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Nuclear and Mitochondrial DNA Analysis of a 2,000-Year-Old Necropolis in the Egyin Gol Valley of Mongolia

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DNA was extracted from the skeletal remains of 62 specimens excavated from the Egyin Gol necropolis, in northern Mongolia. This burial site is linked to the Xiongnu period and was used from the 3rd century B.C. to the 2nd century A.D. Three types of genetic markers were used to determine the genetic relationships between individuals buried in the Egyin Gol necropolis. Results from analyses of autosomal and Y chromosome short tandem repeats, as well as mitochondrial DNA, showed close relationships between several specimens and provided additional background information on the social organization within the necropolis as well as the funeral practices of the Xiongnu people. To the best of our knowledge, this is the first study using biparental, paternal, and maternal genetic systems to reconstruct partial genealogies in a protohistoric necropolis.

Introduction

In recent years, molecular studies have become widely employed to investigate parentage relationships within burial groups (Fily et al. 1998; Stone and Stoneking 1999; Schultes et al. 2000; Clisson et al. 2002), because morphological indicators of kinship are much less precise than the genetic data potentially available by analysis of ancient DNA. Understanding genetic relationships within and between burial sites helps us to understand the organization of sepulchral places and the origin of human remains recovered (e.g., unrelated individuals or members of a single or a limited number of family groups). This should be the first step of any work devoted to the history of settlement based on the investigation of remains from a cemetery, because every external inclusion in a group of subjects sharing a common parentage may introduce a bias (Crubézy et al. 2000).

In the present study, we examined biological kinship in a necropolis from the Xiongnu period, a culture known mainly through the graves discovered in 1943 by a joint Mongolian-Russian expedition in the Noin-Ula Mountains in northern Mongolia (Rudenko 1970) but also through other funerary sites of the Selenge Basin (Konovalov 1976). The Xiongnu were an ancient nomadic Turkomongolian tribe who were first described in Chinese manuscripts as early as the 4th century B.C. (Minajev 1996). In the 3rd century B.C., Xiongnu tribes rose to great power and created the first empire governed by Central Asian nomads. They ruled over a territory that extended from Lake Baikal in the north to the Gobi desert in the south and from western Manchuria in the east to the Pamirs in the west. During the newly established Han dynasty (206 B.C. to A.D. 220), China expanded its borders, and the Xiongnu empire lost ground (Marx 2000).

According to radiocarbon dating, the Egyin Gol site was used from the 3rd century B.C. to the 2nd century A.D. (i.e., over the whole Xiongnu period). It is located in northern Mongolia, in a cold environment favorable to a good preservation of the DNA (Burger et al. 1999; Leonard et al. 2000). We studied genetic diversity at the Egyin Gol site, first by use of autosomal STRs. Autosomal STRs consist of tandemly organized repeats of short nucleotide patterns (2–6 bp), which are transmitted according to a Mendelian mode of inheritance. These genetic markers took precedence in our study, owing to their excellent power of discrimination for the study of close parentage relationships. They also represent propitious markers for ancient DNA studies because of their small size and because they allow detection of sample contamination (Hummel et al. 2000). Moreover, they can be simultaneously amplified, reducing to an absolute minimum the amount of sample material necessary for kinship analysis. Although both maternal and paternal genetic contributions can be assessed with autosomal markers, such as STRs, we also studied the genetic diversity by typing the nonrecombining part of the Y chromosome, as well as the hypervariable region I (HVI) of the mtDNA. We studied paternal and maternal transmitted polymorphisms to complete the data ob-

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tained by autosomal STR analyses and, above all, to confirm the authenticity of the molecular data obtained from the ancient Egyin Gol specimens. These polymorphisms also provided additional information on the genetic history of the Xiongnu tribes.

Material and Methods

Site

The necropolis is located in the Egyin Gol valley near the Egyin Gol river, ~10 km from its confluence with the Selenge, a main tributary of Lake Baikal (fig. 1. The valley's position is −49° 27′ N, 103° 30′ E, and it has a continental climate, with an average annual temperature of −1°C. The winter (October to April) is cold (with temperatures often dropping to −30°C in January and February), whereas Summer (July to September) is pleasant (with temperatures sometimes as high as 22°C). Precipitation is light (300–400 mm per year). Because of its relatively high altitude (885 m), the valley floor is covered with snow from mid-November to April, and ice thickness on the Selenge reaches 1.8 m during this period. Permafrost was found in some areas by the geologists who were present on the site.

From 1997 to 1999, the burial site was wholly excavated by a French-Mongolian expedition, under the sponsorship of UNESCO, after preliminary boring revealed the excellent preservation of the graves (Crubézy et al. 1996). The necropolis comprised a total of 103 graves, among which 84 were excavated by the archaeological mission. The 19 remaining graves had been explored before the arrival of the mission in Mongolia, and no data were available on these spots. Graves were organized on both sides of a small depression on the river valley, in four sectors that were designated "A," "B," "C," and "D" (figs. 2, 3, and 4). The southern sector (A) was composed of four double graves (32/32A, 33/33A, 37/37A, and 38/38A), each of which contained two sets of remains that were probably buried within the same period (Murail et al. 2000). Grave 27 was surmounted by a standing stone and was found to conceal exceptional furniture. In eight graves (18, 47, 49, 54, 59, 69, 83, and 85), secondary deposits (bones of very young children) were found beside the deceased.

The associated funeral material was of great interest and allowed us to link the necropolis to the Xiongnu culture (Crubézy et al. 1996). Bone samples from 31 specimens scattered across the necropolis were dated by carbon 14 (^{14}C) determinations. The projection of the 31 mean values corresponding to each radiocarbon datum were linearly extrapolated, by use of UNIRAS software, to establish clines, which are represented by shades of gray on the necropolis map (fig. 5). This diagram suggests a topographical development of the burial ground, with a progressive expansion from south to north. Indeed, grave 28, slightly remote in the southern sector, was found to be the oldest of the necropolis, followed by grave 27 and the double burials. Therefore, sector A is probably the oldest, even though some graves located around it appear more recent. Sectors B and C seem more recent, although some graves situated near the center of sector B might have been implanted earlier.

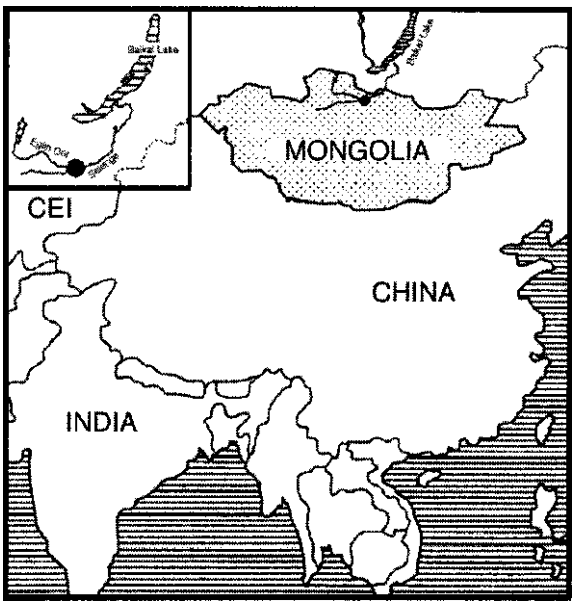

Figure 1 Location of the Egyin Gol site

The graves were at a depth of 2–5 m and were delimited by stones set in circles with diameters of several meters. They were protected by several layers of stones included in a loessial sediment. Chests and coffins were perfectly visible and relatively well preserved, as were most of the artifacts made of perishable matter (e.g., horn and bone) that were found in the graves.

Samples

Excavation of the 84 unexplored graves resulted in the recovery of 99 human skeletons (including double graves and secondary deposits). In most instances, complete and articulated skeletons were recovered, but, in some cases (e.g., secondary deposits or looted graves), numerous bones were missing or severely damaged. Most of the skeletal material was in an excellent state of preservation, as was confirmed by the mineral/organic composition of the bones, which did not differ significantly from that of contemporary bones. For instance, the mean ± SD crystallinity index was 0.07 ± 0.02, close to that of ice-preserved ancient bones (Person et al. 1996). Mean ± SD percentages of carbon and nitrogen were 13.8% ± 0.8 and 4.2% ± 0.2, respectively,

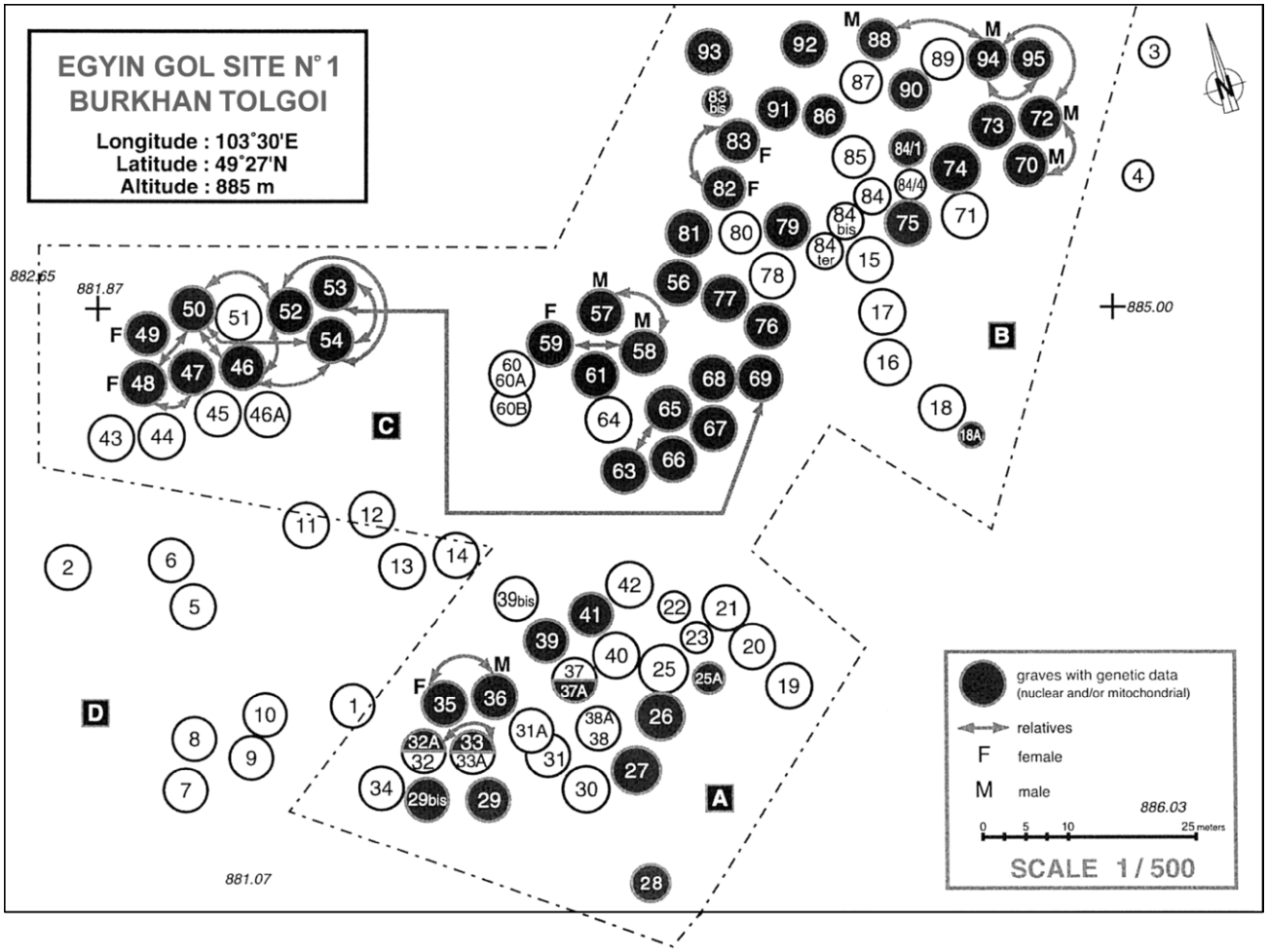

Figure 2 Map of the necropolis showing the autosomal STR data. Graves are represented by circles. Letters A, B, C, and D refer to the four sectors distinguished in the present study. Dotted lines define the boundary of these sectors.

quite similar to those of reference material obtained from surgical samples.

Sex was established according to the methodology developed by Murail et al. (1999). Age at death was estimated using dental calcification for the children and epiphyseal fusion for the adolescents (Crubézy et al. 2000). The age distribution of the skeletons did not correspond to expected human mortality patterns (for a 30-year life expectancy), since the 0–9-year-old group was underrepresented. Moreover, the total number of subjects was surprisingly low for such a long period of use (at least 400 years). These findings suggest that only specific members of the Xiongnu community were buried in this necropolis.

Samples for DNA analysis were collected from such skeletal elements as astragalus, calcaneus, rib, vertebrae, and teeth during the first year of the excavation; samples from more substantial long cortical bones, such as femur, tibia, and humerus, were collected during the next 2 years. After authorization from the Mongolian authorities, bone samples from 80 skeletal remains (taken, when possible, in duplicate) were transferred to Strasbourg, France, under appropriate storage conditions. On arrival in the laboratory, highly damaged bones (showing extreme fragility and porosity) and severely deteriorated teeth were excluded from the genetic analysis.

DNA Extraction and Purification

DNA was extracted from 79 bone samples corresponding to 62 individuals (some individuals were typed from two independent samples).

To eliminate surface contamination, the outer surface of the bones was removed to almost 2–3 mm of depth with a sanding machine (Dremel). Powdered bone was generated by grinding bone fragments under liquid nitrogen in a 6800 Freezer Mill (Fischer Bioblock) or with a drill fitted with a surgical trepan to avoid overheating.

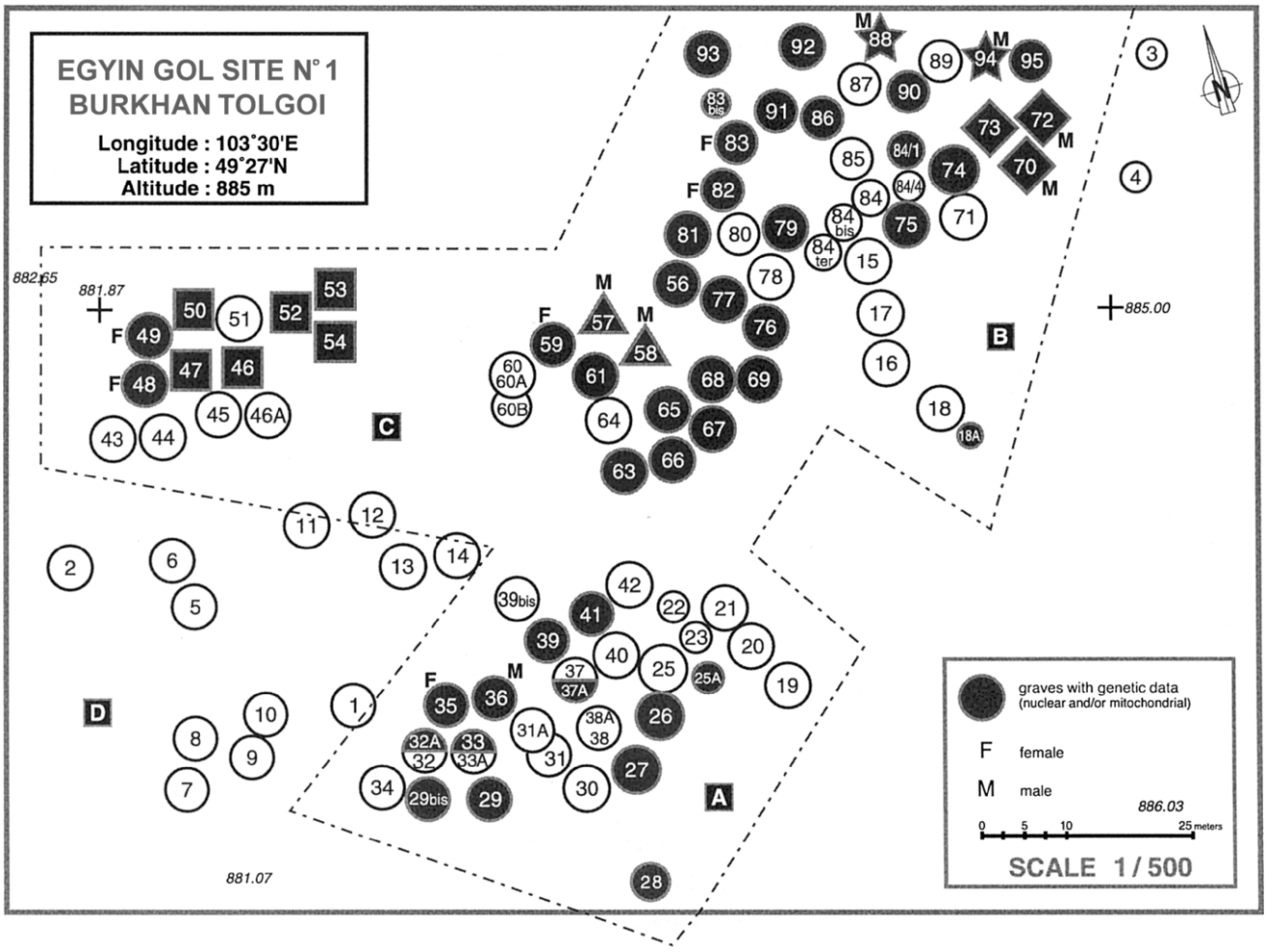

Figure 3 Map of the necropolis showing the Y chromosome STR data. Graves containing specimens of the same patrilineage are represented by an identical geometric figure.

DNA was carefully extracted according to a published protocol (Fily et al. 1998). In brief, 2 g of the pulverized material was incubated at 50°C overnight in 5 ml of a solution containing 5 mmol EDTA, 2% SDS, 10 mmol Tris HCl (pH 8.0), 0.3 mol sodium acetate, and 1 ml proteinase K/ml. A phenol/chloroform/isoamyl alcohol (25/24/1, v/v) extraction was performed on the supernatant. The aqueous phase was then purified with the Cleanmix kit (Talent), which relies on the strong affinity of DNA to silica in the presence of guanidium thiocyanate. After the elution step with 400 μl sterile water, the DNA was concentrated by passing through a Microcon YM30 filter (Millipore).

To ensure the accuracy and reliability of the results, all samples were amplified (for each marker) at least six times (more when apparent homozygotes were found by autosomal STR analysis) from three independent DNA extracts and, when possible, from two different bones of the same individual.

Autosomal STR Analysis

Autosomal STRs were amplified using the *AmpFl*STR profiler Plus kit (Applied Biosystems). Nine STRs (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820) and the sex determination marker amelogenin were simultaneously amplified.

PCRs were performed according to the manufacturer's protocol (Applied Biosystems), except that 37 cycles were used instead of 28 in a reaction volume of 10 μl, thereby reducing the volume of the DNA samples and improving the amplification yield.

For three samples (57, 58, and 59), further analyses

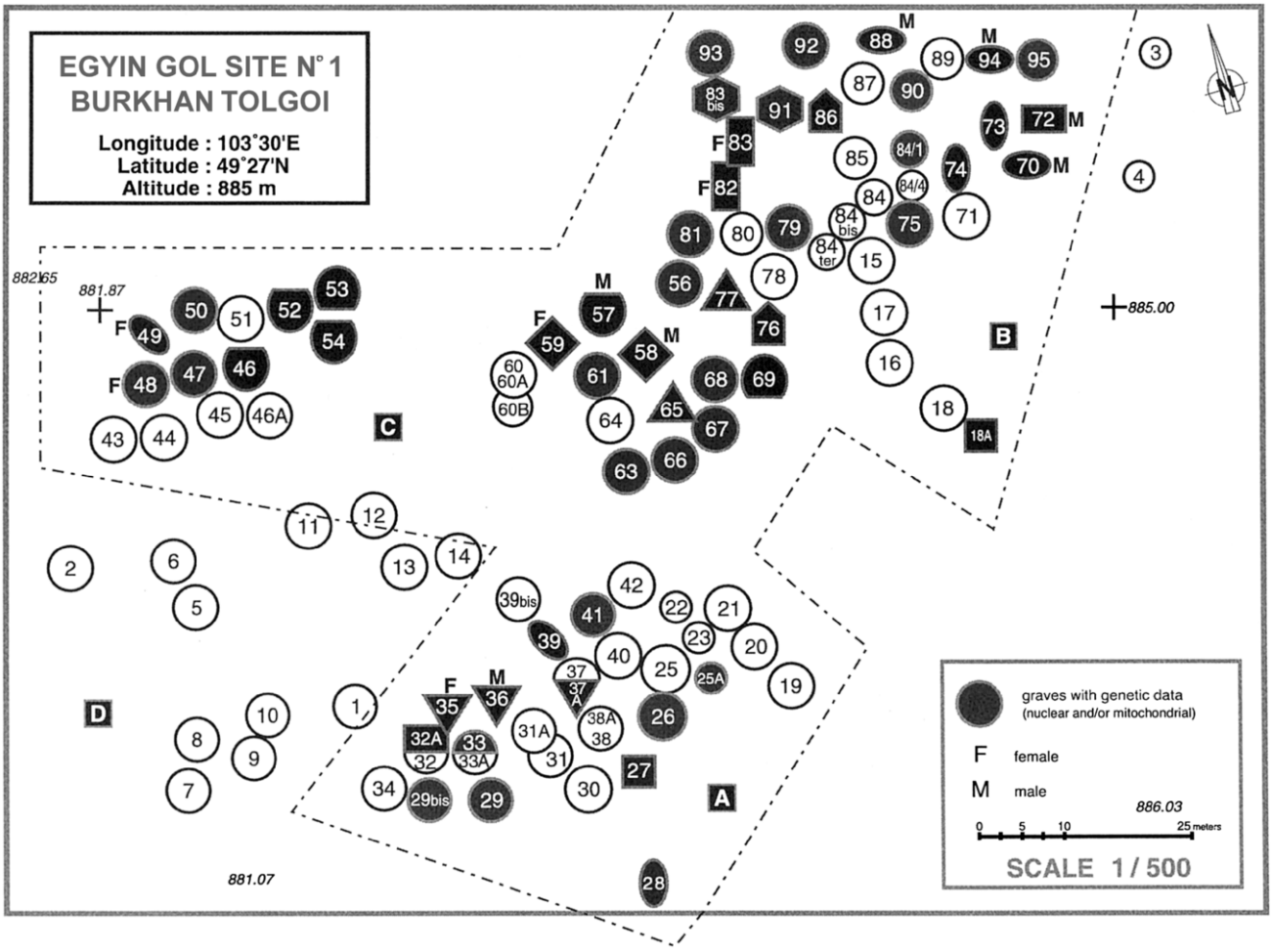

Figure 4 Map of the necropolis showing the mtDNA sequences data. Graves containing specimens of the same matrilineage are represented by an identical geometric figure.

were performed using the *AmpFl*STR SGM Plus kit (Applied Biosystems), which allows the simultaneous amplification of 10 STR loci (4 more than with the previous kit). The genetic relationships between individuals were tested by pairwise comparison of the profiles.

Y Chromosome STR analysis

The DNA of male individuals was analyzed at eight Y chromosome STR loci. Six of them (DYS19, DYS389-II, DYS390, DYS391, DYS393, and DYS385) were coamplified in a multiplex reaction, using the Y-Plex6 kit, according to the manufacturers' recommendations (ReliaGene Technologies). The two others (YCAII and DYS392) were amplified by singleplex PCR. Primer sequences were those described by de Knijff et al. (1997). For PCR amplification (using a Biometra thermocycler), the following conditions were used: predenaturation at 94°C for 3 min; 30 annealing cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 90 s; and a final extension at 72°C for 7 min. The allele nomenclature was the one recommended by the International Society of Forensic Genetics (Gill et al. 2001).

mtDNA Analysis

The HVI of the mitochondrial control region was amplified and sequenced from nucleotide positions 16009 to 16390 (Anderson et al. 1981), using primers L15989 and H16410 (Gabriel et al. 2001). When no amplification was obtained with these primers, presumably because of DNA degradation, the additional primers H16239 (Ivanov et al. 1996) and L16190 (Gabriel et al. 2001) were used to amplify the HVI fragment in two steps. PCR was performed with Ampli*Taq* Gold polymerase, as follows: predenaturation at 94°C for 10 min; 38 annealing cycles at 94°C for 30 s, 48°C or 51°C for 30 s, and 72°C for 45 s; and final extension at 72°C for

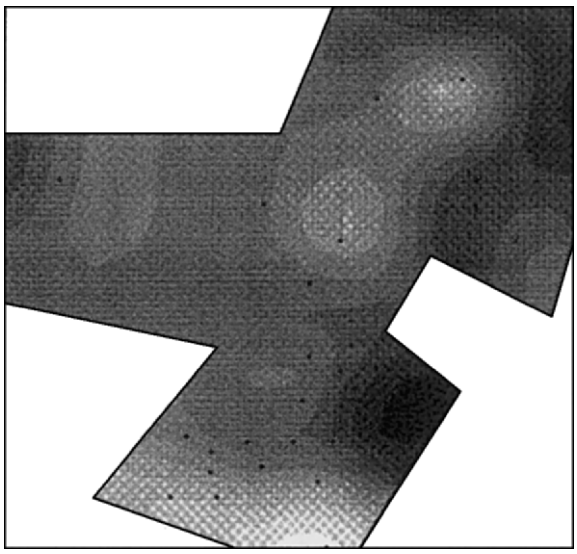

Figure 5 Radiocarbon dating map

10 min. Amplification products were checked on a 1% agarose gel and purified with Microcon-PCR filters (Millipore). The sequence reaction was performed with the same primers on each strand with the ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems).

Amplification Product Analysis

PCR products were analyzed on an ABI Prism 3100 (Applied Biosystems) automated DNA sequencer. Fragment sizes were determined automatically by use of GeneMapper software and were typed by comparison with allelic ladders (provided in the kits or obtained by the mixture of previously sequenced samples for the most common alleles). mtDNA sequences were analyzed using the Sequencing Analysis and Sequence Navigator software packages.

Measures Taken to Avoid Contamination

Because the possibility of performing genetic analyses had been considered before beginning the archaeological work, precautions were taken to reduce contamination during excavation and curation, for example, samples were handled with gloves by a reduced number of anthropologists wearing face masks. To check for possible modern contamination, the DNA extracted from saliva samples of all people handling the material or working in the laboratory was genetically typed and then compared with the profiling results of all ancient samples.

The entire process of DNA extraction and PCR amplification was performed in an isolated laboratory dedicated to work with ancient DNA, where all staff wore lab coats, face masks, and gloves and where strict cleaning procedures were respected (frequent treatment with DNAse Away and UV light and frequent change of gloves). Autoclaved disposable plasticware, dedicated reagents, and pipettes with aerosol-resistant tips were used; extraction and template blanks were included in every PCR assay; and positive PCRs were never performed. Multiple extractions from the same samples were undertaken at different times, and PCR products were never brought into the ancient DNA laboratory.

Results

Autosomal STR Analysis

Of the 62 individual remains analyzed by multiplex amplification, 8 DNA samples (from graves 32, 34, 51, 60, 78, 83bis, 84bis, and 85 [fig. 2]) appeared severely degraded, since no amplifiable product could be obtained (from at least three independent extracts). One sample (from grave 18) was excluded from further analyses, because it was considered a likely case of contamination (the multiallelic profile matched that of one of the staff, despite multiple independent extractions of this vertebral sample). Four other DNA samples (from graves 26, 27, 67, and 81) were found to contain too few template DNA molecules to provide reproducible results (data not shown). The remaining extracted samples gave 49 more or less complete allelic profiles. Consensus data are reported in table 1. In most cases, these 49 DNA profiles were obtained from diaphyses, but vertebrae provided the genetic profiles in 4 cases, calcaneus in 1 case, and clavicle in 1 case. Long cortical bones (such as femur, tibia, and humerus) thus appeared to be good sources of ancient DNA, whereas rib samples and other thin bones did not. When apparent homozygotes were obtained, amplifications were repeated as many as eight times to avoid the possibility that one allele of an heterozygote was not detected.

Morphological and molecular typing results for sex determination were in accordance with each other, which is indicative of authentic ancient DNA extracts. We used the amelogenin locus to deduce the sex of six juvenile skeletons for which morphological indicators of sex were absent: three were male (graves 18A, 36, and 84.1), and three others were female (graves 41, 83, and 91). For three other adolescent remains (graves 74, 75, and 76), even molecular determination of sex was ambiguous, despite nearly complete STR profiles.

Comparison of the profiles in pairs allowed us to identify a family composed of the father (grave 57), the mother (grave 59), and one child (grave 58). For these three specimens, all nine STR loci were amplified; and, at each locus, alleles of the child profile could have been assigned to the genotypes from either grave 57 or grave 59 (table 1). To confirm this result, further analysis was

Table 1

Consensus Allelic Profiles of 49 Specimens Recovered from the Egyin Gol Necropolis

		Allele(s) at Marker								
Grave	Amel[a]	D3S1358	VWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
18A	XY	15?/16	14/17	19/25	11/13	?	14/17	12/12	8/11	?
25A	XY	15/16	16/17	20/21	13/13	30/31	14/19	10/11	10/11	10/11
28	XY	15/16	14/15	23/26	10/11	30/31	14/16	9/11	10/11	10/11
29	XY	15/16	19?	24/25	13/16	31.2/?	13/14	10/12	8/8	8/8
29bis	XY	14/15	18/19	22/22	?/14	30/32.2	14/15	11/13	11/12	8/11
32A	XY	15/16	15/17	22/23	13/14	29/30	16/16	11/11	9/9	11/13
33	XY	15/15	17/18	22/23	13/13	28/30	12/16	11/11	9?	11?
35	XX	15/17	15/17	22/24	14/16	28.2/33.2	13/15	11/12	11/11	12/12
36	XY	15/16	15/17	22/25	14/16	28.2/31	13/15	11/11	8/11	8/12
37A	XX	15/16	18/18	24/25	13/14	29/30	?/21	8/11	9/?	10/12
39	XX	16/17	17/19	22/23	13/14	28/30	14/15	8/11	8/8	8/12
41	XX	15/16	16/17	24/28	?	29/32.2	16/16	10/11	8/12	8/8
46	XY	16/18	16/18	23/24	12/13	29/30	13/14	10/12	9/13	10/12
47	XY	14/15	16/17	22/23	13/14	30/33.2	14/14	11/13	8/9	8/10
48	XX	15/16	16/17	22/24	10/13	30/31.2	14/14	11/12	9/12	8/12
49	XX	15/16	16/17	24/24	13/15	28/32.2	?	11/11	8/11	10/11
50	XY	16/18	17/18	23/24	13/14	29/30	14/17	10/12	9/10	10/11
52	XY	16/18	16/18	24/24	14/14	29/29	13/14	11/12	9/11	8/10
53	XY	15/17	16/17	23/24	12/13	29/32.2	15/22	10/11	10/13	11/11
54	XY	15/16	16/18	20/24	12/13	29/29	13/17	11/12	10/13	8/11
56	XX	15/17	14/17	23/25	14/16	30/31	16/20	10/11	8/12	10/10
57	XY	16/16	16/17	23/24	13/14	30/30	15/16	11/11	10/11	8/9
58	XY	15/16	14/17	22/23	12/14	30/31	13/16	11/12	8/11	8/12
59	XX	15/15	14/18	22/22	12/15	30/31	13/15	12/12	8/10	10/12
61	XX	15/15	18/18	21/23	8/10	?/30	12/21	11/12	8/13	8/11
63	XX	15/17	16/17	24/26	12/16	30/32.2	15/16	7/12	10/10	10/11
65	XY	15/16	17/19	21/26	14/16	29/32.2	14/15	12/13	10/12	8/10
66	XX	15/16	18/19	23/24	10/16	29/30	14/16	12/12	9/12	8/10
68	XX	15/16	17/18	24/24	13/15	30/32.2	21/22	11/13	9/11	11/13
69	XY	15/17	15/16	23/23	10/12	29/30	15/15	13/13	10/10	10/11
70	XY	15/16	16/17	22/23	13/14	30/32.2	17/19	9/11	8/10	8/10
72	XY	15/16	16/17	23/24	10/14	30/32.2	14/17	11/11	10/10	10/10
73	XY	16/17	17/19	18/22	13/13	30.2/32.2	14/19	10/13	8/8	11/11
74	?	15/17	16/19	21/24	12/13	29/30	13/15	10/11	8/9	8/10
75	?	16/18	14/16	22/26	14/15	29/31	16/17	9/11	10/11	9/12
76	?	15/16	15/16	21/24	13/14	29/29	14/22	10/10	(8)/11[b]	8/10
77	XX	15/17	18/19	21/25	10/12	32.2/32.2	14/16	11/12	9/11	8/11
79	XX	16/18	18/19	21/24	14/14	31.2/32	14/15	11/12	8/9	8/12
82	XX	16/16	14/17	19/24	13/14	30/32.2	13/14	10/11	11/14	10/13
83	XX	16/17	16/17	23/?	12/14	29/30	18/?	10/10	10/14	10/10
84.1	XY	15/16	16/17	19/25	13/14	28/28	?/17	11/12	10/11	11/12
86	XX	15/16	16/17	24/25	13/14	30/31.2	13/15	13/13	8/9	12/13
88	XY	15/17	15/17	23/24	12/14	29/30	14/14	10/11	8/10	8/12
90	XX	15/16	18/18	23/23	13/14	31/32.2	16/16	11/12	10/11	11/12
91	XX	15/16	16/17	23?/26	13/14	29/30	16/17	9/?	10/11	9/10
92	XY	16/16	15/16	21/25	12/16	29/30	19/20	10/11	10/11	10/11
93	XX	16/17	16/18	22/24	13/13	29/31.2	15/22	11/12	8/10	8/8
94	XY	16/17	14/17	18/24	12/14	30/31	14/15	11/11	10/10	10/12
95	XY	16/17	16/17	21/24	12/13	29/30	15/21	11/11	8/8	8/12

Note.—Question marks denote alleles that could not be clearly amplified for the locus in question.

[a] Amel = amelogenin.

[b] Allele noted in parentheses to indicate that ambiguity could not be eliminated even after reiteration of the experimentation.

Table 2

Y Chromosome STR Haplotypes Determined for 27 of the Ancient Male Specimens

	Allele(s) at Marker							
Grave	DYS19	DYS390	DYS391	DYS392	DYS393	YCAII	DYS385	DYS389II
25A	14	–	11	14	14	–	–	–
26	14	24	10	11	14	18/22	12/19	–
27	–	–	10	11	14	22/22	–	–
28	15	24	10	11	14	19/19	–	30
32A	–	23	10	–	14	–	–	–
36	16	23	10	11	13	–	–	–
46	15	24	10	11	13	22/23	12/15	29
47	15	24	10	11	13	22/23	12/15	–
50	15	24	10	11	13	22/23	12/15	29
52	15	24	10	11	13	22/23	12/15	29
53	15	24	10	11	13	22/23	12/15	–
54	15	24	10	11	13	22/23	–	–
57	17	23	10	11	14	22/24	11/20	29
58	17	23	10	11	14	22/24	11/20	29
65	16	24	11	11	13	–	–	–
69	14	23	11	13	13	18/21	11/13	–
70	16	25	11	11	13	19/23	11/14	31
72	16	25	11	11	13	19/23	–	–
73	16	25	11	11	13	19/23	–	–
76	14	–	–	13	13	23/23	–	–
81	14	23	–	14	13	–	–	–
84.1	14	24	10	16	14	18/20	–	–
84bis	–	23	11	–	11	19/19	–	–
88	14	25	–	14	15	18/23	14/14	–
94	14	25	10	14	15	18/23	14/14	–
92	13	24	10	15	13	19/20	15/17	29
95	15	24	11	14	12	19/21	13/20	28

Note.—Dash denotes that an allele could not be amplified for the locus in question.

performed on these three samples, using the *AmpFl*STR SGM Plus Kit (PE Biosystems). This additional analysis confirmed and completed previous results, with one allele contributed from each parent (data not shown), and proved the parental relationships.

It was also possible to determine other familial relationships; for instance, the child from grave 36 is probably the son of the female individual buried near him (grave 35), since the genotypes of these two subjects shared a common allele at each of the nine loci tested. No putative father was found among the profiles of table 1. In the same manner, the genetic profile of the male skeleton retrieved from grave 50 shared one allele at each locus with individuals from graves 46, 52, and 54 and is probably the father of these three individuals. It also shared eight alleles with individuals from graves 48, 65, and 66. Individual profiles from graves 46 and 48 and from 47 and 48 also indicated a parent/child relationship, with one common allele at each locus, as did profiles from the following pairs of graves: 63 and 65, 32A and 33, 70 and 72, 72 and 94, 88 and 94, and 93 and 95. Other individuals could have been closely related parents: the two adolescents from graves 74 and 76 shared one allele at seven or eight of the nine STR markers, as did those from the following sets of graves: 46, 52, and 54; 53 and 54; 50 and 52; 82 and 83; 53 and 69; 65 and 66; and, finally, 94 and 95. The incomplete genotyping of some samples probably hampered the search for other familial relationships.

Y Chromosome STR Analysis

To identify male lineages, an analysis of polymorphic STR systems located on the male-specific part of the Y chromosome was performed. Eight Y-specific STRs were typed and used to construct haplotypes. Of the 35 individuals who were male or whose sex could not be determined, 27 could be typed at more than three loci (table 2). Among them, 18 different haplotypes could be identified (even when incomplete, most haplotypes could be differentiated). The loci DYS385 and DYS389II often could not be amplified, probably because they are expressed in the higher molecular weight range. Such an inverse dependence of the amplification efficiency on the size of the segment to be amplified is typical of DNA retrieved from ancient remains and results from damage and degradation of the DNA.

The most common haplotype was observed in six male specimens buried in the C sector (graves 46, 47, 50, 52, 53, and 54 [fig. 3; table 2]), suggesting a grouping of

individuals belonging to the same paternal lineage. Three of these individuals (graves 46, 52, and 54), shared, in addition, the same mtDNA sequence (see below); they were therefore considered to be brothers. Since their autosomal allelic profile showed one allele in common, at each locus, with that of the male from grave 50, the latter was considered to be their father. The others (from graves 47 and 53) were probably more distant paternal relatives (half-brothers, nephew and uncle, or grandfather and grandson).

The study of these uniparentally inherited STR markers also showed that the individual in grave 58 had the same Y haplotype as his putative father in grave 57: all seven regions of the Y chromosome tested matched, confirming the autosomal typing results (paternity). Three other adult individuals, buried in the northern part of the necropolis (graves 70, 72, and 73) were found to share an identical six-locus haplotype (fig. 3; table 2). Two of them (from graves 70 and 72), who shared at least one common allele at each locus (see "Autosomal STR Analysis" section), may be considered to be a father and son. Close to them, two other specimens (graves 88 and 94) sharing an identical six-locus haplotype (table 2) and half of their autosomal alleles (table 1) were supposed to be genetically linked by a father/son relationship.

Some DNA samples failed to yield any amplification results. Among them were the DNA extracted from the adolescent remains from graves 74 and 75. Since the sex of both specimens could not be clearly established (either morphologically or genetically), one can suppose that these adolescents were female individuals. Conversely, the individual from grave 76 gave incomplete but consistent results, suggesting that this specimen was a male.

mtDNA Analysis

Sequence variation in the HVI was investigated in 56 of the ancient specimens. Reproducible HVI sequences were obtained for 46 of them. Among these 46 individuals, a total of 28 different sequences, defined by 44 variable positions, were identified (table 3). The most frequent mtDNA type was scored in four individuals: three of them (from graves 46, 52, and 54) belong to the C sector and were considered to be brothers, since they also shared an identical Y haplotype (table 2); the fourth (from grave 57) was the father of the little family identified in the middle of the necropolis. Twelve other mtDNA types were shared by at least two individuals, the remaining 15 mtDNA types being represented by just one individual. Two of these unique mtDNA types (from graves 48 and 61) differed from the most frequent one (from graves 46, 52, 54, and 57) by a single mutation and may be considered to arise from the same maternal lineage.

Differences between sequences could be mostly attributed to transitional substitutions (90%) and concerned mainly the pyrimidines; however, at position 183, transversions occurred in several different sequences. The C→T transition at position 16223 (nucleotide position in the reference sequence of Anderson et al. [1981]) was shared by most of the ancient specimens (35/46 individuals), as was the T→C transition at position 16362 (27/46 individuals). Two instances of a transition and transversion at the same site were also observed: position 16129 showed both G→A and G→C mutations, and position 16232 showed both C→T and C→A mutations, as previously reported by Kolman et al. (1996). Insertion of a C residue was found once, between positions 16193 and 16194.

Polymorphic sites shared by two individuals allowed us to confirm or to reconsider close genetic affinities between some specimens. For instance the individuals from graves 59 and 58 showed an identical HVI sequence, confirming the maternal relationship deduced from the autosomal STR typing. The child of grave 36 had the same mtDNA sequence as her presumed mother (grave 35) and the female specimen from grave 37A (double grave) (fig. 4). Other complete matches were noted between individuals from graves 83bis and 91; 65 and 77; 32A and 72; 28, 73, and 74; 70, 88, and 94; 53 and 69; 83 and 82; and 76 and 86 (table 3), even though autosomal STR data did not always clearly show any parental relationship. The male individuals from graves 88 and 94, who were thought to be a father-son pair, since they share at least one allele at every locus (see the "Autosomal STR Analysis" section) and an identical five-locus haplotype, may in fact be brothers, since their mitochondrial haplotype is identical. Similarly, the two adolescents from graves 74 and 76 who were thought to be siblings are obviously not, since they do not share an identical HVI sequence.

Heteroplasmies were found within the mtDNA sequences of individuals from graves 39, 41, and 18A. Specimens from graves 39 and 18A were grouped, respectively, with individuals from graves 49 and 27, since the remaining nucleotides perfectly matched each other; a comparison with nuclear data to decide whether or not graves 18A and 27 contained maternal relatives was not possible, because of the incompleteness of the autosomal DNA profiles. Individuals from graves 39 and 49 were considered to be maternal relatives on the basis of the genotyping results.

Although the mtDNA sequences obtained could not be assigned with certainty to mtDNA haplogroups (since they encompassed only the HVI of the control region), three (A, C, and D) of the four major haplogroups observed in Native American (Torroni et al. 1993) and Siberian (Starikovskaya et al. 1998; Schurr et al. 1999) populations were detected in the ancient samples tested

Table 3

mtDNA HVI Sequences of the Egyin Gol Specimens

			Nucleotide Differences from the Reference Sequence																																							
			16066	16069	16082	16086	16092	16093	16126	16129	16136	16179	16183	16189	16192	16193i	16213	16217	16223	16227	16232	16239	16243	16245	16249	16256	16261	16270	16274	16278	16288	16290	16291	16293	16298	16300	16304	16311	16319	16327	16357	16362	16365	16368
Grave	Sex	Hap[a]	A	C	C	T	T	T	T	G	T	C	A	T	C	–	G	T	C	A	C	C	T	C	T	C	C	C	G	C	T	C	C	A	T	A	T	T	G	C	T	T	C	T
68	F	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.
83bis	I	A	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	C	.	.
91	I	A	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	C	.	.
93	F	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	A	.	.	.	.	.
37A	F	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	C	.	.
35	F	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	C	.	.
36	I	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	C	.	.
63	F	A	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	A	.	.	C	.	.
84.1	M	B4b	.	.	.	C	.	.	.	.	C	.	C	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
39	F	C	.	.	.	.	.	Y	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.
49	F	C	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.
50	M	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	T	.	.	.	.
56	F	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	T	C	.	.	.
47	M	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	.	.	.
66	F	C	.	.	.	.	.	C	.	A	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.
61	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
41	M	D4	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
65	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
77	F	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
32A	M	D4	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
72	M	D4	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
48	F	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C
46	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
52	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
54	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
57	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
28	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.
73	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.
74	I	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.
70	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
88	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
94	M	D4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
95	M	U2	.	.	.	.	.	.	.	C	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
53	M	D5/ D5a	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
69	M	D5/ D5a	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
25A	M	G2a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.
83	I	F1b	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
82	F	F1b	.	.	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
58	M	F1b	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.
59	F	F1b	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.
27	M	J1	.	T	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
18 A	M	J1	.	T	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
92	M	M	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
90	F	M	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
76	F	U5a1a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
86	F	U5a1a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.

NOTE.—Polymorphic nucleotide sites are numbered according to the report by Anderson et al. (1981). Dots (.) indicate identity with the reference sequence. Dash (–) indicates nucleotide insertion between nucleotide positions 16193 and 16194.

[a] Hap = haplogroup.

(with haplogroup D being the most prevalent). A few sequences belonging to subclusters B4b, D5 or D5a, F1b, J1, G2a, U2 or U5a1a—and some that probably belonged to cluster M—were also observed (Richards et al. 2000; Yao et al. 2002). No member of the major European cluster H, which occurs in >40% of most European populations (Richards et al. 1996) was found. Interestingly, some of the haplotypes reported here are similar to those found in previous studies of the area (Kolman et al. 1996; Comas et al. 1998).

Discussion

In the present article, partial genealogical reconstruction was obtained using biparental, paternal, and maternal genetic systems in a sample of 62 human skeletal remains

exhumed from a cemetery dating from >2,000 years ago. To the best of our knowledge, no equivalent molecular analysis has been undertaken so far. Such a study was possible because the Egyin Gol necropolis was mainly composed of relatively well-preserved skeletons. Indeed, the climatic conditions (cold and dry) and the archaeological context (architectural structure of the graves) encountered at this site had undoubtedly protected the recovered specimens against DNA degradation. Regarding DNA retrieval, PCR amplification results showed three kinds of samples, as described elsewhere (Burger et al. 1999): (i) samples in which sufficient DNA molecules were preserved and for which definite and reproducible genotypes or haplotypes could be determined; (ii) samples in which DNA could be detected sporadically but without reproducible results; and (iii) samples in which no DNA (or almost none) was preserved.

The choice of autosomal STR markers as a first approach for analyzing individual remains of the Egyin Gol necropolis was based on (i) their high discriminatory power, in comparison with mtDNA analysis, as a means to investigate close familial relationship; (ii) their small size, which facilitates amplifications in old or degraded DNA; (iii) the possibility of amplifying several of them simultaneously from minute amounts of DNA; and (iv) their ability to indicate a result's authenticity (notably by comparing amplified products to the profiles of all persons involved in the investigation).

The multiallelic DNA profiles obtained for 49 of the ancient specimens were compared with each other. A direct parental link was considered plausible if a pair shared an allele at each of the nine loci tested; in this manner, a total of nine pairs were identified as representing possible parent-child relationships. Moreover, we could identify three children thought to have a common parent (individuals from graves 46, 52, and 54, fathered by the individual from grave 50). The traditional parentage trio, with both parents, was encountered only once. This is not surprising, considering that not all profiles were complete and that the number of inhumations that occurred throughout more than four centuries (the duration of the necropolis's use) is relatively small.

To verify the accuracy of the biological relationships deduced from autosomal STR data and to gain a higher power of discrimination, the study of nonrecombining marker systems, such as Y chromosome STRs and mtDNA was undertaken. These analyses confirmed the child or sibling status of some individuals (graves 35 and 36; 57, 58, and 59; 50, 46, 52, and 54; 70 and 72; and 88 and 94) and the close genetic relationship between some others (graves 53 and 54, 82 and 83, and 53 and 69). In one case, however, discordant results between the biparental and the two uniparental systems were observed. Indeed, the multiallelic profiles obtained from the skeletal remains recovered from graves 94 and 95 supported a biological relationship between them. Nevertheless, because these two "relatives" had neither the same Y haplotype nor the same mtDNA sequence, we had to consider the possibility that the two specimens were genealogically unrelated (unless each of the two sets of parents were siblings). For other pairs of individuals (from graves 47 and 48, 63 and 65, 65 and 66, and 93 and 95), the validation of a close parental link was not possible without knowing which was the parent and which was the child.

Among other results, the Y chromosomal STR analysis revealed that one of the defined topographical sectors was exclusively composed of males of the same patrilineage (the individuals from graves 48, 49, and 51 were female, and no bone samples were available for specimens from graves 43–46A). The other males found to share the same Y haplotype (graves 57 and 58; 88 and 94; and 70, 72, and 73) were also buried close to each other. Such a grouping of male relatives has never been demonstrated before for ancient specimens and provides an insight into the funeral practices of ancient Eurasian tribes.

The maternal genetic inheritance, which was tested through sequencing of the mtDNA HVI, revealed some biological links. For example, children from graves 83bis and 91 might be considered to be relatives, since they share an identical HVI sequence (table 3). Other maternal links were revealed, such as those between individuals from graves 65 and 77; 32A and 72; 28, 73, and 74; 70 and both 88 and 94; and 18A and 27. In some cases, heteroplasmies were observed. The possibility that these heteroplasmies resulted from contamination was invalidated by the fact that identical results were obtained from DNA samples extracted and amplified in triplicate at different time intervals.

Nevertheless, the pitfall of contamination from extraneous human DNA is a major concern for researchers working with human remains (Handt et al. 1994; Kolman and Tuross 2000) and should not be underrated. Since some erroneous ancient DNA results have been published, a number of "criteria of authenticity" need to be fulfilled before results from ancient DNA analyses can be taken to be genuine (Handt et al. 1994; Cooper and Poinar 2000). In the present study, extensive precautions (described in the "Material and Methods" section) were taken to avoid the amplification of contaminating contemporary DNA molecules. Despite the fact that not all reported criteria of authenticity could be met, the possibility that our data arose from contaminating DNA was considered highly unlikely for the following reasons: (i) reproducible PCR results were obtained from multiple extractions and amplifications of the same samples made at different times; (ii) multiallelic profiles were not mixtures of different individuals'

DNA and were not found to correspond to someone involved in the present work (except once); (iii) the results of both sex typing methods (morphologic and genetic) were in accordance with each other; (iv) an inverse relationship between amplification efficiency and length of the amplification products was observed, especially with STR markers; (v) the crystallinity index and the carbon/nitrogen ratio determinations indicated no significant alteration of the bones (Nielsen-Marsh et al. 2000); (vi) a concordance was observed between data obtained with the markers inherited biparentally, paternally, and maternally; (vii) mtDNA analysis of the ancient sample revealed that most of the haplogroups present were of Asian origin and that European maternal lineages identical to those of the excavators or laboratory personnel (all of whom were of European origin) were absent; (viii) the 16223 thymine-cytosine transition was found in 76% of the ancient Egyin Gol samples, a result close to that of Kolman et al. (1996), who found it in 65% of Mongolian samples (compared with 7% of European samples), and (ix) the level of genetic diversity detected in the protohistoric population, as well as some of the haplotypes reported, are similar to those obtained in modern Mongolian populations (authors' unpublished data; Kolman et al. 1996).

Nevertheless, the test that confers the greatest level of robustness is duplicate analysis by two independent laboratories. This was not feasible in the present study, because of the large number of subjects tested. Cloning of the PCR products was not conceivable for the same reason and is not really adapted to the study of STR. Methods such as amino acid racemization and DNA quantitation were not applied, mainly because they do not allow the distinction between contaminated and uncontaminated samples (Kolman and Tuross 2000). Moreover, the proposed use of amino acid racemization to estimate DNA survival in archaelogical bones is challenged by some authors (Collins et al. 1999). The fact that the multiallelic profiles were repeatable from the same—and different—DNA extracts of a specimen allowed us to consider that the number of starting templates was high enough to obtain reliable results and to analyze mtDNA (for which the contamination problem is worse). On the other hand, we subscribed to other important criteria, such as the reiteration of the extraction and amplification steps, the sexing of the samples tested, and, foremost, the use of a molecular combined approach. Moreover, the bone samples studied are not fossil remains and, consequently, are not prone to high rates of DNA alteration.

A majority (89%) of the Xiongnu sequences can be classified as belonging to an Asian haplogroup (A, B4b, C, D4, D5 or D5a, or F1b), and nearly 11% belong to European haplogroups (U2, U5a1a, and J1). This finding indicates that the contacts between European and Asian populations were anterior to the Xiongnu culture, and it confirms results reported for two samples from an early 3rd century B.C. Scytho-Siberian population (Clisson et al. 2002).

The genetic data obtained in the present study, in addition to the topographical and radiocarbon data, suggested hypotheses concerning the social history of the necropolis. Around the 3rd century B.C., the grave of an adult male (grave 28) had been dug on the southern part of the Egyin Gol valley (A sector). At a short distance from him, a privileged man was also buried (grave 27), as were other individuals, including those found in double graves (graves 32/32A, 33/33A, 37/37A, and 38/38A). Some of these surrounding graves could be sacrificial burials, as has been reported elsewhere for one of them (Murail et al. 2000). This tradition of having double graves near an opulent one in cemeteries containing individuals of high social class is well documented, notably in the Sakka (another group of nomadic people of the Eurasian steppes) and the Pazyryk cultures (Francfort et al. 2000). This ritual, at the first developmental step of the cemetery, suggests that the cultural influence of the "old Scythian spirit" was already present in some nomadic families at the beginning of the Xiongnu empire. Although close genetic relationships could not be clearly established between ancient specimens of the A sector (because of the lack of amplification results), a parent/child link was nevertheless shown between individuals in graves 32A and 33, suggesting the possibility of burials based on familial relationships.

Some years later, a new sector of interment seems to have been created (sector B). In this second sector, some individuals shared mtDNA sequences with individuals from the most ancient graves. (Thus, individuals from graves 73 and 74, 18A, and 72 were found to share mtDNA sequences with those from graves 28, 27, and 32A, respectively.) This result suggests that maternal relatives of the individuals first buried might participate in the extension of a new cemetery area. It should be noted that, except for individuals from grave 18A, these maternal relatives were buried close to each other. Two of them (from graves 73 and 72) were from the same paternal lineage. We can imagine that the creation of this new burial area could be the result of tensions between members of the ruling family. The fact that no double graves were built could reflect the cultural rupture with the "old Scythian spirit."

From the 2nd century B.C. to the 1st century A.D., the social organization of the necropolis cannot be clearly deduced from the genetic data. Male and female individuals from different paternal and maternal lineages were buried from south to north in the A sector and from north to south in the B sector. Genetic analyses revealed several familial groups buried close to each

other, notably, one consisting of a father, mother, and son. Since these three individuals were probably not buried at the same time (the son appeared to be as old as his parents), the family grouping was organized at least one generation earlier. From these results, it seems reasonable to speculate that the Xiongnu buried relatives together although the practice was not systematic. During this period the site might have been the cemetery of a social group with a significant genetic diversity, of which only certain members were buried (the small number of inhumations that occurred throughout more than three centuries suggests that this Xiongnu tribe probably had other sepulchral places).

After the fusion of the A and B sectors, new graves were dug in the west. These graves correspond to a group of genetically linked individuals, since they belong to a single paternal lineage. Interestingly, this paternal lineage has been, at least in part (6 of 7 STRs), found in a present-day Turkish individual (Henke et al. 2001). Moreover, the mtDNA sequence shared by four of these paternal relatives (from graves 46, 52, 54, and 57) were also found in a Turkish individuals (Comas et al. 1996), suggesting a possible Turkish origin of these ancient specimens. Two other individuals buried in the B sector (graves 61 and 90) were characterized by mtDNA sequences found in Turkish people (Calafell 1996; Richards et al. 2000). These data might reflect the emergence at the end of the necropolis of a Turkish component in the Xiongnu tribe.

In conclusion, our study shows how the use of genetic markers of different mutability might provide an insight into the history of past necropolises. It also provides genetic data on ancient Eurasian specimens that could help to confirm or disprove models developed from modern genetic data to explain population history. Finally, it provides an excellent tool to select samples of interest for interpopulation analyses.

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