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ORIGINAL INVESTIGATION

Genetic kinship and admixture in Iron Age Scytho-Siberians

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Abstract

Scythians are known from written sources as horse-riding nomadic peoples who dominated the Eurasian steppe throughout the Iron Age. However, their origins and the exact nature of their social organization remain debated. Three hypotheses prevail regarding their origins that can be summarized as a "western origin", an "eastern origin" and a "multi-regional origin". In this work, we first aimed to address the question of the familial and social organization of some Scythian groups (Scytho-Siberians) by testing genetic kinship and, second, to add new elements on their origins through phylogeographical analyses. Twenty-eight Scythian individuals from 5 archeological sites in the Tuva Republic (Russia) were analyzed using autosomal Short Tandem Repeats (STR), Y-STR and Y-SNP typing as well as whole mitochondrial (mtDNA) genome sequencing. Familial relationships were assessed using the Likelihood Ratio (LR) method. Thirteen of the 28 individuals tested were linked by first-degree relationships. When related, the individuals were buried together, except for one adult woman, buried separately from her mother and young sister. Y-chromosome analysis revealed a burial pattern linked to paternal lineages, with men bearing closely related Y-haplotypes buried on the same sites. Inversely, various mtDNA lineages can be found on each site. Y-chromosomal and mtDNA haplogroups were almost equally distributed between Western and Eastern Eurasian haplogroups. These results suggest that Siberian Scythians were organized in patrilocal and patrilineal societies with burial practices linked to both kinship and paternal lineages. It also appears that the group analyzed shared a greater genetic link with Asian populations than Western Scythians did.

L. Mary and V. Zvenigorosky contributed equally to this work.

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Introduction

For millennia, the vast Eurasian steppe, stretching from the Northern Black Sea to the Altai Mountains, was occupied by various nomadic populations that have had a significant impact on the cultural history of the Eurasian continent. Among these populations, the Scythians were probably the most famous. They are known from ancient texts (Achaemenid, Greek and Chinese sources) (Grousset 1965) and through the excavation of their burial mounds (kurgans), including the rich Scythian royal tombs (Clisson et al. 2002).

From an archeological and historical point of view, the term "Scythians" refers to Iron Age nomadic or semi-nomadic populations characterized by the presence of three types of artifacts in male burials: typical weapons, specific horse harnesses and items decorated in the so-called "Animal Style". This complex of goods has been termed the "Scythian triad" and was considered to be characteristic of nomadic groups belonging to the "Scythian World" (Yablonsky 2001). This "Scythian World" includes both

the Classic (or European) Scythians from the North Pontic region (7th–3th century BC) and the Southern Siberian (or Asian) populations of the Scythian period (also called Scytho-Siberians). These include, among others, the Sakas from Kazakhstan, the Tagar population from the Minusinsk Basin (Republic of Khakassia), the Aldy-Bel population from Tuva (Russian Federation) and the Pazyryk and Sagly cultures from the Altai Mountains.

Despite the Scythians having left an important archeological heritage, their origins remain controversial and three hypotheses are currently debated (Bashilov and Yablonsky 2001). The first hypothesis suggests that the Scythians originated in local Bronze Age populations of the North Pontic Region (NPR) and spread into Asia. However, historical and archeological data support a Central Asian origin for the Scythian populations: the first written mention of Scythians is attributed to Greek historian Herodotus, for whom the Scythians had come from Asia. This second hypothesis was supported by the fact that the oldest kurgans with evidence of the Scythian Triad had been discovered in Central Asia (e.g., Arzhan-1, late ninth century BC) (Gryaznov 1981; Zaitseva et al. 2005). The third hypothesis states that it is not possible to trace back to a unique origin for the Scythians and that the "Scythian World" was a collection of various, genetically distinct populations sharing common cultural traits. This last hypothesis is strongly supported by recent paleogenomic studies, through whole genome sequencing (WGS) (Damgaard et al. 2018) and/or whole mitochondrial genome analysis (Unterländer et al. 2017; Juras et al. 2017). Scythians appear to be genetically heterogeneous, with a genetic pool composed of local Bronze Age populations and evidence of Eastern/Western Eurasian admixture in their maternal lineages.

If the origins of the Scythians and their genetic affinities with other ancient nomadic populations of the Eurasian steppe have attracted considerable interest in recent years, only few studies have focused on questions relating to their social or familial structures and have examined genetic kin relationships between buried individuals (Clisson et al. 2002; Ricaut et al. 2004a; Pilipenko et al. 2015).

In this work, we first aim to address the question of the familial and social organization of Scytho-Siberian groups by studying the genetic relationship of 29 individuals from the Aldy-Bel and Sagly cultures using autosomal STRs. Through the analysis of the whole mitochondrial (mtDNA) genome and the Y-chromosome STR and SNP of these individuals, we intended to confirm the relationships deduced from the autosomal DNA makers and also to assess the ethno-geographic origins of the individuals studied.

Materials and methods

Samples

Human remains from 29 individuals (2 teeth per individual) were obtained from 5 archeological sites located in the valley of the Eerbek river in Tuva Republic, Russia (Fig. 1). All the mounds of this archeological site were excavated but DNA samples were not collected from all of them. ^{14}C dates mainly fall within the Hallstatt radiocarbon calibration plateau (ca. 800–400 cal BC) where the chronological resolution is poor. Only one date falls on an earlier segment of calibration curve: Le9817–2650 ± 25 BP, i.e. 843–792 cal BC with a probability of 94.3% (using the OxCal v4.3.2 program). This sample (Bai-Dag 8, Kurgan 1, grave 10) is not from one of the graves studied but was used to date the kurgan as a whole. The relative chronology of the kurgans was determined based on typology. Kurgans Bai-Dag 6–6 and 8–1, Eerbek 2–2, 2–3 and Eki-Ottug 2–1 were attributed to various stages of the Aldy-Bel culture (Grach 1980), whereas Eki-Ottug 1–6 and 1–12 were attributed to early stages of the Sagly culture (archeological and ^{14}C dating, Table 1). During this time, burial rites have evolved from individual burials inside one fence to collective burials inside a single pit. These collective burials have all been looted, leading to skeletal displacements (Supplementary Fig. 1) and difficulties in the morphological determination of the sex of each individual. Detailed information on the samples is given in Table 1.

DNA extraction

Teeth were first cleaned with bleach, rinsed with ultrapure water and exposed to UV light for 30 min on each side for decontamination purposes. The teeth were then ground in a liquid nitrogen mill (6870 SamplePrep Freezer Mill®) and DNA was extracted from 280 to 320 mg of tooth powder according to a protocol previously described (Mendisco et al. 2011).

DNA quantification

DNA quantification was realized using the Quantifiler™ Trio DNA Quantification kit (ThermoFisher Scientific, TFS). A multiplex RT-PCR reaction was performed to quantify three products: 2 autosomal products of different sizes (80 and 214 base pairs) and 1 Y-chromosomal product. The gDNA (genomic DNA) concentration of each sample was considered equal to the concentration in autosomal short fragments, according to the manufacturer's protocol. The calculated concentration in autosomal products was then

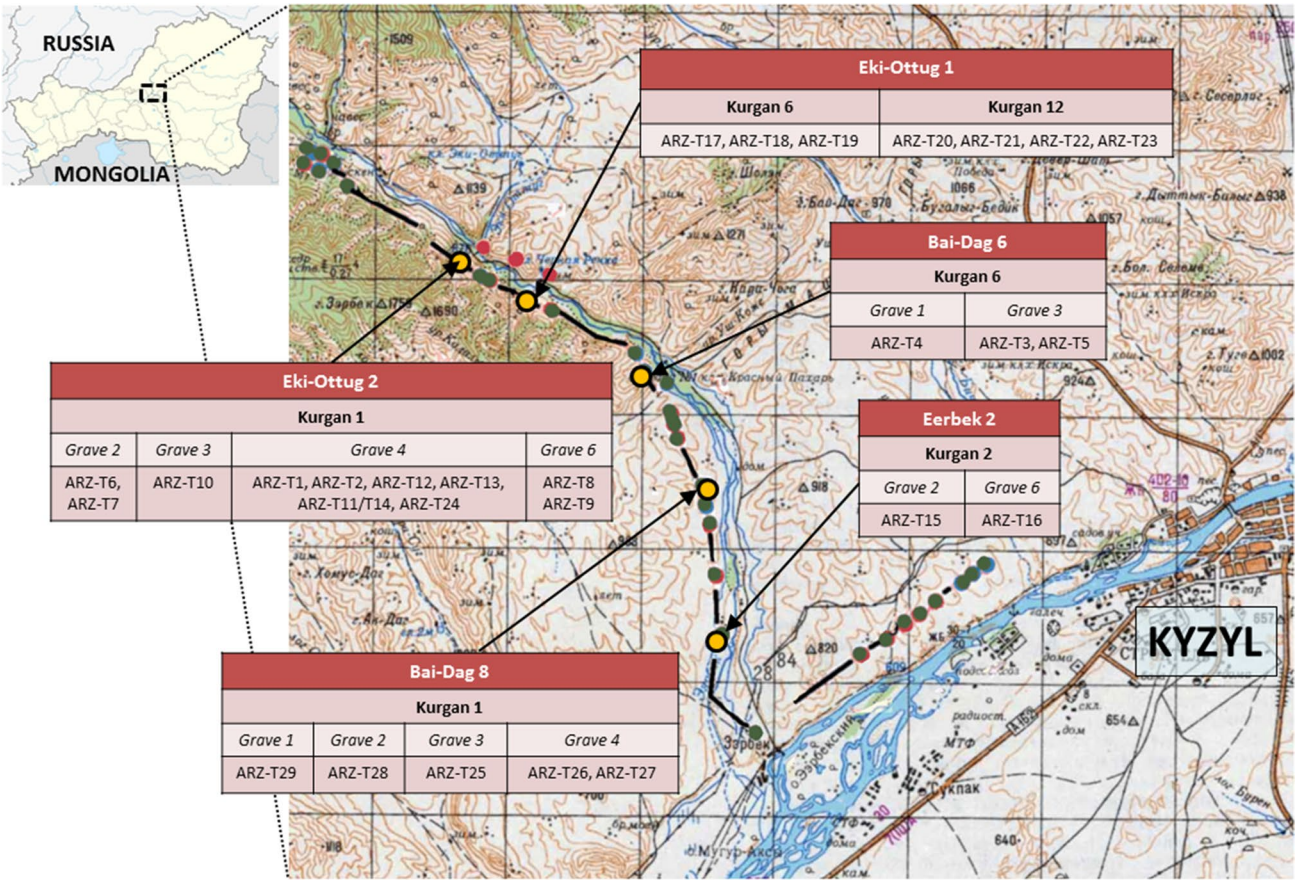

Fig. 1 Map of the archeological sites and localization of each sample in the excavated graves and kurgans

used to estimate the degradation index (i.e., the ratio of the concentration of the shorter product divided by the concentration of the longer one). The higher above 1 the index, the more degraded the DNA. We obtained ratios between 1.99 and 56.82 and concentrations between 0.005 and 0.78 ng/µL (Supplementary Table 1).

Autosomal STR analysis and kinship estimation

Twenty-one autosomal STRs, 1 Y-chromosomal STR (Y-STR), 1 Y chromosome indel polymorphism and the sex determining marker amelogenin were amplified using the GlobalFiler® kit (TFS). Since the design of the primers varies from one STR kit to another and may produce shorter fragments for the same STR, we secondarily used the MiniFiler® STR kit (TFS) or the Investigator® 24plex QS STR kit (Qiagen) when the GlobalFiler® kit failed to amplify the longest STR fragments because of DNA degradation. We followed the manufacturers' protocols, except for the number of PCR cycles, which was increased from 29 to 32 (GlobalFiler® kit) and from 30 to 32 (Investigator® 24plex QS). Capillary electrophoresis was performed on the 3500 Genetic Analyzer (TFS) and data analysis was performed with the GeneMapper™ software (TFS). Probabilities of kinship were estimated by pairwise comparison of the autosomal STR profiles using ML-Relate and FAMILIAS software (Kalinowski et al. 2006; Kling et al. 2014).

Y-chromosomal analysis

Twenty-seven Y-STRs were amplified using the YFiler Plus® kit (TFS) from the DNA of the male samples. We followed the manufacturer's protocol, except for the number of PCR cycles which was increased from 27 to 30. We used 2 online tools to determine Y-chromosome haplogroups from the haplotypes determined from Y-STR data: Y Haplogroup Predictor (http://www.hprg.com/hapest5/) and Y-DNA Haplogroup Predictor NEVGEN (http://www.nevgen.org/). Based on these results, we selected a set of Y-chromosomal SNP to confirm the haplogroups identified in our samples, according to the 2018 ISOGG nomenclature (https://isogg.org/tree/ISOGG_YDNATreeTrunk.html). SNP typing was performed using a set of primers detailed in Supplementary Table 2.

Table 1 Archeological localization of the samples, morphological and genetic sex determination of the individuals, ^{14}C dating and cultural affiliations

	Sample	Site-Kurgan-grave	Morphological age and sex estimation	Genetic sex determination	Laboratory index	^{14}C dating (in YBP) (non calibrated)
Aldy-Bel culture	ARZ-T2	Eki-Ottug 2-1-4	F? 20–25	M	Other tombs in the same kurgan	
	ARZ-T24	Eki-Ottug 2-1-4	M?	M	Le-9850	2350 ± 75 (human bone)
	ARZ-T1	Eki-Ottug 2-1-4	M? 14–15	M	Le-9860	2490 ± 45 (wood)
	ARZ-T12	Eki-Ottug 2-1-4	F 25–30	M	Le-9861	2460 ± 20 (wood)
	ARZ-T13	Eki-Ottug 2-1-4	M > 40	M	Le-9862	2280 ± 25 (wood)
	ARZ-T11/T14	Eki-Ottug 2-1-4	F? 25–30	F	Le-9863	2370 ± 25 (wood)
	ARZ-T6	Eki-Ottug 2-1-2	M > 50	M	Le-9873	2500 ± 20 (wood)
	ARZ-T7	Eki-Ottug 2-1-2	F > 50	M	Le-9874	2480 ± 35 (wood)
	ARZ-T8	Eki-Ottug 2-1-6	M 35–40	M	Le-9875	2340 ± 50 (wood)
	ARZ-T9	Eki-Ottug 2-1-6	F > 50	M	Le-9876	2375 ± 20 (charcoal)
					Le-9877	2375 ± 30 (wood)
					Le-9878	2440 ± 40 (wood)
					Le-9880	2530 ± 30 (wwod)
					Le-9881	2460 ± 45 (wood)
	ARZ-T10	Eki-Ottug 2-1-3	M > 40	F	Le-9848	2330 ± 55 (human bone)
	ARZ-T15	Eerbek 2-3-1	M 40–45	M	Unknown	
	ARZ-T16	Eerbek 2-2-6	F 45–50	F	Le-9838	2380 ± 70 (human bone)
Sagly culture	ARZ-T17	Eki-Ottug 1–6	F 45–50	F	Le-9856	2360 ± 40 (wood)
	ARZ-T18	Eki-Ottug 1–6	M 30–35	M	Le-9857	2420 ± 25 (wood)
	ARZ-T19	Eki-Ottug 1–6	M 35–40	M	Le-9865	2400 ± 30 (wood)
	ARZ-T20	Eki-Ottug 1–12	M 25–30	M	Le-9866	2430 ± 30 (wood)
	ARZ-T21	Eki-Ottug 1–12	M 30–40	M		
	ARZ-T22	Eki-Ottug 1–12	F 30–40	F		
	ARZ-T23	Eki-Ottug 1–12	M 16–18	M		
Aldy-Bel culture	ARZ-T25	Bai-Dag 8-1-3	M 25–35	M	Other tombs in the same kurgan	
	ARZ-T26	Bai-Dag 8-1-5	F 25–30	F	Le-9817	2650 ± 25 (charcoal)
	ARZ-T27	Bai-Dag 8-1-5	?	?	Le-9818	2500 ± 45 (wood)
	ARZ-T28	Bai-Dag 8-1-2	M > 50	M	Le-9820	2450 ± 50 (wood)
	ARZ-T29	Bai-Dag 8-1-1	F > 50	F		
	ARZ-T3	Bai-Dag 6-6-3	F? 15–19	F	Le-9830	2490 ± 70 (human bone)
	ARZ-T5	Bai-Dag 6-6-3	F 40–50	F		
	ARZ-T4	Bai-Dag 6-6-1	F? 15–19	F	Le-9833	2370 ± 120 (human bone)

? sex is undetermined

YBP years before present

Whole mitochondrial genome analysis

Whole mitochondrial genome analysis was performed on a PGM™ IonTorrent (TFS) using the Precision ID mtDNA panel (TFS). Library preparation was performed using the Ion AmpliSeq library kit (TFS). We used the "conservative" method for the initial multiplex PCR step performed in two reaction pools. A volume of 3 µL of DNA was used per reaction pool and the number of PCR cycles was adapted to low DNA concentrations according to the manufacturer's protocol. The amplicons were partially digested using a FuPa enzyme to remove PCR primers. Then, a mix of P1 and A adapters with sample-specific barcodes was ligated to the amplicons, followed by a step of library purification using AMPure™ XP magnetic beads. The two pools of libraries were quantified by qPCR using the Ion Library TaqMan Quantification kit and diluted to a concentration of 8 pM. The following emulsion PCR step was performed on a OneTouch 2 instrument (TFS). Emulsion PCR products were then purified to remove reagents and unbound samples using the Ion OneTouch ES system (TFS). Sequencing primers and Control Ion Spheres were annealed to the enriched library and the sequencing polymerase was added before loading the mix onto an Ion 316 Chip and performing the run on the Ion Torrent PGM (TFS).

All samples were analyzed using the software Torrent_Suite v4.6. Differences were reported relative to the reference mitogenome (rCRS) using the plug-in variantCaller

v4.6.18-1. The resulting pileups from the bamfiles were visually inspected using the IGV software (http://software.broadinstitute.org/software/igv/). Variants that did not fit one of the following criteria were filtered out, to avoid artifacts and variants in the heteroplasmic state: strand bias > 50%, allele coverage > 50 reads, observed frequency > 50% (Wai et al. 2018). VCF files were then analyzed using the online software HaploGrep 2 (Weissensteiner et al. 2016) to obtain a determination of the mitochondrial haplogroup for each sample, based on the latest version of PhyloTree (http://www.phylotree.org/).

Precautions taken to avoid contamination

Pre and post-PCR laboratories are located on different floors. Pre-PCR laboratories are dedicated to the analysis of ancient DNA, under positive pressure and UV light. Between each experiment, benches and supplies were cleaned with bleach, ultrapure water and DNA contamination removal solution and placed under UV light. The manipulators wore appropriate equipment (a facial mask, a mobcap, clean room overalls and gloves). The genetic profile of all people in contact with the samples was determined and compared with the DNA profile obtained for each ancient individual. Multiple DNA extractions and multiple PCR amplifications were performed for each tooth (Supplementary Tables 3 and 6) and a negative control was included in each experiment (one extraction or amplification blank for every four samples).

Results

Autosomal STR analysis and kinship estimation

We obtained autosomal STR profiles for 28 out of 29 samples (22 complete profiles, 5 incomplete profiles with 1–5 STR missing and 1 very partial profile). Complete STR typing results and consensus data are presented in Supplementary Tables 3 and 4. These results indicate that DNA is particularly well-preserved for almost all samples. Only DNA from sample ARZ-T27 appeared too severely degraded to achieve any STR amplification. This failure may be explained by the incomplete closing of the roots of the two teeth tested, favoring contamination and DNA degradation.

Typing of the amelogenin locus revealed that 11 individuals were females and 17 were males. The significant discordance (4/28) between molecular and morphological sex determination (Table 1) can be explained by the displacement of skeletal remains (all graves show substantial evidence of looting, Supplementary Fig. 1), causing morphological sex determination to be attempted on isolated skulls. Since autosomal STR profiles were mostly complete and extraction and amplification blanks were always negative, we assumed that molecular sex determination provided the more reliable results.

Pairwise comparison of all profiles revealed a complete match between ARZ-T11 and ARZ-T14, suggesting that samples T11 and T14 belonged to a single individual, which archaeological researchers confirmed was likely. Kinship determinations were thus attempted using 27 autosomal STR genotypes.

Likelihood Ratio (LR) analyses (Supplementary Tables 5a, b) indicated the presence of familial relationships in four out of five archaeological sites (Fig. 2). First-degree relationships are generally reliably detected by the LR method applied to STR (Zvénigorosky 2018). Seven parent/child pairs were identified, including a man and his two parents (respectively, ARZ-T20, ARZ-T17 and ARZ-T19), as well as a mother and two daughters (ARZ-T5, ARZ-T3 and ARZ-T26). Additionally, three full-sibling pairs were detected, with one individual, ARZ-T18, associated with the ARZ-T20, ARZ-T17, ARZ-T19 trio (Fig. 2), as the brother of ARZ-T19, the paternal uncle of ARZ-T20 and the brother-in-law of ARZ-T17 (in a broad sense, since this is a non-biological relationship).

More distant kinship cannot reliably be detected using these methods on an isolated pair in a small archaeological population (Zvénigorosky et al. 2016). We, however, detected several concordant second-degree relationships (connected pairs where the LR value of a second-degree relationship for each pair is superior to 1). Kurgan 12 on the Eki-Ottug 1 site contained the remains of a woman, ARZ-T22, her son, ARZ-T21 and a man unrelated to her, ARZ-T20. LR was compatible with ARZ-T21 sharing a second-degree relationship with the man ARZ-T20 and with his parents ARZ-T17 and ARZ-T19. The most parsimonious interpretation of these results is that ARZ-T17 and ARZ-T19 were the grandparents of ARZ-T21, while ARZ-T20 was the uncle of ARZ-T21. Consequently, ARZ-T18 would have been the great uncle (a third-degree relationship) of ARZ-T21, a conclusion also compatible with LR calculations.

Y-chromosomal lineage analysis

Male lineages were characterized through the analysis of 27 STR of the non-recombining region of the Y chromosome (NRY) to determine haplotypes. We obtained complete or near-complete Y-STR haplotypes for 16 of the 17 male individuals analyzed (complete STR typing results and consensus data are presented in Supplementary Tables 6 and 7). Three haplotypes were not unique among the samples (they were shared by two or more individuals).

In two cases, shared Y-haplotypes conflicted with kinship determined by LR. Samples ARZ-T2 and ARZ-T6 were identified as brothers by LR but they did not share a Y-haplotype. However, those two haplotypes only differed

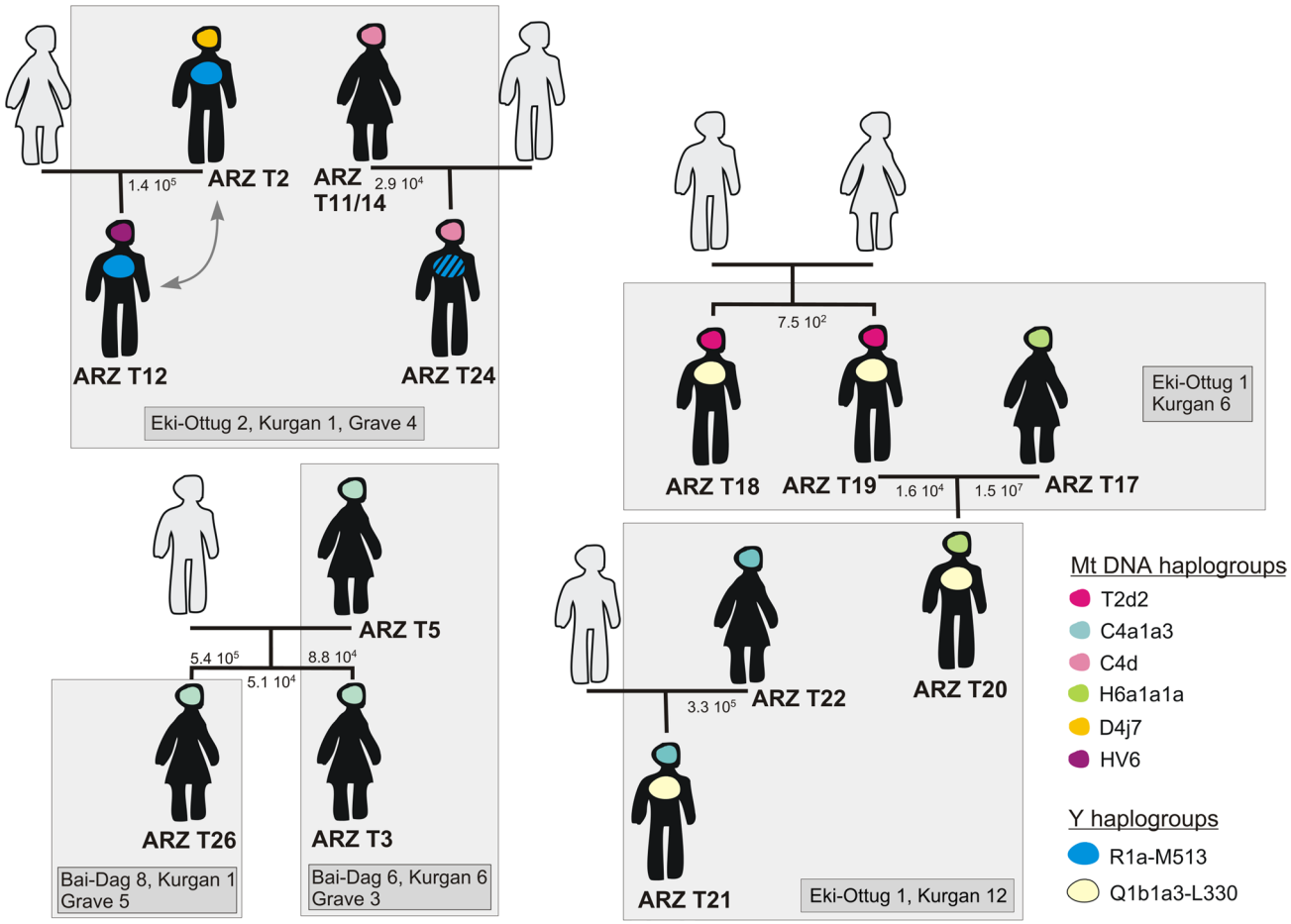

Fig. 2 Genealogies reconstructed from LR estimations. Gray silhouettes represent unknown individuals. The Y-haplotype of ARZ-T24 differs from other R1a-M513 males represented by a single one-step mutation

at one locus (DYS389I), the corresponding alleles differing by a one-step mutation (alleles 14 and 15). It is, therefore, possible that ARZ-T2 and ARZ-T6 are not brothers but rather half-brothers or that a one-step mutation occurred in the father of ARZ-T2 and ARZ-T6. If the latter is true then ARZ-T2, T6, T7, T12 and T24 share a common Y-haplotype.

Another father/son pair that included one of the two aforementioned individuals (ARZ-T2 and ARZ-T12) might differ at one biallelic locus (DYS387S1), with one individual carrying allele 35 and the second carrying alleles 35 and 38. However, given the degradation of DNA, it is very probable that the longer fragment corresponding to allele 38 could not be amplified in ARZ-T2 and that ARZ-T2/ARZ-T12 is a father/son pair.

There were no other discrepancies between LR kinship determinations and Y-chromosome lineages: the remaining father/son pair shared a haplotype, as did the remaining pair of brothers. The 16 male individuals analyzed, therefore, carried either 9 or 10 different haplotypes (depending on the reliability of the discrepancy between ARZ-T2 and ARZ-T12).

Y-chromosome haplogroups were first assigned using the ISOGG 2018 nomenclature. In order to improve the precision of haplogroup definition, we also analyzed a set of Y-chromosome SNP (Supplementary Table 2). Nine samples belonged to the R1a-M513 haplogroup (defined by marker M513) and two of these nine samples were characterized as belonging to the R1a1a1b2-Z93 haplogroup or one of its subclades. Six samples belonged to the Q1b1a-L54 haplogroup and five of these six samples belonged to the Q1b1a3-L330 subclade. One sample belonged to the N-M231 haplogroup. For sample ARZ-T16, DNA degradation did not permit Y-chromosome haplogroup determination.

The distribution of these haplogroups in the population must be confronted with the prevalence of kinship among the samples. Although five individuals belonged to haplogroup Q1b1a3-L330, three of them (ARZ-T18, ARZ-T19 and ARZ-T20) were paternally related (Fig. 2). It must, therefore, be considered that haplogroup Q1b1a3-L330

is present in three independent instances (given that the remaining two instances exhibit no close familial relationship with other samples or one another). All five were buried on the Eki-Ottug 1 archaeological site (although in two different kurgans).

In the same way, although two groups, of two and three individuals, shared haplotypes belonging to the R1a-M513 haplogroup, these groups likely include a father/son pair (ARZ-T2 and ARZ-T12). Therefore, among nine R1a-M513 men, we found six independent haplotypes, one being present in two independent instances. All R1a-M513 haplotypes, however, including those attributed to the R1a1a1b2-Z93 subclade, only differed by one-step mutations, across 5 loci at most. All R1a-M513 individuals were buried on the same site, Eki-Ottug 2, in a single Kurgan.

We searched previously published data (gathered in an in-house database) for ancient individuals bearing those same haplogroups and subclades (Supplementary Table 8). R1a-M513 was present in Neolithic and LNBA (Late Neolithic/Early Bronze Age) individuals from Northern Europe and Western Russia (as far East as the Ural Mountains). In the Bronze Age, it was still found in more eastern sites, between Samara (400 km west of the Ural, north of the Caspian Sea) and the Tarim Basin in China. Iron Age individuals north-west of the Caspian Sea (Astrakhan Oblast), Samara, Southern Siberia and Mongolia also carried the R1a-M513 haplogroup.

The more specific subclade R1a1a1b2-Z93 was not found in Neolithic and LNBA individuals but it was carried by Bronze Age individuals in Samara and the Altai Mountains, as well as Iron Age individuals from Kazakhstan, Samara and the Tuva Republic.

Haplogroup N-M231 was only found in China during the Neolithic and Bronze Age and in the Russian Altai Mountains during the Iron Age.

Haplogroup Q1b1a-L54 (including subclade Q1b1a3-L330) was only found in Bronze Age individuals from the Russian Altai Mountains.

mtDNA genome

We obtained 26 complete mitochondrial genomes with an average coverage depth between 697X and 1512X, as well as one genome with an average coverage depth of 298X (ARZ-T8). We dismissed samples ARZ-T16 and ARZ-T27 because the quality of the DNA extracted was insufficient. These overall high values attest to the relatively good preservation of DNA in our samples.

Mitochondrial haplogroup diversity was higher than Y-chromosome haplogroup diversity (Supplementary Table 9). We identified 16 different haplotypes, belonging to 14 different haplogroups (Supplementary Table 9). All first-degree relationships identified through autosomal STR-typing were supported by shared mitochondrial haplotypes. This was relevant for five mother/child relationships (ARZ-T17/ARZ-T20, ARZ-T22/ARZ-T21, ARZ-T14/ARZ-T24, ARZ-T5/ARZ-T3/ARZ-T26) and a brother/brother relationship (ARZ-T18 and ARZ-T19). Pairs ARZ-T14/ARZ-T24 and ARZ-T21/ARZ-T22 are cases where a man and a woman are linked by a parent/child relationship. Because it is uncertain whether they were buried simultaneously, both cases could be father/daughter pairs or mother/son pairs. However, given shared mitochondrial haplotypes in both cases, Fig. 2 represents these pairs as mother/son pairs, the more parsimonious alternative.

Contrary to what we observed for Y-chromosome haplogroups, burial location did not appear directly linked to maternal lineages, since we found both unrelated individuals with the same mitochondrial haplogroup buried in the same kurgan and closely maternally related individuals (with identical haplotypes) buried on two different archaeological sites.

We searched for matching haplotypes in previously published complete mitochondrial genome data (in an in-house database of more than 30,000 haplotypes) to assess the affinity of Arzhan samples with other ancient populations. Six haplotypes showed less than six mismatches. They had been assigned to haplogroups H, HV6, HV14a, H6a1a, U5a2a1 and U4a1a. For the Neolithic period (including Copper Age cultures), matching haplotypes were found throughout Europe, from Spain and England to the Ukraine and Turkey (Fig. 3). They included matches for Arzhan individuals of all aforementioned haplogroups, except U4a1a.

Among Bronze Age samples, we found matching haplotypes for Arzhan individuals of all six haplogroups, from Portugal and England to Samara, in Russia (Fig. 3). The two individuals found in the region of Samara carried haplogroup H6a1a and U5a2a1 and were attributed to the Srubnaya culture (Mathieson et al. 2015).

There were close matches for three haplotypes among Iron Age samples in the literature, in Sardinia, Italy, Hungary, Moldova and Latvia (Fig. 3). The corresponding Arzhan individuals belonged to haplogroups H, H6a1a and U5a2a1. It must be noted that one of these individuals was an Iron Age Scythian from Moldova (Juras et al. 2017) that differed from ARZ-T4 by only two polymorphisms.

All matching haplotypes were, therefore, found West of the Ural Mountains and belonged to haplogroups within the mitochondrial R-clade (H, HV and U). Twelve Arzhan samples also bore haplogroups within the mitochondrial M-clade (C, D and G), but no matches could be found in the literature. There is however a bias to this result, with only 54 ancient mitochondrial genomes belonging to haplogroups C, D and G published in the literature and 718 ancient mitochondrial genomes belonging to haplogroups H, HV and U. Because some haplogroups (C, D, G and others) are more closely associated to Eastern Eurasia, this bias is directly

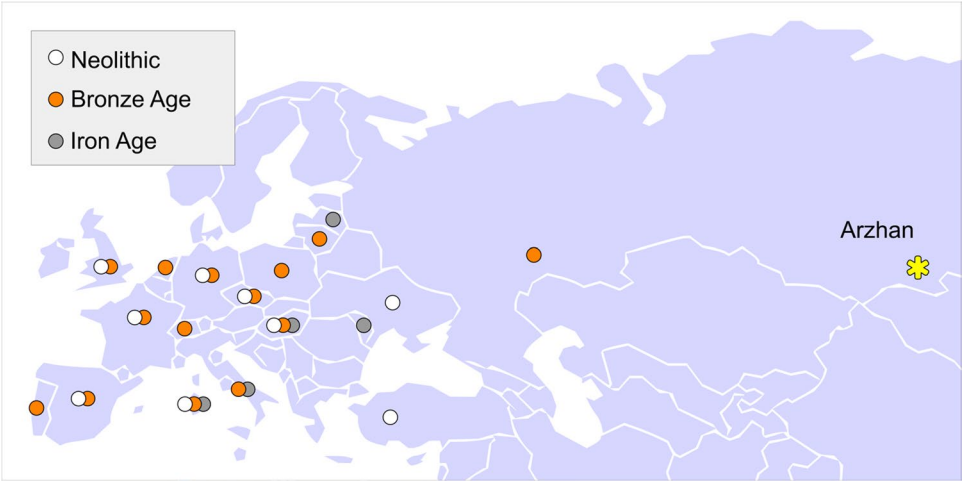

Fig. 3 Map of matching Arzhan haplotypes in previously published ancient complete mitochondrial genome data (Bollongino et al. 2013; Brotherton et al. 2013; Haak et al. 2015; Lipson et al. 2017; Mathieson et al. 2015; Matisoo-Smith et al. 2018; Mittnik et al. 2018; Nepararaczki et al. 2017; Nikitin et al. 2017; Olalde et al. 2018; Olivieri et al. 2017; Schuenemann et al. 2011; Vai et al. 2019)

linked to the relative lack of published ancient genomic data from Asian archeological sites (or a lack of archeological excavations).

Discussion

Burial patterns suggest patrilocality and indicate the presence of specific kinship structures

The typing of STR markers is possible even when attempted using degraded DNA (Ricaut et al. 2004b; Amory et al. 2007; Harder et al. 2012) and the discriminative power of these markers allows reliable distinction between all individuals. STR profiles can also be reliably used to identify parent/child pairs and, in most cases, full-sibling pairs (Zvénigorosky 2018). Kinship analyses performed on 21 autosomal STR markers have, therefore, allowed us to detect first-degree relationships between the individuals buried at archeological sites in the Arzhan complex, along the river Eerbek. We were also able to confirm second-degree relationships when they coincided with parent/child or full-sibling pairs and suggest second-degree relationships when uniparental lineages were concordant.

Paternal lineages, characterized by Y-chromosome haplotypes, were strictly specific to different archaeological sites. All carriers of one haplotype belonging to the Q1b1a3-L330 haplogroup were buried at Eki-Ottug 1, with at least two of them sharing no detectable (close) familial relationship. Bearers of two haplotypes belonging to the R1a-M513 haplogroup were all buried at Eki-Ottug 2. Those haplotypes likely corresponded to more than one lineage, but we have shown that the haplotypes of at least three individuals on this site, ARZ-T6, ARZ-T7 and ARZ-T24, were identical. Because LR results also indicate that ARZ-T6 and ARZ-T2 were closely related, it is possible that the discrepancy between their haplotypes is due to a one-step mutation at one locus in their father. In that case, five individuals of the same paternal line would have been buried at Eki-Ottug 2. This pattern, with all men from one line buried in one location, is typical of patrilocal societies, where newlywed women are expected to reside in their husband's homestead. Consequently, female lines are dispersed with each new generation and male descent groups, sometimes referred to as "clans", become characteristic of one location or one nomadic group (Chaix et al. 2007).

Maternal lineages, characterized by mitochondrial haplotypes, were more diverse and one was present at two sites, Bai-Dag 6 and Bai-Dag 8. This is again consistent with the broader cultural context of Southern Siberia (Chaix et al. 2007), with Y-lineage diversity lower than mitochondrial lineage diversity due to the presence of male clans and female postnuptial mobility between those clans. The burial of three women, ARZ-T5 and her daughters ARZ-T26 and ARZ-T3, is exemplary of this cultural phenomenon. The woman T5 died after the age of 40 and was buried in the same grave as T3, her teenage daughter. She, however, had an older daughter, T26, who died around the age of 25 and was buried at a different site along the river Eerbek. This is concordant with ARZ-T26 having moved to a different group after marriage and ARZ-T3 having died unmarried, due to her young age.

Patrilocality is also apparent in the case of the Eki-Ottug 1 site. In Kurgan 12, ARZ-T21 was the son of ARZ-T22, both having died around the age of 35. This implies that T21 was buried in the same kurgan as his mother, even though he was old enough to have been married. Furthermore, we analyzed the remains of a second man from the same Kurgan, ARZ-T20. This individual carried the same Y-haplotype as T21 and his parents and uncle were buried in Kurgan 6 on the same site. We have shown that T20 was likely the uncle of T21, that T17 and T19 were likely his grandparents and T18 his great-uncle. Two reasons could explain the absence of the father of T21 in our study: either his remains were present in Kurgan 12 but they were not

recovered (archaeological researchers indicate that this is a possibility), or he was not buried in Kurgan 12. If the latter is the case, we can envisage that T20 was the biological uncle of T21 and his adoptive father through a levirate marriage (where a widow marries her late husband's brother), which has been documented in Iron Age and later cultures of the region (Gmyrya 1995). Levirate marriage is also typical of patrilineal societies. This hypothesis would be supported by the discovery of a child of ARZ-T20 and ARZ-T22, either at Eki-Ottug 1 or elsewhere. In any case, it appears that Eki-Ottug 1 includes a six-member family of three men and three women, carrying only one Y-chromosome lineage and three mitochondrial lineages, as expected in a society with patrilocal practices and patrilineal burial preferences.

European and Asian Scythians have a different mtDNA gene pool

Genetic analyses of maternal lineages revealed the presence of both western (H, H14b2, H6a1a1, HV6, HV14a, T2d2, U4a1a, U5a2a1 and U5a1f1) and eastern (D4j7, C4a1a3, C4d, F1b1b and G2a1g) Eurasian lineages equally distributed in our Scytho-Siberian sample ($n = 13/26$ for each) (Fig. 4; Supplementary Table 9). This pattern of admixture was compared with that of a European Scythian group composed of 19 NPR Scythians from Moldova and Ukraine (Juras et al. 2017), 2 Scythians from Moldova (Krzewińska et al. 2018) and 1 Scythian individual from southern Ural (Mathieson et al. 2015) for whom complete mtDNA genomes were also obtained. In this group ($n = 22$), western and eastern mitochondrial haplogroups, respectively, account for about two-thirds ($n = 16/22$) and one-third of the lineages ($n = 6/22$) showing a higher representation of European lineages for an equivalent sample size. Detailed analysis of these haplogroups revealed that only two of them (U5a2a1 and H) are shared between the two Scythian groups, revealing important maternal gene pool differences between them.

Moreover, comparison of whole mtDNA sequences for matching haplotypes in published data showed that the U5a2a1 haplotypes found in the two Scythian groups differ from only two positions. Therefore, ARZ-T4 carried a maternal lineage very close to that of a NPR Scythian (SCY193) (Juras et al. 2017). Near-identical haplotypes were also present in two Bronze Age individuals from the Netherlands (one mismatch), the Czech Republic (four mismatches),

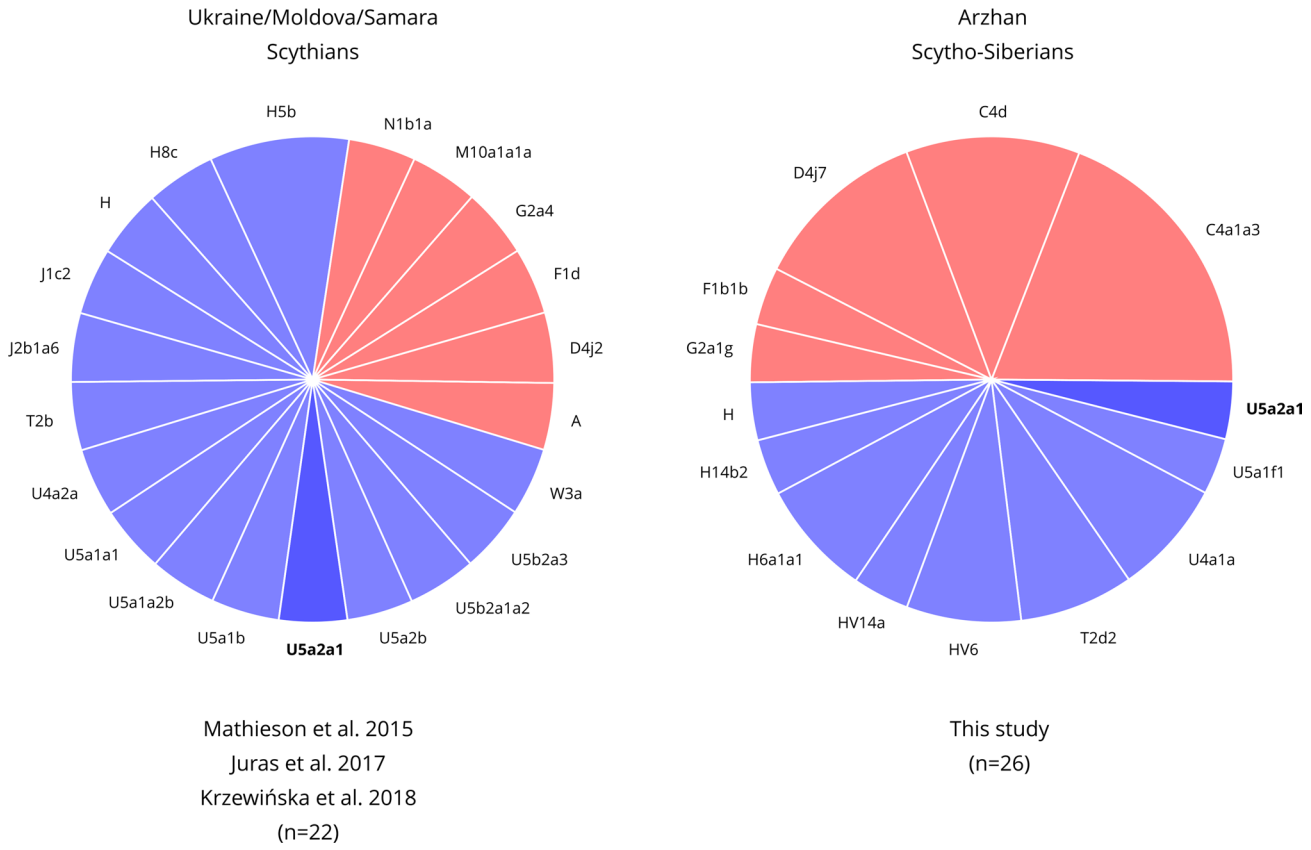

Fig. 4 Proportions of Scythian mtDNA haplogroups. Western (blue) and eastern (pink) Eurasian lineages are equally distributed in the Arzhan Scytho-Siberian sample. The U5a2a1 haplogroup shared between the two Scythian groups studied is in bold

Poland and Portugal (six mismatches) (Olalde et al. 2018), two Bronze Age individuals from Latvia and Lithuania (two mismatches) (Mittnik et al. 2018) and one Late Bronze Age Srubnaya individual (three mismatches) (Mathieson et al. 2015). The coalescence time of U5a2a1 was estimated to be 6377.15 ± 1149.2 YBP which corresponds to the end of the Late Neolithic and the beginning of the Bronze Age in Eurasia (Ning et al. 2015). Also according to Ning et al. (2015), haplogroup U5a2a, which was notably found in a Late Bronze Age individual from the Krasnoyarsk region in Southern Siberia (Keyser et al. 2009), may have a Volga-Ural origin, which is in agreement with our data.

The second mitochondrial haplogroup shared between both Scythian groups was haplogroup H carried by ARZ-T8 and SCY005 (Juras et al. 2017). However, closer comparison showed seven mismatches between the two haplotypes (individual SCY005 is, therefore, not included in Fig. 3). Because haplogroup H and its subclades are frequently found in Europe from the Neolithic period to the Iron Age, 126 individuals whose mitochondrial haplotypes were similar (fewer than six mismatches) to ARZ-T8 were identified across all three periods (Neolithic, Bronze Age and Iron Age) from Spain to the Ukraine, including three Iron Age individuals from Sardinia, Hungary and Latvia (Matisoo-Smith et al. 2018; Mittnik et al. 2018; Vai et al. 2019). Therefore, although a European Scythian and a Scytho-Siberian shared haplogroup H, this does not constitute a definitive connection between their populations.

It, therefore, appears that the mtDNA pool of the Scytho-Siberians analyzed presents significant Western/Eastern Eurasian admixture and that, although it shares that characteristic with European Scythians, there are significant differences in the proportions of Eastern and Western haplogroups. Scytho-Siberians carried more lineages associated with Eastern Eurasia than their European counterparts, testifying to greater Asian influence or ancestry. The lack of whole mitochondrial genomes from ancient remains for more eastern regions or, more generally, for large parts of the Eurasian steppe, prevents us from drawing more precise conclusions concerning Scythian admixture history in Asia.

European and Asian Scythians have a different Y-chromosomal gene pool

To date, few Y-chromosomal lineages have been reported for Scythian or Scytho-Siberian individuals (fewer than twenty Y-haplogroups published). In the present work, the combined use of STRs and SNPs allowed us to obtain Y-haplogroups for 16 Scytho-Siberian male individuals. More than half of them were carriers of the R1a-M513 haplogroup ($n = 9/16$) including two individuals belonging to the R1a1a1b2-Z93 haplogroup or one of its subclades.

Haplogroup R1a-M173 was previously reported for 6 Scytho-Siberian individuals from the Tagar culture (Keyser et al. 2009) and one Altaian Scytho-Siberian from the Sebÿstei site (Ricaut et al. 2004a), whereas haplogroup R1a1a1b2-Z93 (or R1a1a1b-S224) was described for one Scythian from Samara (Mathieson et al. 2015) and two Scytho-Siberians from Berel and the Tuva Republic (Unterländer et al. 2017). On the contrary, North Pontic Scythians were found to belong to the R1b1a1a2 haplogroup (Krzewińska et al. 2018), showing a distinction between the two groups of Scythians.

Haplogroup R1a is a major clade of human Y chromosomal haplogroups that is distributed across Eurasia and showed a major expansion during the Bronze Age (Hollard et al. 2018). It has previously been found in Bronze Age individuals from the Andronovo culture in Southern Siberia (Keyser et al. 2009), in Bronze Age Sintashta individuals (Allentoft et al. 2015) and late Bronze Age Srubnaya individuals (Krzewińska et al. 2018; Mathieson et al. 2015) from Russia, as well as in Bronze Age individuals from the Tarim Basin (Li et al. 2010). This contrasts with the R1b-related haplogroups carried by most of the Afanasievo and Yamnaya males reported to date (Haak et al. 2015; Hollard et al. 2018) and supports the notion of a paternal lineage shift during the Bronze Age (Hollard et al. 2018).

Subclade R1a1a1b2-Z93 has been described as the Asian branch of the R1a1a haplogroup (Pamjav et al. 2012). It was previously found in Bronze and Iron Age individuals from Central Asia and the Altai region (Hollard et al. 2014; Mathieson et al. 2015; Unterländer et al. 2017) and is absent in all ancient central Europeans studied to date.

The seven other male individuals studied in this work were found to carry Eastern Eurasian Y haplogroups Q1b1a and one of its subclades ($n = 6$) and N ($n = 1$). Haplogroup Q1b1a-L54 was previously described in four males from the Bronze Age in the Altai Mountains (Hollard et al. 2014, 2018) and was clearly associated with Siberian populations (Regueiro et al. 2013).

The N-M231 haplogroup emerged from haplogroup K in Southern Asia around 21,000 years BCE, maybe in Southern China (Shi et al. 2013; Ilumäe et al. 2016). Previous studies attested to its presence in samples from Neolithic and Bronze Age in China (Li et al. 2011; Cui et al. 2013). Waves of northwestern expansion of this haplogroup are described as beginning during the Paleolithic period (Derenko et al. 2006; Shi et al. 2013) but traces of this expansion in archeological samples were reported only in two Scytho-Siberian males from the Altai (Pilipenko et al. 2015).

Thus, these results are similar to what we have described for maternal lineages with Scytho-Siberians carrying more paternal lineages associated with Eastern Eurasia than Western Eurasia. The absence of R1b lineages in the Scytho-Siberian individuals tested so far and their presence in the North Pontic

Scythians suggest that these 2 groups had a completely different paternal lineage makeup with nearly no gene flow from male carriers between them.

Conclusion

In this work, we report the first genetic evidence of patrilocal and patrilineal burial practices in Scytho-Siberians from the Tuva Republic. We show that while closely related individuals such as brothers or parents and their underage children are buried together (in the same kurgans or on the same burial sites), sisters appear to be buried separately after they have reached adulthood. This is consistent with the postnuptial mobility of women, which defines patrilocality. This is also supported by the exclusive association of certain Y-chromosome lineages with specific archaeological sites, originating from the burial at the same location, one generation after another, of all members of a paternal "clan". Conversely, in societies with patrilocal practices, mitochondrial lineages are more diverse and are associated with more than one locality or group, which is also supported by the results of this study.

We have also determined that mitochondrial and Y-chromosome lineages among our samples were almost equally divided between haplogroups of Eastern and Western Eurasian origins. Previously studied Scythians from the North Pontic Region showed fewer Eastern Eurasian paternal and maternal lineages. There were also few genetic links between these individuals and their Siberian contemporaries analyzed in this study. While many mitochondrial haplogroups are shared by Western and Eastern Scythians, haplotype-level analyses identified only one shared maternal lineage, underlining the importance of these fine-scale analyses in unveiling ancient population interactions. It, therefore, appears that, although Pontic and Siberian Scythians belonged to the "Scythian world", they do not share common genetic origins, be it from the east or west of the Eurasian steppe. Rather, our results are consistent with a local Bronze Age origin for the Scytho-Siberians of the Arzhan archeological complex.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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