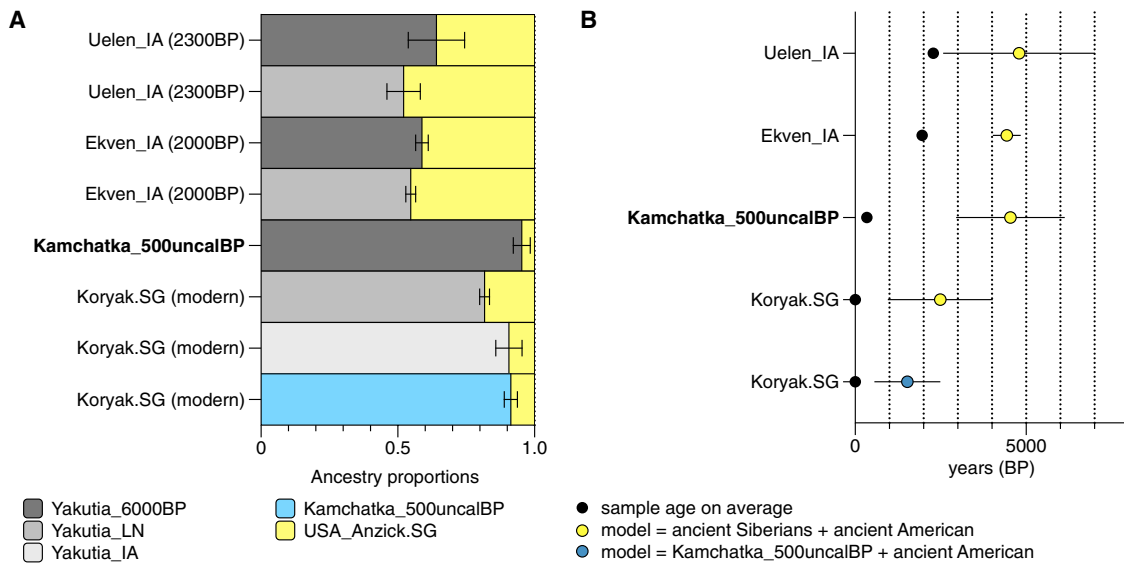
Dzungaria\_EBA2) ([Data S2D](#)). DATES estimates that this 2-source admixture occurred around  $158 \pm 43$  years before the age of Dzungaria\_EBA1 and within a few generations ( $1 \pm 3$ ) before Dzungaria\_EBA2 ([Data S2D](#)). We also tested the robustness of these 2-source admixture models by adding Tarim\_EMBA1 to the set of outgroups but found that any combination of Altai hunter-gatherer groups with Afanasievo are rejected due to the close genetic affinity between Tarim\_EMBA1 and Dzungaria\_EBA1. However, we also find that, for Tarim\_EMBA1, the 2-source admixture models between AG3 and either UKY, Kolyma\_M, or Baikal\_EBA are rejected when Altai\_7500BP is added to the outgroup set ([Data S2D](#)). This is consistent with the high shared genetic affinity between Altai hunter-gatherers and Tarim\_EMBA1 seen in outgroup  $f_3$  statistics ([Figure S2](#)). On the other hand, we are also able to describe Altai\_7500BP and Altai\_6500BP as 2-source mixtures of Tarim\_EMBA1 and UKY or Kolyma\_M ([Data S2H](#)). These models are confirmed even when Botai, West\_Siberia\_N, or EHG are added to the outgroups list, implying that Tarim\_EMBA1 might be an alternative ANE-related proximal source for Altai hunter-gatherers. However, Tarim\_EMBA1 could also be successfully modeled in a reverse way—that is, as a mixture in almost equal parts of AG3 and Altai\_HG ancestry, which statistically fits even when Baikal\_EBA is added to the outgroup set ([Data S2D](#)). We also show that Altai hunter-gatherers carry runs of homozygosity segments ([Figure S5](#)) and low pairwise mismatch rates ([Data S2L](#)), suggesting a small effective population size but not to the extent of the population bottleneck previously reported for the Tarim\_EMBA1 individuals.<sup>12</sup> Overall, we show that the gene pool of Altai hunter-gatherers is a suitable ancestry proxy for Bronze Age Inner Asian groups and that such ancestry was preserved in substantial amounts over large spatial and temporal distances. Additional genetic as well as archaeological investigations have the potential to clarify the directionality of the described admixture patterns.

### Widespread ANA ancestry from the Far East to the Altai

Contrary to the main Altai\_HG cluster, another newly reported Altai hunter-gatherer individual, found in the

Nizhnetytkesken Cave-I and dated to 6,500 BP, is shifted toward the eastern part of the west-to-east Eurasian cline ([Figure 1B](#)). This is confirmed in pairwise  $f_4$ -statistics in the form  $f_4(\text{Nizhnetytkesken}_6500\text{BP}, \text{Altai}_7500\text{BP}/\text{Altai}_6500\text{BP}/\text{Altai}_5500\text{BP}; \text{test population, Mbuti})$  where Nizhnetytkesken\_6500BP shows increased genetic affinity than the other Altai hunter-gatherer groups to ancient populations carrying high proportions of ANA-related ancestry ([Figure S4](#)). Using Altai\_7500BP as the local ancestry source, Nizhnetytkesken\_6500BP can be modeled with a substantial ANA-related contribution, estimated to be  $47.7\% \pm 5.2\%$  using Baikal\_EN or  $37.4\% \pm 4.3\%$  using DevilsCave\_N ([Data S2B](#)). This admixture event is dated to  $52 \pm 11$  generations before the age of Nizhnetytkesken\_6500BP, corresponding to  $\sim 8,000 \pm 300$  BP ([Data S2B](#)). As mentioned before, the westernmost presence of ANA ancestry was reported in the Baikal region and in Central Mongolia.<sup>4,7</sup> This newly sequenced individual—archeologically distinct from the other contemporaneous hunter-gatherers from the same region—reveals the existence of ANA ancestry as far west as the Altai by at least 6,500 years ago.

In this study, we also report genomic data from a Russian Far East individual. As shown in PCA and outgroup- $f_3$  statistics, the 7,000-year-old Letuchaya Mysh individual shows the highest genetic similarities with previously published ANA-related populations from the Far East, such as DevilsCave\_N dated to 6,700 BP<sup>6</sup> and Boisman\_MN dated to 6,300 BP ([Figures 3](#) and [S4](#)).<sup>20</sup> Intriguingly, we find that LetuchayaMysh\_7000BP is not symmetrically related to DevilsCave\_N. Instead, this individual shows extra affinity with hunter-gatherers associated with the Jomon culture from Japan, dated to as early as 9,000 BP ([Figure S4](#)).<sup>21,22</sup> A *qpAdm* analysis confirms that LetuchayaMysh\_7000BP can be modeled as deriving  $29.7\% \pm 9.8\%$  of its genome from Jomon-related ancestry ([Figure 3](#); [Data S2B](#)). Interestingly, we observe a very similar admixture proportion in a single individual from the Middle Neolithic Boisman group (labeled as “Boisman\_MN\_outlier” here) ([Figure 3](#); [Data S2B](#) and [S1B](#)).<sup>20</sup> These findings suggest the existence of previously unappreciated genetic links between hunter-gatherer groups in the Japanese Archipelago and the Russian Far East as early as 7,000 BP.



**Figure 4. Multiple waves of Native American-related ancestry into North Asia**

(A) Ancestry modeling of Kamchatka\_500uncalBP, as well as related ancient and present-day northeastern Asian populations.

(B) Genetic dating of the Native American-related gene flow into ancient and present-day populations reported in (A).

Horizontal bars represent  $\pm 1$  standard error.

See also [Data S1](#) and [S2](#).

### Multiple gene-flow events across the Bering Sea

The paleo-Siberian ancestry was spread over vast areas of Siberia, spanning from the Altai (as shown here in the Neolithic Altai hunter-gatherers) through Yakutia to northeastern Siberia.<sup>6,14</sup> Previous studies revealed that the northeastern Siberia Kolyma\_M individual and the Yakutia\_6000BP population share close genetic ties with modern-day Chukotko-Kamchatkan speakers from the Kamchatka peninsula and with Native Americans.<sup>6,14</sup> In this study, we report genome-wide data of three individuals from the Kamchatka Peninsula dated to 500 uncalibrated years BP (STAR Methods). In outgroup  $f_3$ -statistics, this group presents high genetic affinity with modern-day Kamchatkan populations (such as Koryak and Itelmen), as well as with Kolyma\_M and the 4,000-year-old Saqqaq individual from Greenland (Figure S4). Therefore, we analyzed the Kamchatka\_500uncalBP group, together with ancient populations from Yakutia (Yakutia\_6000BP, Yakutia\_LN, and Yakutia\_IA), the Siberian coast of the Bering Sea (Ekven\_IA and Uelen\_IA), and Greenland (Saqqaq.SG), to investigate past population dynamics in northeastern Siberia and Beringia.

Populations in northern Siberia went through remarkable genetic transitions between 6,000 and 2,000 years ago,<sup>14</sup> as indicated by a PCA cline formed by Yakutia\_6000BP, Yakutia\_LN (~4,100–4,800 BP), and Yakutia\_IA (~2,500 BP) shifting over time toward modern-day northeastern Siberian populations like Even and Evenk (Figure 1B).<sup>14</sup> Our re-analysis of previously published genomes shows that the genetic transition in Yakutia between 6,000 BP (Yakutia\_6000BP) and 4,000 BP (Yakutia\_LN) is associated with nearly 50% increase in ANA ancestry using DevilsCave\_N as proxy, while the transition from 4,000 to 2,500 years ago (Yakutia\_IA) is possibly mediated by admixture with northeastern Siberian ancestry maximized in the present-day Nganasan population. The latter shift is evident in PCA space

(Figure 1B), but to be confirmed, it would require additional data from Iron Age Yakutia (besides the single low-coverage Yakutia\_IA genome) and from Arctic Siberia (i.e., where modern-day Nganasan groups live) (Data S2C). In addition, the demographic history of northeastern Siberian groups also involves backward gene flow of Native American ancestry over the Bering Sea. Using the late Neolithic Yakutia group as an ancestry baseline, we estimate a Native American-related gene flow contributing nearly 50% to the genetic ancestry of Iron Age groups located on the Bering Sea shore, such as Ekven\_IA (~2,000 BP) and Uelen\_IA (~2,300 BP) (Data S2C).<sup>6,64</sup> To model the genetic profile of Kamchatka\_500uncalBP, we thus explored a combination of ancient Native American and northeastern Siberian ancestries. Consistent with  $f_4$  statistics, the Kamchatka\_500uncalBP group can be successfully modeled in *qpAdm* as a 2-source admixture between Yakutia ancestries and various ancient Native American populations (Figure 4; Data S2C and Data S2G). When using Yakutia\_6000BP as an ancestry baseline, Kamchatka\_500uncalBP requires an additional  $8.5\% \pm 4.1\%$  Native American ancestry represented by the Kennewick genome (Figure 4; Data S2C). This genetic contribution ranges from 6.9% to 10.8% when using more proximal sources for the Native American-related ancestry, such as ancient Aleut, Athabaskan, and Old Bering Sea individuals (Data S2C).<sup>64</sup>

The newly reported Kamchatka\_500uncalBP data was finally used to investigate the genetic formation of present-day populations in the Kamchatka peninsula. We found that Koryaks carry additional Native American-related ancestry when compared to Kamchatka\_500uncalBP, corresponding to an extra  $9.8\% \pm 3.3\%$ , or  $17.7\% \pm 3.5\%$  compared to Yakutia\_6000BP (Figure 4; Data S2C). Other present-day populations from the Kamchatka and Chukotka peninsulas, such as Itelmen and



Chukchi,<sup>23</sup> also carry non-negligible amounts of Native American-related ancestry, ranging from 5% to 20% when using Kamchatka\_500uncalBP as an ancestry baseline (Data S2C). Finally, we estimated the admixture time of the Native American-related ancestry in ancient and present-day groups from northeastern Siberia. This backflow is dated to between 5,500 and 4,400 BP for the ancient populations, while the estimation is only 1,500 BP for the present-day Koryak population (Figure 4; Data S2F). Considering the additional amount of Native American ancestry in the latter population, compared to Kamchatka\_500uncalBP, this suggests continuous or repeated gene flow events from North America or Beringia into the Kamchatka Peninsula during the last few centuries.

## DISCUSSION

In this study, we report genome-wide data from Altai hunter-gatherers, dated between 7,500 BP and 5,500 BP, carrying a previously uncharacterized gene pool. This Middle Holocene Siberian ancestry reveals distinct ties with the Upper Palaeolithic UKY<sup>7</sup> and Mesolithic Kolyma<sup>6</sup> individuals from Siberia. Our analysis shows that Altai hunter-gatherers may represent a better genetic proxy than UKY for the ANE-related ancestry in later Inner Asian and Siberian populations, providing a link between Neolithic/Bronze Age Baikal populations and EBA Tarim Basin groups. The observation that Altai hunter-gatherers harbor closer genetic affinities with Tarim\_EMBA1, Botai, and West\_Siberia\_N than with AG3 suggests that such derived ANE-related ancestry was shared between Central Asian and southern Siberian groups from at least the Early Holocene onward. Admixture dating shows that the formation of the Altai hunter-gatherer gene pool started as early as 11,000 years ago (Figure S3). This temporal horizon is older than the estimated formation time not only of the EN and EBA Baikal gene pools<sup>7</sup> but also of the EBA Tarim Basin ancestry.<sup>12</sup> The latter was proposed to represent a substratum of ANE ancestry in Central Asia that genetically contributed to subsequent Middle to Late Bronze Age Xinjiang populations.<sup>12,24</sup> However, the wide confidence interval in the admixture dates of Altai hunter-gatherers, and the fact that they can be modeled with the EBA Tarim Basin genomes as one of the ancestry sources, poses a challenge on resolving the directionality of the inferred gene flow. Additional data from ANE-ancestry-related populations, paired up with archaeological investigations in the region, are essential to answer this question. Taken together, we report genetic connections between the Altai and Baikal regions, as well as with the Dzungaria and Tarim Basins of Inner Asia, from the Middle Holocene onward. The Altai hunter-gatherer genetic profile was largely preserved locally in the gene pool of EBA Okunevo-associated pastoralists, despite the shift in subsistence strategy. In the Altai, instead, the Neolithic genetic ancestry was substantially reduced by the Middle-Late Bronze Age, possibly resulting from both eastern and western population movements into the region.

We also show that the formation of the Altai hunter-gatherer genetic cluster is not related to the expansion of ANA ancestry. However, a contemporaneous individual from the same region (Nizhnytykesken\_6500BP) carries a substantial amount of ANA ancestry, ~1,500 km more to the west than

previously reported. Such a distinct genetic profile indicates that ANA ancestry arrived in the Altai region before the spread of the Afanasievo culture, possibly associated with different cultural features as observed for the Nizhnytykesken\_6500BP individual (STAR Methods). The presence of this “genetic outlier” might inform us about previously unappreciated long-distance connectivity with the east. This is also the case for the newly produced 7,000-year-old genome-wide data from the Russian Far East which, contrary to most previously sequenced Neolithic individuals from the same region, carry Jomon-related genetic ancestry. Interestingly, another “outlier” individual also dated to ~7,000 BP, from the geographically close Boisman site, harbors a very similar genetic composition, revealing unexpected genetic links between hunter-gatherer groups from the Japanese Archipelago and the Russian Far East.

In conclusion, our examination of the newly generated genomic data, in combination with previously reported ancient individuals spanning from Central Asia to North America, uncovered complex population movements of hunter-gatherer groups across North Asia from the Early Holocene onward. Finally, we revealed that the gene pool of present-day Kamchatkan populations was shaped by a prolonged period of Native American-related gene flow over multiple millennia.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2022.11.062>.

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#### AUTHOR CONTRIBUTIONS

C.P., K.W., and J.K. designed and supervised the research. Y.F.K., A.A.T., Y.V.F., N.F.S., K.Y.K., A.L.K., S.S., S.S.T., M.P.T., A.V.Z., M.P., T.K., and A.B. provided skeletal material and archaeological contexts. R.R. and V.S. performed genetic laboratory work. K.W., H.Y., V.S., C.J., and C.P. performed genetic data analysis. K.W., H.Y., C.J., J.K., and C.P. wrote the paper with contributions from all co-authors.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

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#### REFERENCES

1. Fu, Q., Posth, C., Hajdinjak, M., Petr, M., Mallick, S., Fernandes, D., Furtwängler, A., Haak, W., Meyer, M., Mittnik, A., et al. (2016). The genetic history of Ice age Europe. *Nature* 534, 200–205.
2. Raghavan, M., Skoglund, P., Graf, K.E., Metspalu, M., Albrechtsen, A., Moltke, I., Rasmussen, S., Stafford, T.W., Jr., Orlando, L., Metspalu, E., et al. (2014). Upper Palaeolithic Siberian genome reveals dual ancestry of Native Americans. *Nature* 505, 87–91.
3. Narasimhan, V.M., Patterson, N., Moorjani, P., Rohland, N., Bernardos, R., Mallick, S., Lazaridis, I., Nakatsuka, N., Olalde, I., Lipson, M., et al. (2019). The formation of human populations in South and Central Asia. *Science* 365, eaat7487.
4. Jeong, C., Wang, K., Wilkin, S., Taylor, W.T.T., Miller, B.K., Bemmann, J.H., Stahl, R., Chiavelli, C., Knolle, F., Ulziibayar, S., et al. (2020). A Dynamic 6, 000-Year genetic History of Eurasia's Eastern Steppe. *Cell* 183, 890–904.e29.
5. Siska, V., Jones, E.R., Jeon, S., Bhak, Y., Kim, H.-M., Cho, Y.S., Kim, H., Lee, K., Veselovskaya, E., Balueva, T., et al. (2017). Genome-wide data from two early Neolithic East Asian individuals dating to 7700 years ago. *Sci. Adv.* 3, e1601877.
6. Sikora, M., Pitulko, V.V., Sousa, V.C., Allentoft, M.E., Vinner, L., Rasmussen, S., Margaryan, A., de Barros Damgaard, P., de la Fuente, C., Renaud, G., et al. (2019). The population history of northeastern Siberia since the Pleistocene. *Nature* 570, 182–188. <https://doi.org/10.1038/s41586-019-1279-z>.
7. Yu, H., Spyrou, M.A., Karapetian, M., Shnaider, S., Radzevičiūtė, R., Nägele, K., Neumann, G.U., Penske, S., Zech, J., Lucas, M., et al. (2020). Paleolithic to Bronze Age Siberians reveal connections with First Americans and across Eurasia. *Cell* 181, 1232–1245.e20.
8. Skoglund, P., Northoff, B.H., Shunkov, M.V., Derevianko, A.P., Pääbo, S., Krause, J., and Jakobsson, M. (2014). Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. *Proc. Natl. Acad. Sci. USA* 111, 2229–2234.
9. Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S.A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., et al. (2015). Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* 528, 499–503.
10. Allentoft, M.E., Sikora, M., Sjögren, K.G., Rasmussen, S., Rasmussen, M., Stenderup, J., Damgaard, P.B., Schroeder, H., Ahlström, T., Vinner, L., et al. (2015). Population genomics of Bronze Age Eurasia. *Nature* 522, 167–172.
11. de Barros Damgaard, P., Martiniano, R., Kamm, J., Moreno-Mayar, J.V., Kroonen, G., Peyrot, M., Barjamovic, G., Rasmussen, S., Zacho, C., Baimukhanov, N., et al. (2018). The first horse herders and the impact of early Bronze Age steppe expansions into Asia. *Science* 360, eaar7711. <https://doi.org/10.1126/science.aar7711>.
12. Zhang, F., Ning, C., Scott, A., Fu, Q., Björn, R., Li, W., Wei, D., Wang, W., Fan, L., Abuduresule, I., et al. (2021). The genomic origins of the Bronze Age Tarim Basin mummies. *Nature* 599, 256–261.
13. Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., and Reich, D. (2012). Ancient admixture in human history. *Genetics* 192, 1065–1093.
14. Kılınc, G.M., Kashuba, N., Koptekin, D., Bergfeldt, N., Dönertaş, H.M., Rodríguez-Varela, R., Shergin, D., Ivanov, G., Kichigin, D., Pestereva, K., et al. (2021). Human population dynamics and *Yersinia pestis* in ancient northeast Asia. *Sci. Adv.* 7, eabc4587. <https://doi.org/10.1126/sciadv.abc4587>.
15. Rasmussen, M., Sikora, M., Albrechtsen, A., Korneliusen, T.S., Moreno-Mayar, J.V., Poznik, G.D., Zollikofer, C.P.E., de León, M.P., Allentoft, M.E., Moltke, I., et al. (2015). The ancestry and affiliations of Kennewick Man. *Nature* 523, 455–458.
16. Rasmussen, M., Anzick, S.L., Waters, M.R., Skoglund, P., DeGiorgio, M., Stafford, T.W., Jr., Rasmussen, S., Moltke, I., Albrechtsen, A., Doyle, S.M., et al. (2014). The genome of a Late Pleistocene human from a Clovis burial site in western Montana. *Nature* 506, 225–229.
17. Svyatko, S.V., Mallory, J.P., Murphy, E.M., Polyakov, A.V., Reimer, P.J., and Schulting, R.J. (2009). New radiocarbon dates and a review of the chronology of prehistoric populations from the minusinsk basin, Southern Siberia, Russia. *Radiocarbon* 51, 243–273.
18. Hollard, C., Zvenigorosky, V., Kovalev, A., Kiryushin, Y., Tishkin, A., Lazaretov, I., Crubézy, E., Ludes, B., and Keyser, C. (2018). New genetic evidence of affinities and discontinuities between bronze age Siberian populations. *Am. J. Phys. Anthropol.* 167, 97–107. <https://doi.org/10.1002/ajpa.23607>.
19. Jeong, C., Wilkin, S., Amgalantugs, T., Bouwman, A.S., Taylor, W.T.T., Hagan, R.W., Bromage, S., Tsolmon, S., Trachsel, C., Grossmann, J., et al. (2018). Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe. *Proc. Natl. Acad. Sci. USA* 115, E11248–E11255.
20. Wang, C.-C., Yeh, H.-Y., Popov, A.N., Zhang, H.-Q., Matsumura, H., Sirak, K., Cheronet, O., Kovalev, A., Rohland, N., Kim, A.M., et al. (2021). Genomic insights into the formation of human populations in East Asia. *Nature* 591, 413–419. <https://doi.org/10.1038/s41586-021-03336-2>.
21. Cooke, N.P., Mattiangeli, V., Cassidy, L.M., Okazaki, K., Stokes, C.A., Onbe, S., Hatakeyama, S., Machida, K., Kasai, K., Tomioka, N., et al. (2021). Ancient genomics reveals tripartite origins of Japanese populations. *Sci. Adv.* 7, eabh2419.
22. McColl, H., Racimo, F., Vinner, L., Demeter, F., Gakuhari, T., Moreno-Mayar, J.V., van Driem, G., Gram Wilken, U., Seguin-Orlando, A., de la Fuente Castro, C., et al. (2018). The prehistoric peopling of Southeast Asia. *Science* 361, 88–92.
23. Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P.H., Schraiber, J.G., Castellano, S., Lipson, M., et al. (2014). Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 513, 409–413.
24. Kumar, V., Wang, W., Zhang, J., Wang, Y., Ruan, Q., Yu, J., Wu, X., Hu, X., Wu, X., Guo, W., et al. (2022). Bronze and Iron Age

- population movements underlie Xinjiang population history. *Science* 376, 62–69.
25. Motuzaitė Matuzevičiūtė, G., Kiryushin, Y.F., Rakhimzhanova, S.Z., Svyatko, S., Tishkin, A.A., and O’Connell, T.C. (2016). Climatic or dietary change? Stable isotope analysis of Neolithic–Bronze Age populations from the Upper Ob and Tobol River basins. *Holocene* 26, 1711–1721.
  26. Kiryushin, K.Y., Kiryushin, Y.F., Solodovnikov, K.N., Frolov, Y.V., and Schmidt, A.V. (2021). Results of radiocarbon dating of early burials in the Firsovo archaeological area, Barnaul stretch of the Ob. *Arheol. étnogr. antropol. Evrazii* 49, 24–31.
  27. Kiryushin, K.Y., Volkov, P.V., Pugachev, D.A., and Semibratov, V.P. (2006). The Novoaltaysk-Razvilka- Eneolithic cemetery in Barnaul Priob’ye // Preservation and study of the cultural heritage of Altai territory. *Barnaul: Azbuka XV*, 222–228.
  28. Kiryushin, Y., Kiryushin, K., Schmidt, A.V., and Abduganayev, M. (2012). Teeth of animals in burials of the Tuzovskie Bugry-1 Graveyard as an indicator of ethno-cultural processes on the territory of Altai in the 3rd millennium BC. *Archaeol. Ethnol. Anthropol. Eurasia* 40, 59–66.
  29. Kiryushin, Y., Kiryushin, K., Schmidt, A.V., Kuzmenkin, D.V., and Abduganayev, M. (2011). Mollusc shells in burials of the Tuzovskie Bugry-1 cemetery as an indicator of ethno-cultural processes on the territory of South Siberia and Central Asia. *Archaeol. Ethnol. Anthropol. Eurasia* 39, 37–45.
  30. Abduganayev, M.T., Kiryushin, Y.F., Pugachev, D.A., and Schmidt, A.V. (2000). Preliminary Results Of Researches of Tuzovskie Bugry I Burial Ground // Problems Of Archaeology, Ethnography, Anthropology Of Siberia And Adjacent Territories. VI. (Institute of Archaeology and Ethnography, Siberian Branch of the Russian Academy of Sciences), pp. 206–210.
  31. Kim, A.R., and Chikisheva, T.A. (1995). Pogrebenie iz Nizhnetytkeskenskoi peschery I – pervaya doafanasevskaya mogila na territorii Gornogo Altaya. In *Arkheologiya Nizhnetytkeskenskoi peschery I* (Izd), pp. 95–117.
  32. Chikisheva, T.A. (2000). New anthropological data on Neolithic and Bronze Ages populations of the Altai. *Archaeol. Ethnol. Anthropol. Eurasia* 1, 139–148.
  33. Kiryushin, Y.F., Kungurov, A.L., and Stepanova, N.F. (1995). *Arkheologiya Nizhnetytkeskenskoi peschery-1* (Altai Gos. Univ.).
  34. Smith, B.H. (1984). Patterns of Molar Wear in Hunter-Gatherers and Agriculturalists. *Am. J. Phys. Anthropol.* 63, 39–56.
  35. Edgar, H. (2017). *Dental Morphology for Anthropology. An illustrated Manual* (Routledge).
  36. Scott, G.R., and Turner, C.G. (1997). *The anthropology of modern human teeth: dental morphology and its variation in recent human populations* (Cambridge University Press).
  37. Popov, A.N., Chikisheva, T.A., and Shpakova, E.G. (1997). Boismanskaya archeological culture of the Southern Primorye (based on materials of the multi-layer site of Boimana 2) (Institute of Archaeology and Ethnography SB RAS).
  38. Zubova, A. (2018). Neolithic population of the Southern Primorye and its affinities with the indigenous population of the Far East (based on dental non-metric traits from the Boysman-2 burial ground sample). *Camera Praehistorica* 1, 117–128.
  39. Reimer, P.J., Austin, W.E.N., Bard, E., Bayliss, A., Blackwell, P.G., Bronk Ramsey, C., Butzin, M., Cheng, H., Edwards, R.L., Friedrich, M., et al. (2020). The IntCal20 Northern hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* 62, 725–757.
  40. Rohland, N., Glocke, I., Aximu-Petri, A., and Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat. Protoc.* 13, 2447–2461.
  41. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., and Reich, D. (2015). Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20130624.
  42. Gansauge, M.-T., Aximu-Petri, A., Nagel, S., and Meyer, M. (2020). Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* 15, 2279–2300. <https://doi.org/10.1038/s41596-020-0338-0>.
  43. Fu, Q., Hajdinjak, M., Moldovan, O.T., Constantin, S., Mallick, S., Skoglund, P., Patterson, N., Rohland, N., Lazaridis, I., Nickel, B., et al. (2015). An early modern human from Romania with a recent Neanderthal ancestor. *Nature* 524, 216–219.
  44. Fu, Q., Meyer, M., Gao, X., Stenzel, U., Burbano, H.A., Kelso, J., and Pääbo, S. (2013). DNA analysis of an early modern human from Tianyuan Cave, China. *Proc. Natl. Acad. Sci. USA* 110, 2223–2227.
  45. Peltzer, A., Jäger, G., Herbig, A., Seitz, A., Kniep, C., Krause, J., and Nieselt, K. (2016). EAGER: efficient ancient genome reconstruction. *Genome Biol.* 17, 60.
  46. Schubert, M., Lindgreen, S., and Orlando, L. (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Res. Notes* 9, 88.
  47. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760.
  48. Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., and Orlando, L. (2013). mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* 29, 1682–1684.
  49. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079.
  50. Renaud, G., Slon, V., Duggan, A.T., and Kelso, J. (2015). Schmutzi: estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA. *Genome Biol.* 16, 224.
  51. Korneliusson, T.S., Albrechtsen, A., and Nielsen, R. (2014). ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinf.* 15, 356.
  52. Poznik, G.D. (2016). Identifying Y-chromosome haplogroups in arbitrarily large samples of sequenced or genotyped men. Preprint at bioRxiv. <https://doi.org/10.1101/088716>.
  53. Weissensteiner, H., Pacher, D., Kloss-Brandstätter, A., Forer, L., Specht, G., Bandelt, H.-J., Kronenberg, F., Salas, A., and Schönherr, S. (2016). HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* 44, W58–63.
  54. Moreno-Mayar, J.V., Potter, B.A., Vinner, L., Steinrücken, M., Rasmussen, S., Terhorst, J., Kamm, J.A., Albrechtsen, A., Malaspina, A.-S., Sikora, M., et al. (2018). Terminal Pleistocene Alaskan genome reveals first founding population of Native Americans. *Nature* 553, 203–207.
  55. Raghavan, M., Steinrücken, M., Harris, K., Schiffels, S., Rasmussen, S., DeGiorgio, M., Albrechtsen, A., Valdiosera, C., Ávila-Arcos, M.C., Malaspina, A.-S., et al. (2015). Population genetics. Genomic evidence for the Pleistocene and recent population history of Native Americans. *Science* 349, aab3884.
  56. Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D.C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K., et al. (2016). Genomic insights into the origin of farming in the ancient Near East. *Nature* 536, 419–424.
  57. Yang, M.A., Gao, X., Theunert, C., Tong, H., Aximu-Petri, A., Nickel, B., Slatkin, M., Meyer, M., Pääbo, S., Kelso, J., and Fu, Q. (2017). 40, 000-Year-Old Individual from Asia Provides Insight into Early Population Structure in Eurasia. *Curr. Biol.* 27, 3202–3208.e9.
  58. Fu, Q., Li, H., Moorjani, P., Jay, F., Slepchenko, S.M., Bondarev, A.A., Johnson, P.L.F., Aximu-Petri, A., Prüfer, K., de Filippo, C., et al. (2014). Genome sequence of a 45, 000-year-old modern human from western Siberia. *Nature* 514, 445–449.

59. Haak, W., Lazaridis, I., Patterson, N., Rohland, N., Mallick, S., Llamas, B., Brandt, G., Nordenfelt, S., Harney, E., Stewardson, K., et al. (2015). Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* 522, 207–211.
60. Jeong, C., Balanovsky, O., Lukianova, E., Kahbatkyzy, N., Flegontov, P., Zaporozhchenko, V., Immel, A., Wang, C.-C., Ixan, O., Khussainova, E., et al. (2019). The genetic history of admixture across inner Eurasia. *Nat. Ecol. Evol.* 3, 966–976.
61. Mallick, S., Li, H., Lipson, M., Mathieson, I., Gymrek, M., Racimo, F., Zhao, M., Chennagiri, N., Nordenfelt, S., Tandon, A., et al. (2016). The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* 538, 201–206.
62. Patterson, N., Price, A.L., and Reich, D. (2006). Population structure and eigenanalysis. *PLoS Genet.* 2, e190.
63. Ringbauer, H., Novembre, J., and Steinrücken, M. (2021). Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* 12, 5425.
64. Flegontov, P., Altınışık, N.E., Changmai, P., Rohland, N., Mallick, S., Adamski, N., et al. (2019). Palaeo-Eskimo genetic ancestry and the peopling of Chukotka and North America. *Nature* 570, 236–240.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Biological samples</b>		
Human archaeological skeletal material	This study	FRS001
Human archaeological skeletal material	This study	FRS002
Human archaeological skeletal material	This study	NVR001
Human archaeological skeletal material	This study	TZB001
Human archaeological skeletal material	This study	TZB002
Human archaeological skeletal material	This study	NIZ001
Human archaeological skeletal material	This study	LetuchayaMysh
Human archaeological skeletal material	This study	KMT001
Human archaeological skeletal material	This study	KMT002
Human archaeological skeletal material	This study	KMT003
<b>Chemicals peptides and recombinant proteins</b>		
USER enzyme	New England Biolabs	Cat# M5505
Uracil Glycosylase inhibitor UGI	New England Biolabs	Cat# M0281
1x Tris-EDTA pH 8.0	AppliChem	Cat# A85690500
0.5 M EDTA pH 8.0	Life Technologies	Cat# AM9261
10x Buffer Tango	Life Technologies	Cat# BY5
Isopropanol	Merck	Cat# 1070222511
Ethanol	Merck	Cat# 1009832511
Proteinase K	Sigma Aldrich	Cat# P2308
Guanidine hydrochloride	Sigma Aldrich	Cat# G3272
3M Sodium Acetate pH 5.2	Sigma Aldrich	Cat# S7899
Tween-20	Sigma Aldrich	Cat# P9416
5M NaCl	Sigma Aldrich	Cat# S5150
ATP 100 mM	Thermo Fisher Scientific	Cat# R0441
1 M Tris-HCl pH 8.0	Thermo Fisher Scientific	Cat# 15568025
dNTP Mix	Thermo Fisher Scientific	Cat# R1121
Bst DNA Polymerase2.0 large frag.	New England Biolabs	Cat# M0537
BSA 20mg/mL	New England Biolabs	Cat# B9000
T4 Polynucleotide Kinase	New England Biolabs	Cat# M0201
T4 DNA Polymerase	New England Biolabs	Cat# M0203
20% SDS	Serva	Cat# 39575.01
Dynabeads MyOne Streptavidin T1	Thermo Fisher Scientific	Cat# 65602
<b>Critical commercial assays</b>		
High Pure Viral Nucleic Acid Large Volume Kit	Roche	Cat# 5114403001
HiSeq 4000 SBS Kit 50/75 cycles	Illumina	Cat# FC-410-1001/2
DyNAmo Flash SYBR Green qPCR Kit	Thermo Fisher Scientific	Cat# F415L
MinElute PCR Purification Kit	QIAGEN	Cat# 28006
Quick Ligation Kit	New England Biolabs	Cat# M2200L
Oligo aCGH/Chip-on-Chip Hybridization Kit	Agilent Technologies	Cat# 5188-5220
<b>Deposited data</b>		
Raw and analyzed data	This study	ENA: PRJEB55777
<b>Software and algorithms</b>		
EAGER v1.92.55	Peltzer et al. <sup>25</sup>	<a href="https://github.com/apeltzer/EAGER-GUI">https://github.com/apeltzer/EAGER-GUI</a>
AdapterRemoval v2.2.0	Schubert et al. <sup>26</sup>	<a href="https://github.com/MikkelSchubert/adaptremoval">https://github.com/MikkelSchubert/adaptremoval</a>

(Continued on next page)

**Continued**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
BWA v0.7.12	Li and Durbin <sup>27</sup>	<a href="http://bio-bwa.sourceforge.net">http://bio-bwa.sourceforge.net</a>
dedup v0.12.1	Peltzer et al. <sup>25</sup>	<a href="https://github.com/apeltzer/DeDup">https://github.com/apeltzer/DeDup</a>
samtools v1.3	Li and Durbin <sup>28</sup>	<a href="http://www.htslib.org/doc/samtools.html">http://www.htslib.org/doc/samtools.html</a>
HaploGrep 2 v2.1.19	Weissensteiner et al. <sup>29</sup>	<a href="https://haplogrep.imed.ac.at/category/haplogrep2/">https://haplogrep.imed.ac.at/category/haplogrep2/</a>
yHaplo	Poznik <sup>30</sup>	<a href="https://github.com/alexhbnr/yhaplo">https://github.com/alexhbnr/yhaplo</a>
Eigensoft v7.2.1	Patterson et al. <sup>31</sup>	<a href="https://github.com/DReichLab/EIG">https://github.com/DReichLab/EIG</a>
DATES v753 <sup>11</sup>	Narasimhan et al. <sup>11</sup>	<a href="https://github.com/priyamoorejani/DATES">https://github.com/priyamoorejani/DATES</a>
admixture v5.1	Patterson et al. <sup>13</sup>	<a href="https://github.com/DReichLab/AdmixTools">https://github.com/DReichLab/AdmixTools</a>
Schmutzi	Renaud et al. <sup>32</sup>	<a href="https://bioinf.eva.mpg.de/schmutzi">https://bioinf.eva.mpg.de/schmutzi</a>
ANGSD v0.910	Korneliussen et al. <sup>33</sup>	<a href="http://www.popgen.dk/angsd/index.php/ANGSD">http://www.popgen.dk/angsd/index.php/ANGSD</a>
mapDamage v2.0.6	Jonsson et al. <sup>34</sup>	<a href="https://github.com/ginolhac/mapDamage">https://github.com/ginolhac/mapDamage</a>
hapROH	Ringbauer et al. <sup>35</sup>	<a href="https://pypi.org/project/hapROH/">https://pypi.org/project/hapROH/</a>

**RESOURCE AVAILABILITY**

**Lead contact**

Further information and requests for resources should be directed to and will be fulfilled by the lead contact: Cosimo Posth ([cosimo.posth@uni-tuebingen.de](mailto:cosimo.posth@uni-tuebingen.de)).

**Materials availability**

This study did not generate new reagents.

**Data and code availability**

The accession number for all newly reported sequencing data reported in this paper are available from the European Nucleotide Archive: PRJEB55777.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Archaeological information**

**Sample provenience**

In this study, we collected skeletal elements from ten ancient individuals that were housed at the following institutions: the Altai State University and the Altai Museum of Archaeology and Ethnography, the Federal Science Center of East Asian Terrestrial Biodiversity and the Research Institute and Museum of Anthropology in Moscow. All specimens were sampled with the approval of the appropriate institutions for the handling of archaeological samples and/or in collaboration with local scientists and curators listed among the authors of this study. Together, we newly generated genome-wide data for these ten individuals from the Altai, the Russian Far East and Kamchatka Peninsula.

**Samples from the Altai region**

**Cemetery Firsovo-XI (“Altai\_7500BP”).**

- FAS001 (Burial 14, skeleton 2): 5512-5375 cal BCE (uncalibrated 6490 ± 26 BP, MAMS-48705)
- FAS002 (Burial 9): 5521-5378 cal BCE (uncalibrated 6500 ± 24 BP, MAMS-48706)

The site is located on the right bank of the Ob River (opposite Barnaul city; Altai Territory, Russia). Firsovo-XI was first discovered in 1975, then excavated in 1993 and 1996, with fieldwork done by an expedition of the Altai State University. Eight burials of the Neolithic-Chalcolithic period were discovered at the burial site, including five single graves, two paired graves and one collective grave. Skeletons were buried in two rows (not-parallel orientation), and were elongated on their back with the heads were oriented to the northeast. The depth of the graves was from 0.4 to 1.3 m. The only exception is burial No.18, which is the only sitting burial known in the south of Western Siberia and the Altai. The accompanying equipment is represented mainly by bone tools blanks, triangular-shaped arrowheads, scrapers on flakes, axes, bifacial knives. Decorations are presented by necklaces made from bone pendants and from drilled teeth of carnivores and herbivores. All associated remains from this site are stored in the Altai State University, the Altai Museum of Archaeology and Ethnography. Burial 14 skeleton No.2 is dated by AMS to 6684±39BP (UBA-22954).<sup>25</sup> AMS-dates for other burials can be found in Kiryushin et al. 2021.<sup>26</sup>

**Cemetery Novoaltaysk – Razvilka (“Altai\_6500BP”).**

- NVR001 (Burial 2): 4333-4075 cal BCE (uncalibrated 5390 ± 24 BP, MAMS-48707)

The site was discovered and excavated in 2005, human remains were found during work on a highway. The deepness of the grave is 0.42 m from the modern surface. Judging by the preservation of the remains, the skeleton was lying on his back, in an elongated position, with his head facing the north, his hands along the body. Few accompanying objects include stone and bone products: a badger's tusk pendant with a hole drilled in the root, a bird's bone, a flake, a technical splinter, a polished splinted piece, rectangular shells of large river mollusks, animal vertebrae, beaver's teeth, as well as a needle box with ornament made on bird's bones.<sup>26,27</sup>

**Cemetery Tuzovskie Bugry-I** ("Altai\_5500BP").

- TZB001 (Burial 8): 3495-3347 cal BCE (uncalibrated 4606 ± 22 BP, MAMS-48708)
- TZB002 (Burial 33): 3983-3805 cal BCE (uncalibrated 5125 ± 23 BP, MAMS-48709)

This site was excavated in 1995 by D.A. Pugachev, and he discovered three graves. In 2000, an expedition by the Altai State University led by M.T. Abdulganeev excavated the site, and discovered 37 burials. 19 of them belong to the Neolithic to Chalcolithic period. 14 burials were with single skeletons; 4 burials were with two and one was a collective grave. The graves were located in four rows of 5-6 burials in a row, 0.5–1.15 m deep. Most skeletons were partially complete and not all in anatomical connection. The skeletons of the Neolithic period lay stretched on their backs, head to the northeast, arms were stretched along the body. The accompanying inventory is represented mainly by ornaments of clothes and headdresses consisting of teeth of maral (Caspian red deer), elk, mountain goat, badger, groundhog, and kabarga (Siberian musk deer),<sup>28</sup> as well as pendants of rounded and rectangular shape made of river and sea (fossil) shells.<sup>29</sup> Grave goods also included lithic and bone artifacts (arrowheads, scrapers, fishing rod hooks, harpoons, etc.).<sup>30</sup> The associated remains are stored at the Altai State University, the Altai Museum of Archaeology and Ethnography. **Nizhnetykesken Cave-I** ("Nizhnetykesken\_6500BP").

- NIZ001: 4445-4337 cal BCE (uncalibrated 5528 ± 28 BP, MAMS50153)

Nizhnetykesken Cave-I (NTP-I) is a karst cave, located in a mountain range at an altitude of 130 m from the level of the Katun River. The cave is horizontal, the soft sediment area was about 25 square meters. Several cultural layers were discovered in the cave: Middle Age, Early Iron Age, Chalcolithic, Neolithic, and Final Mesolithic. The first signs of burial appeared at the level of the Chalcolithic cultural layer. The dimensions of the pit were 1.2x1 m. The orientation of the skeleton cannot be established. In the southern part of the pit there was the right side of the chest with the spinal column and the right shoulder blade. In the northeastern part laid the lower half of the skeleton in a bent position (five lumbar vertebrae, a pelvis and legs in anatomical connection without feet). On the northeastern side of the skull laid a bent left hand with the rest of the hand oriented in the opposite direction from the legs. The second arm without a hand and with broken epiphyses was found southwest of the legs. Separate fragments of ribs, phalanges of arms and legs were dispersed. All bones belong to a single individual 40-45 of age.<sup>31,32</sup> Accompanying inventory in the burial was distributed by three main clusters. The first cluster consists of 12 stone arrowheads lying flat in 3 layers, in the lower part of the chest. The second cluster is a compact assemblage of various objects: flint arrowheads, one and a half dozen of flakes and tools, several heavily destroyed gopher skulls with lower jaws and isolated jaws and incisors of these animals; several paws of small birds of prey with claws (or rather, with bone claw bases), a bone pin, rods of compound hooks, stumps and bone integral and compound hooks, bone fragments, split teeth of animals - more than 70 objects in total. The third cluster was cleared along the northeastern edge of the grave. Southeast of it, on a rocky ledge of the base of the cave, a polished stone adze and a large compound hook with a sting of musk deer canine were found. At the top of the cluster laid a rod of elk bone, and under it there was a cluster of split long animal bones, split animal teeth, 7 flint arrowheads, a fragment of a biface knife, flakes and a bone product with cuts along the edge. The disarticulated skeleton could be associated with a shamanism practice, since most of the objects likely constitute a single cult complex (shaman costume) that existed among Siberian tribes in the 4<sup>th</sup> millennium BC.<sup>33</sup> The meaning of the costume could be interpreted as the symbol of a transformation from a human into an animal like a bird. Overall, the collection of remains associated with the burial provides new perspectives into the cultural diversity of the Altai regions before Afanasievo. Anthropological observations make it possible to separate the burial in NTP-I from the Paleo-European and the later Afanasievo population of the Altai.<sup>33</sup> The associated remains are stored is at the Altai State University, the Museum of Archaeology and Ethnography.

#### **Samples from the Russian Far East**

**Letuchaya Mysh Cave** ("LetuchayaMysh\_7000BP").

- Letuchaya Mysh (LM16): 4935-4729 cal BCE (uncalibrated 5957 ± 27 BP, MAMS-47131)

The Letuchaya Mysh Cave (N 42°59'58,8" E 133°05'42,2") is located at the right bank of the Partizanskaya River, 30 km from Nakhodka city (Primorsky krai, Russia). The cave emerged in the reefogenic limestone. The entrance is 3 m high and 1.5 m wide and situated 15 m above the basin level. Total length of the cave is 120 m. The tooth used for DNA analyses was found in the deeper section of the cave during exploration pits in 2016. The tooth found in the Letuchaya Mysh Cave (LM16) is a lower right first molar. Post-mortem damages are minor. There is only a network of cracks, with a maximum thickness of 0.3 mm, but no crown and root fragments loss. Occlusal enamel wear rate is grade 4-5 by the Smith's scale.<sup>34</sup> The crown of LM16 consists of six main cusps with Y-pattern. The metaconulid (C7) GRADE 1<sup>35</sup> and the distal trigonid crest are present. The presence of these traits and an additional third root make it possible to classify this molar as east Eurasian, possible sinodont in the C.G. Turner's classification.<sup>36</sup>

The tooth demonstrates morphological affinities with a sample from the Boisman 2 Neolithic site from the Southern Primor-  
ye,<sup>20,37,38</sup> where the high frequencies of a third root, distal trigonid crest and cusp 6 on the lower first molar are also observed.<sup>38</sup>

**Kamchatka Peninsula (Kamchatka\_500uncalBP).**

- KMT001: 1646 ± 68 BP (uncalibrated)
- KMT002: 1578 ± 52 BP (uncalibrated)
- KMT003: 1118 ± 31 BP (uncalibrated)

Paleontological excavations were carried out in 2018 and 2019 by Maria Pevzner and Timur Karimov. The site is located in central Kamchatka, close to the Kamchatka River near the geological section of the Nikolka Mountain (55°27'1.04 "N, 159°41'1.76 "E). Three human bones were discovered during excavations. The bones were studied using morphological methods for estimation of sex and age. The bones are gracile, the minimum number of individuals is two (two different tibia, KMT001 and KMT002), and we do not exclude that the ulna (KMT003) could be from another individual. The human remains belong to adult individuals and, possibly, not younger than 35 years of age. The estimation of sex was problematic due to bone incompleteness. We could propose that a sample KMT001 could belong to a female individual (also confirmed genetically) while sex assignment for the others individuals is questionable.

We carried out radiocarbon dating for some of these individuals. The bone collagen is strongly enriched in  $\delta^{15}\text{N}$  (16.5-17.3), suggesting a diet rich in fish. Along with sample KMT003 a dog mandibula was found dated to 1147±30 <sup>14</sup>C years, also showing a high nitrogen value ( $\delta^{15}\text{N}$  14.1). Due to these characteristics, we have to consider the potential large impact of marine water reservoir effect when calibrating the obtained radiocarbon dates. The lack of estimates for the local reservoir effect in the region does not allow us to determine the exact chronological age of these remains. Therefore, for the ancient Kamchatka individuals we only report the non-calibrated dates and when grouped the average non-calibrated age among the three dated individuals (Kamchatka\_500uncalBP).

## METHODS DETAILS

### Sample collection and radiocarbon dates

In this study, we collected skeletal elements from ten ancient individuals that are housed at the following institutions: the Altai State University and the Altai Museum of Archaeology and Ethnography, the Federal Science Center of East Asian Terrestrial Biodiversity and the Research Institute and Museum of Anthropology in Moscow. We reported nine new direct accelerator mass spectrometry (AMS) <sup>14</sup>C dates that were generated at the radiocarbon laboratory in the Curt-Engelhorn-Center of Archaeometry in Mannheim, Germany. Tooth and bone samples were prepared for radiocarbon dating following the laboratory-specific protocols. <sup>14</sup>C-ages are calibrated using the dataset IntCal20<sup>39</sup> and software OxCal v4.4 and SwissCal 1.0 (L.Wacker, Swiss Federal Institute of Technology in Zürich, Switzerland).

### Ancient DNA sample processing

All samples were processed in the dedicated clean facilities at the Max Planck Institutes for the Science of Human History in Jena and Evolutionary Anthropology in Leipzig, Germany. Bone powder for DNA extraction was obtained by drilling from the dental pulp and from the densest part of long bones. We performed DNA extraction following established protocols for ancient DNA.<sup>40</sup> For the sample from Nizhnetykkesken Cave-I, we built a double-stranded, uracil-DNA-glycosylase-treated ("half-UDG") DNA library to reduce but not completely remove the deaminated bases at the ends of ancient DNA fragments<sup>41</sup> (Data S1A). For the other nine samples, we built single-stranded libraries<sup>42</sup> without UDG treatment ("non-UDG") (Data S1A). The built libraries were shotgun sequenced on Illumina HiSeq4000 platform using 75 bp single-end reads for screening. The ten samples were enriched for a set of 1,237,207 targeted SNPs (1240k capture)<sup>43</sup> across the whole genome and for the entire mtDNA sequence (mtDNA capture).<sup>44</sup> The enriched DNA products were further sequenced on an Illumina HiSeq4000 platform.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Sequence data processing

We processed de-multiplexed 1240k capture DNA sequences using *EAGER* 1.92.55,<sup>45</sup> in which adapters were removed with *AdapterRemoval* 2.2.0,<sup>46</sup> reads were mapped to the human *hg37d5* reference genome with *BWA* 0.7.12 *aln/samse* algorithm,<sup>47</sup> duplicates were removed with *DeDup* 0.12.1 (<https://github.com/apeltzer/DeDup>) and damage patterns were inspected with *mapDamage* 2.0.6<sup>48</sup> (Data S1A). The resulting reads with base quality and mapping quality over 30 were piled up with *samtools mpileup* in *SAMtools* 1.3.<sup>49</sup> The sequences deriving from the mtDNA captured libraries were instead mapped to rCRS reference sequence (GenBank: NC\_012920.1) with *BWA* 0.7.12 *aln/samse* algorithm and realigned with *CircularMapper*.<sup>45</sup> After removing duplication with *DeDup* 0.12.1, the consensus sequences were generated by *schmutzi*.<sup>50</sup>

We called pseudo-haploid genotypes using *pileupCaller* 1.4.0.3 (available at <https://github.com/stschiff/sequenceTools>) on 1,233,013 targeted sites, using random haploid calling and single-stranded mode, which ignores forward reads at C/T polymorphisms and reverse reads at G/A polymorphisms. For individuals with double-stranded libraries, we called pseudo-haploid genotypes using *pileupCaller* 1.4.0.3 (available at <https://github.com/stschiff/sequenceTools>) on 1,233,013 targeted sites, using random haploid calling and double-stranded mode.



### Quality control and uniparental marker analysis

We examined contamination levels by applying *ANGSD*<sup>51</sup> for X-chromosome contamination in males and *schmutzi*<sup>50</sup> for mtDNA contamination in all samples. We find that the Letuchaya Mysh individual has around 40% contamination based on the X-chromosome estimate and 15% contamination based on the mtDNA estimate. Therefore, for the downstream analyses of LetuchayaMysh\_7000BP, we applied *pmdtools* 0.60<sup>8</sup> at threshold 3 to reduce the amount of modern-day contamination that resulted in 242,242 SNPs on the 1240k panel (Table 1). After *pmd* filtering, LetuchayaMysh\_7000BP has lower than 5% contamination based on X-chromosome estimates, which is an acceptable contamination level for further population genetics analyses.

We determined the genetic sex for all individuals by estimating coverage on the X and Y chromosomes in comparison to the coverage on the autosomal chromosomes. We determined Y haplogroups with the *yHaplo* program.<sup>52</sup> We assigned mtDNA haplogroups with *HaploGrep2*.<sup>53</sup>

### Analyzed present-day and ancient genomic data

We merged our newly reported ancient individuals with previously published ancient individuals across Eurasia and North America,<sup>1–7,9–12,14–16,20–22,54–59</sup> together with 225 modern-day populations from the Human Origins dataset<sup>56,60</sup> and the Simons Genome Diversity Panel<sup>61</sup> (Data S1B).

### Principal component analyses (PCA)

We carried out PCA analyses using *smartpca* v16000 from *EIGENSOFT* v7.2.1. package<sup>62</sup> using all autosomal SNPs and projected ancient individuals on eigenvectors computed from present-day Eurasian populations on the Affymetrix Human Origin (HO) array using option “*Isproject*: YES”. Intersecting with SNPs in the HO array, we obtain data for 593,124 autosomal SNPs across all populations analyzed in PCA.

### *f*-statistics

For *f*-statistics and *qpWave*/*qpAdm* analyses (see below), we use up to 1,233,013 SNPs (1240k SNP capture array) across the analyzed ancient and modern individuals, to maximize resolution. We calculate  $f_3$  and  $f_4$  statistics using *qp3Pop* (v435) and *qpDstat* (v755) in the *AdmixTools* v5.1 package.<sup>13</sup> We performed outgroup  $f_3$  with Mbuti.DG as outgroup, to inspect the genetic affinity of our newly reported groups to ancient and present-day East Eurasian populations and ancient Native Americans. We performed symmetric  $f_4$  statistics in form of (Ancient 1, Ancient 2; Test Population; Mbuti.DG) to examine genetic similarity between Ancient 1 and Ancient 2 which are the newly reported ancient groups in this study and closely related published ancient/modern genetic groups.

### qpWave and qpAdm analyses

We applied *qpWave* v410 and *qpAdm* v810 from the *AdmixTools* v5.1 package<sup>13</sup> to assess possible ancestral components in the targeted individuals/populations. We used a set of 10 worldwide populations—“Mbuti.DG, Natufian, Villabruna, Tianyuan, Iran\_N, Mixe.DG, Ami.DG, Onge.DG, Ust\_Ishim, Anatolia\_N”—as the default outgroup list, and added additional populations individually to test specific models. In particular, for testing 2-source admixture for the Altai hunter-gatherers, we used AG3/MA1/Botai/West\_Siberia\_N as the ANE source, and tested the following ten ancient populations as the potential second source, including ANA populations (Baikal\_EN<sup>5,6</sup> and DevilsCave\_N<sup>7</sup>), “paleo-Siberian-related” populations (~14000-year-old UKY from Baikal, Yakutia\_6000BP, Krasnoyarsk Krai\_4500BP, TransBaikal\_8500BP, ~9700-year-old Kolyma\_M from northeastern Siberia),<sup>6,7,14</sup> an ancient Beringian individual (~11000-year-old USR1),<sup>54</sup> and ancient Native Americans (USA\_Anzick.SG, Kennewick\_WA.SG).<sup>15,16</sup>

### Dating admixture

We used *DATES* v753<sup>3</sup> to date the admixture in the newly reported ancient groups/individuals and relevant published genetic groups (Figures 4 and S3). We applied a default bin size of 0.001 Morgan as recommended in the software documentation (flag “*binsize*: 0.001” added).

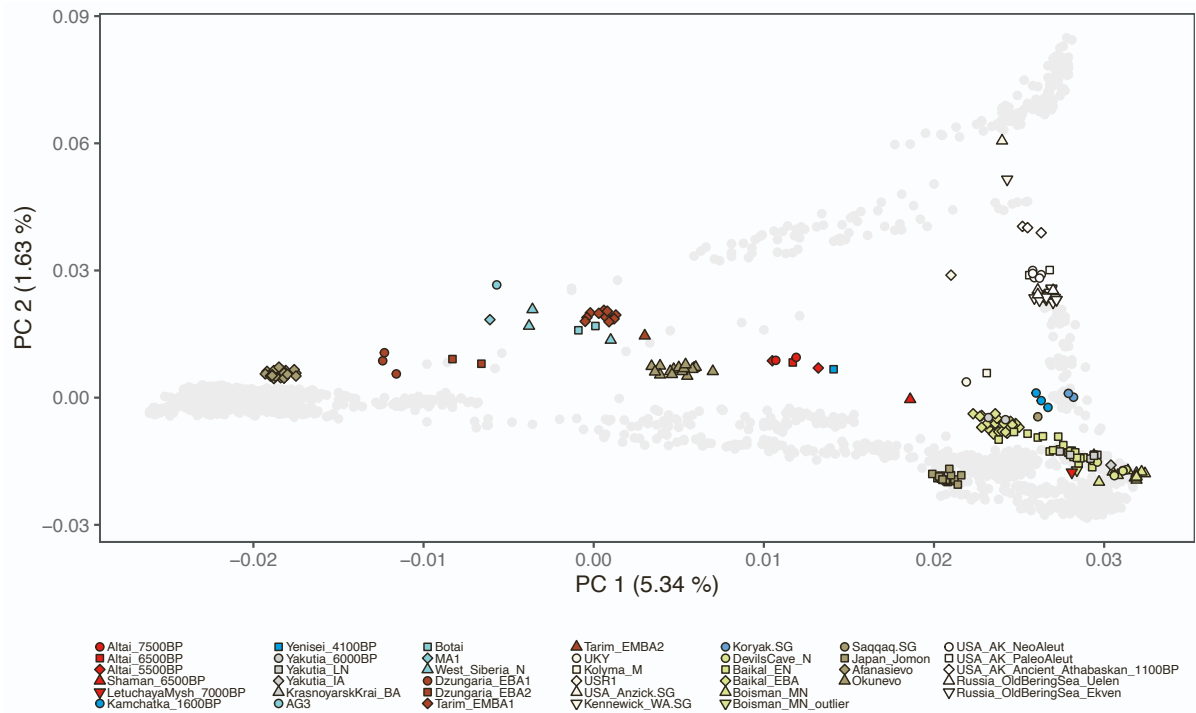
### Run of homozygosity (ROH)

We used *hapROH*<sup>63</sup> to estimate ROH genomic segments in the newly reported ancient individuals, which can inform whether the targeted genome has recent inbreeding patterns or originate from a population with small effective population size. The ROH results is reported in Figure S5.

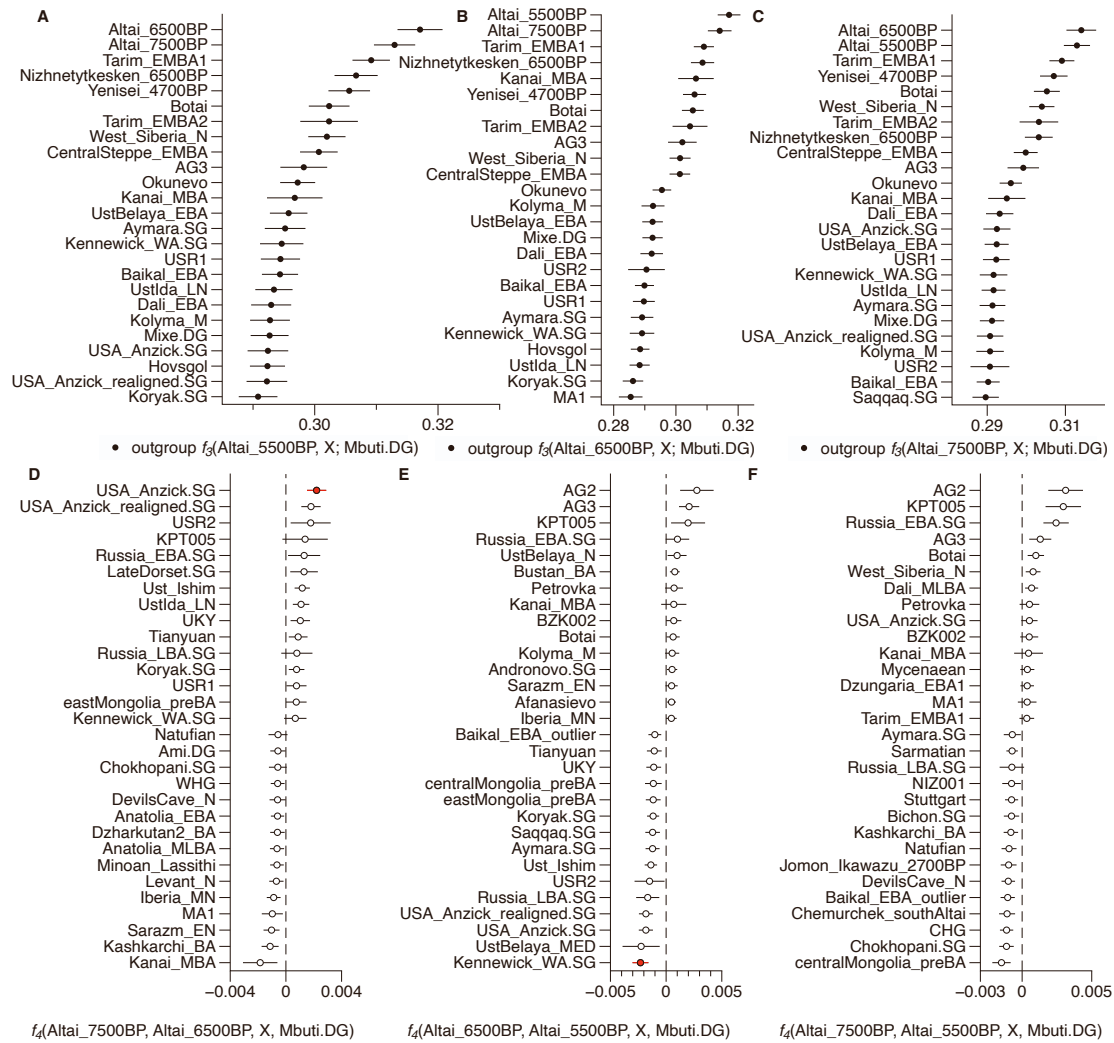
## Supplemental Information

### **Middle Holocene Siberian genomes reveal highly connected gene pools throughout North Asia**

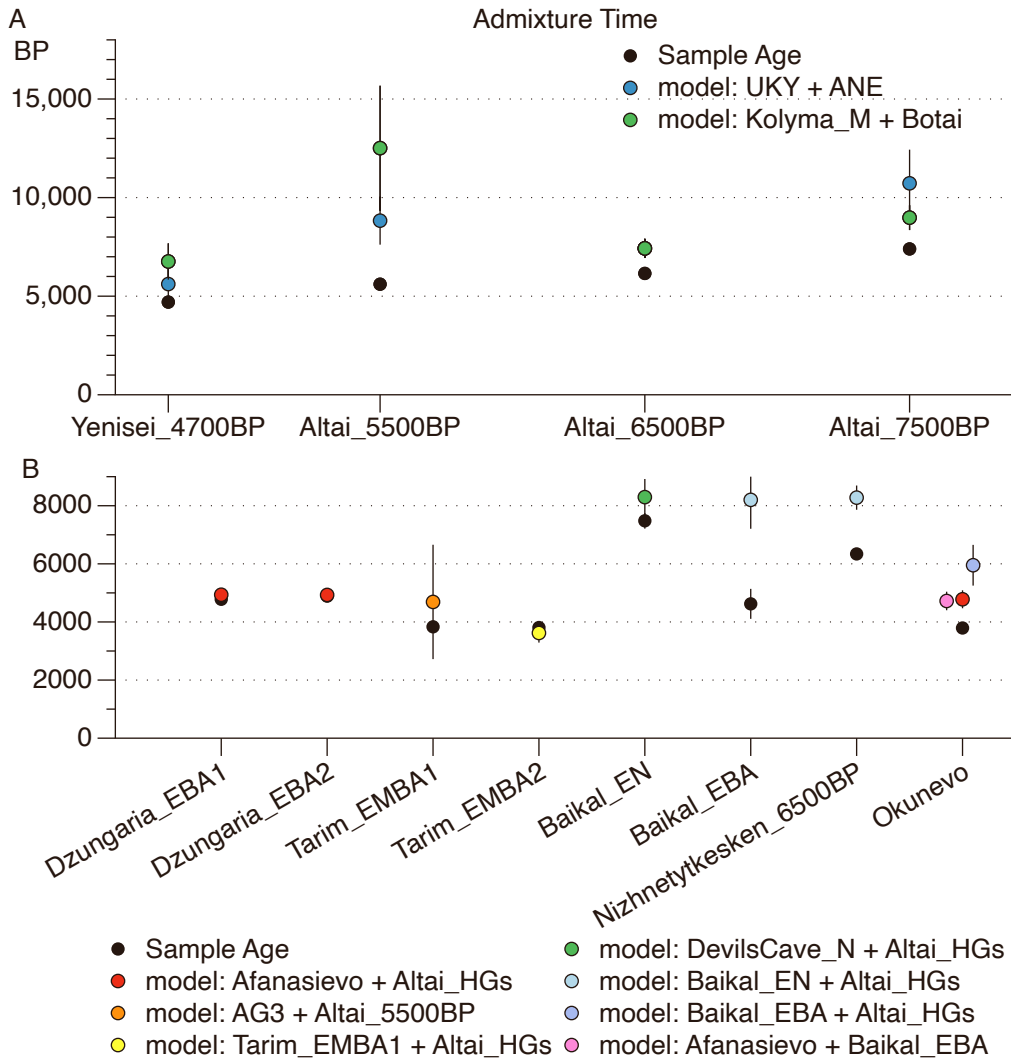
**Ke Wang, He Yu, Rita Radzevičiūtė, Yuriy F. Kiryushin, Alexey A. Tishkin, Yaroslav V. Frolov, Nadezhda F. Stepanova, Kirill Yu. Kiryushin, Artur L. Kungurov, Svetlana V. Shnaider, Svetlana S. Tur, Mikhail P. Tiunov, Alisa V. Zubova, Maria Pevzner, Timur Karimov, Alexandra Buzhilova, Viviane Slon, Choongwon Jeong, Johannes Krause, and Cosimo Posth**



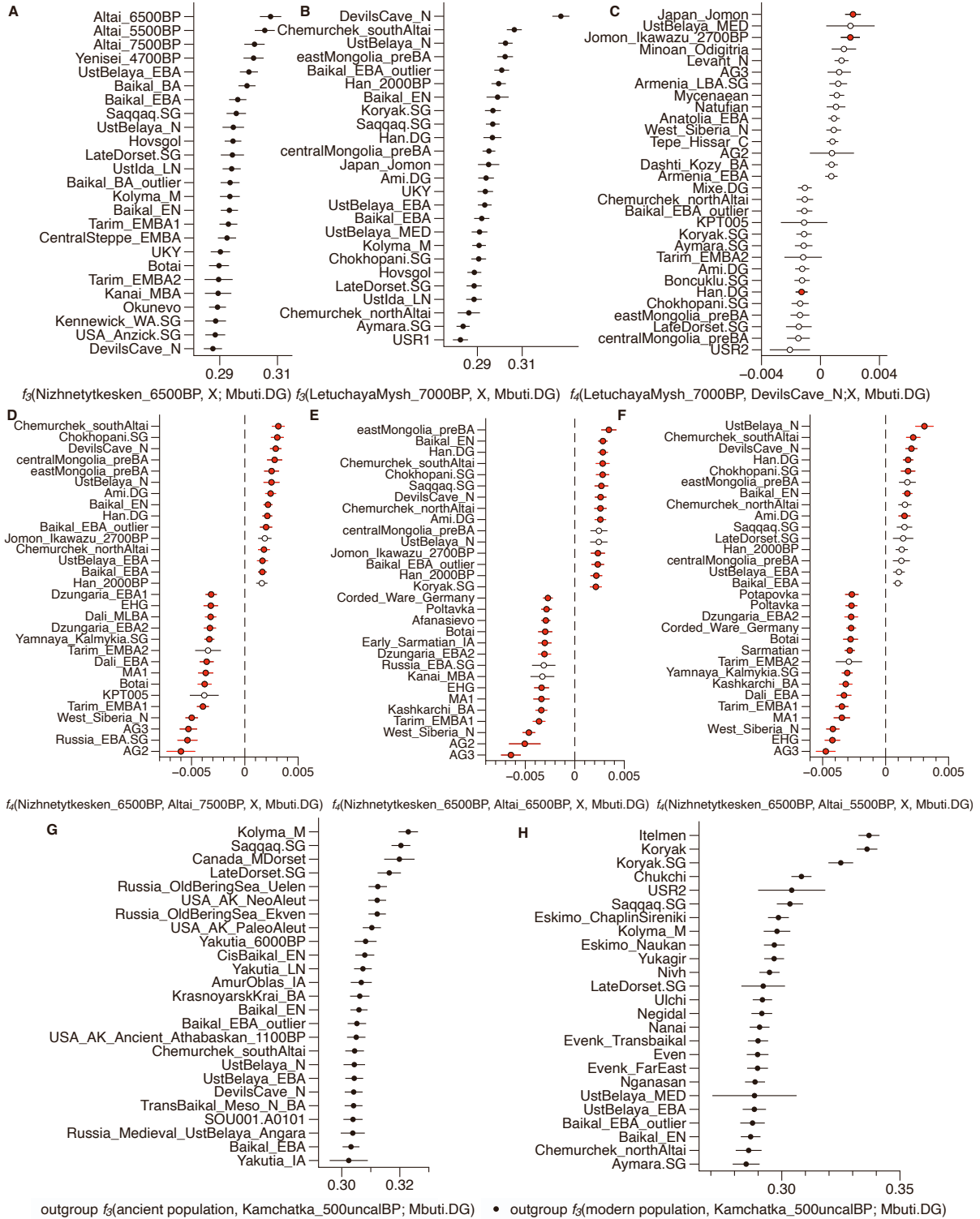
**Figure S1. PCA calculated with present-day Eurasian and Native American populations, related to Figure 1. Ancient individuals are projected into the PC space calculated from the genetic variation of present-day Eurasians and Native Americans (shown in grey dots).**



**Figure S2. Outgroup  $f_3$  statistics and genetic symmetry tests using  $f_4$ -statistics for the Altai hunter-gatherers, related to Figure 2.** Outgroup  $f_3$ -statistics reveal that Altai hunter-gatherer groups share high genetic affinity with populations associated with ANE ancestry. (A) outgroup  $f_3(\text{Altai}_{7500\text{BP}}, \text{population X}; \text{Mbuti.DG})$ . (B) outgroup  $f_3(\text{Altai}_{6500\text{BP}}, \text{population X}; \text{Mbuti.DG})$ . (C) outgroup  $f_3(\text{Altai}_{5500\text{BP}}, \text{population X}; \text{Mbuti.DG})$ . (D).  $f_4(\text{Altai}_{7500\text{BP}}, \text{Altai}_{6500\text{BP}}; \text{population X}, \text{Mbuti.DG})$ . (E).  $f_4(\text{Altai}_{6500\text{BP}}, \text{Altai}_{5500\text{BP}}; \text{population X}, \text{Mbuti.DG})$ . (F).  $f_4(\text{Altai}_{7500\text{BP}}, \text{Altai}_{5500\text{BP}}; \text{population X}, \text{Mbuti.DG})$ . Population X are test populations from ancient Eurasia or America (Table S11). We plot the highest 25 outgroup  $f_3$ -statistics in descending order based on  $f_3$  value, with horizontal bars representing  $\pm 1$  standard error (SE). For  $f_4$ -statistics, we plot the highest 15 and lowest 15  $f_4$  values with horizontal bars representing  $\pm 1$  SE calculated by 5cM block jackknifing. Tests with Z-score  $> |3|$  are highlighted in red.

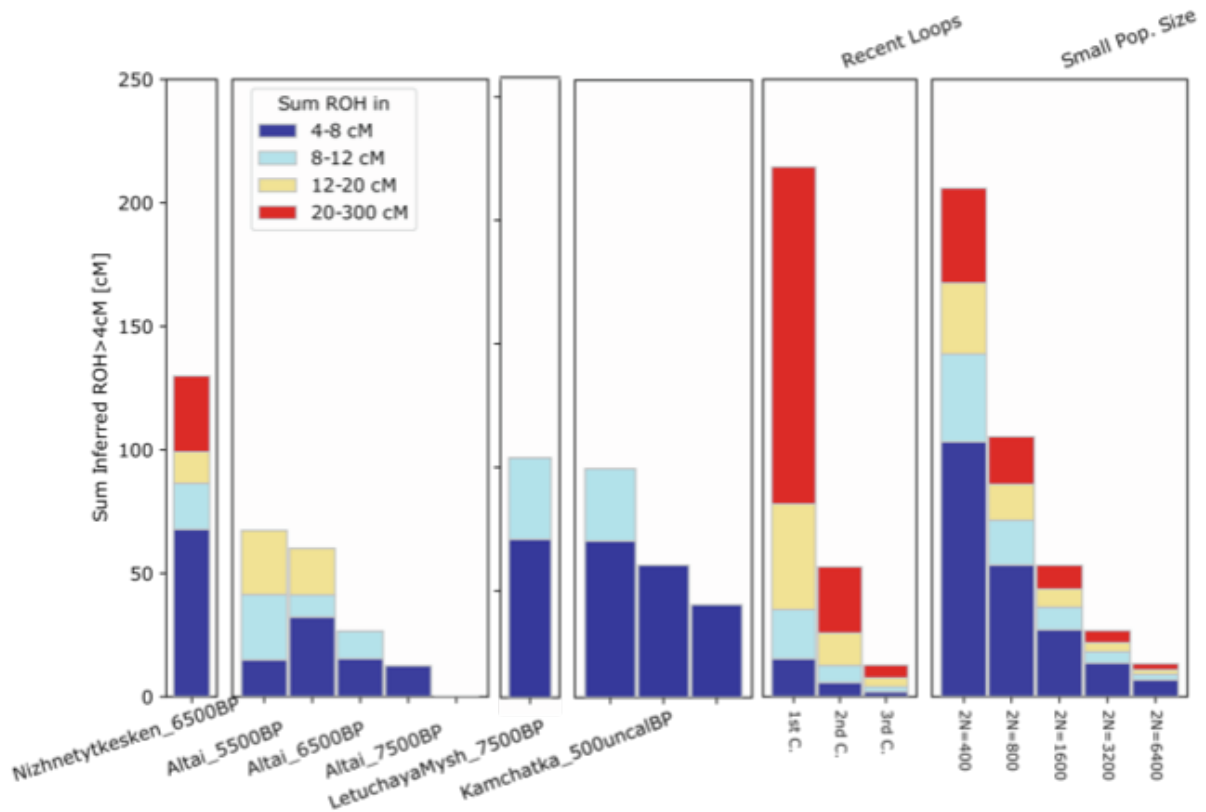


**Figure S3. Estimating admixture dates for Altai hunter-gatherers and related groups, related to Figure 2 and STAR Methods.** (A) Dating results for Altai\_7500BP, Altai\_6500BP, Altai\_5500BP and Yenisei\_4700BP. (B) Dating the admixture event for Baikal\_EN, Baikal\_EBA, Dzungaria and Tarim Basin Bronze Age groups and a Okunevo-related population. See also Data S2.



**Figure S4. Outgroup  $f_3$ -statistics and genetic symmetry tests using  $f_4$ -statistics for Nizhnytykesken\_6500BP and LetuchayaMysh\_7000BP, related to Figure 3. (A) Outgroup  $f_3(\text{Nizhnytykesken}_6500\text{BP}, \text{population X}; \text{Mbuti.DG})$  reveals high genetic affinity to populations with ANA ancestry; (B) Outgroup  $f_3(\text{Letuchayamysh}_7000\text{BP}, \text{population X}; \text{Mbuti.DG})$  reveals high genetic affinity to populations with ANA ancestry; (C)  $f_4(\text{LetuchayaMysh}_7000\text{BP}, \text{DevilsCave}_N; \text{population X}, \text{Mbuti.DG})$  reveals extra genetic affinity with Jomon hunter-gatherers from Japan. (D), (E), (F) show that Nizhnytykesken\_6500BP share close genetic affinity to East Asian populations with high**

ANA ancestry compared to Altai\_7500BP, Altai\_6500BP and Altai\_5500BP. (G) Outgroup  $f_3$ (ancient population X, Kamchatka\_500uncalBP; Mbuti.DG). (H) Outgroup  $f_3$ (population X genotyped on *Human Origins* panel, Kamchatka\_500uncalBP; Mbuti.DG). See also Data S2. We plot the highest 25 outgroup  $f_3$ -statistics in descending order based on  $f_3$  value, with horizontal bars representing  $\pm 1$  standard error (SE). For  $f_4$ -statistics, we plot the highest 15 and the lowest 15  $f_4$ -statistics with horizontal bars representing  $\pm 1$  SE calculated by 5cM block jackknifing and tests with Z-score $>3$  highlighted in red color.



**Figure S5. Run of Homozygosity (ROH) for the newly reported genomic data, related to STAR Methods.** This analysis provides insights on consanguinity and effective population size (examples provided in the two right-most panels). The presence of long ROHs implies close biological relatedness between the parents of the studied individuals<sup>35</sup>. Substantial amount of short ROHs (dark blue segments) hints at a small effective size for the population the target individuals derive from.