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PRESERVATION OF ANCIENT DNA IN HUMAN BONES FROM THE ENEOLITHIC AND BRONZE AGE KURGAN CEMETERES IN YAMPIL REGION, UKRAINE

ABSTRACT

Ancient DNA was analyzed in altogether 28 Late Eneolithic and Bronze Age human skeletons form 4 sites in southern Ukraine. More than 0,3% of human DNA was preserved only in 13 skeletons. The results of our analyses provide evidence that recovery of DNA molecules suitable for genetic analyses is more dependent on the specificity of the archaeological site and is not strongly correlated with particular environmental factors.

Key words: ancient DNA, Middle Dniester Area, barrows

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INTRODUCTION

In recent years ancient DNA (aDNA) has become a powerful tool for bioarchaeological research. It is being used to tackle variety of research questions concerning past migrations, kinship structure of prehistoric societies and human microevolution. Since the first attempts to extract DNA from archaeological samples were published, its diagenesis and the role of environmental factors in its preservation were widely discussed [Allentoft *et al.* 2012]. Determining which factors and in what way affects the DNA degradation would be crucial for estimating the DNA preservation in potential samples and therefore form most cost-effective sampling strategies for ongoing and future research projects.

Despite the fact that the field is now more than 30 years old no consensus on what factor or set of factors is the most crucial for the aDNA preservation. Numerous aspects were discussed: burial and exposure temperature, rapid or slow sedimentation, chemical properties of the soil, pH value, the presence or absence of oxygen, water, ionic radiation and microorganisms. The deposition temperature and its fluctuations seem to have mostly pronounced effect on DNA preservation. This factor seems to be coupled not only with the climatic zone but also the type of the archaeological site and deposition depth both of which can have a buffering effect on the temperature itself [Smith *et al.* 2001]. Nevertheless, the temperature doesn't explain all the variation in aDNA preservation between samples coming from similar contexts. Other factors such as the amount of liquid water and hydrolytic factors are thought to play major role in DNA degradation [Schwarz *et al.* 2009]. And those can be closely connected with the sediment properties such us its permeability and pH [Geigl 2002]. Some researchers believe that those factors might vary within the burials, and more attention should be paid to microenvironments surrounding particular bone parts targeted for sampling [Hagelberg *et al.* 1991]. Other studies show that not the post-excavation factors such as bone treatment and storage could be responsible for rapid DNA degradation in some samples [Pruvost *et al.* 2007].

Here we aimed to compare how various environmental factors connected with soil properties and deposition depth of skeleton remains influenced preservation of DNA in Bronze Age human bones coming from archaeological excavations conducted in the Yampil Region, in western Ukraine.

1. MATERIALS AND METHODS

Materials. All ancient human skeletons were excavated during the *Yampil Expedition* conducted in 2010-2015, in Ukraine and came from archaeological sites in Pidlisivka (*n*=3), Porohy (*n*=12), Prydnistryanske (*n*=7) and Klembivka (*n*=5) (Tab. 1). All of the burials were interred between 4000 BC and 1000 BC in barrows made of loess soil on various depths (Tab. 1). Excavated human skeletons were treated and stored in similar way. Morphological preservation of each individual was described in detail by Litvinova *et al.* [2015].

The features of the soil environment, including pH, soil type and content of calcium carbonates, for the mounds from Pidlisivka, Klembivka and Prydnistryanske, from which the studied skeletons originate, were reconstructed on the basis of the methods described in chapter Jankowski *et al.* [2017] of this study. Collagen extraction efficiency in analyzed human bones was obtained through ^{14}C analyses which were previously conducted and described by Goslar *et al.* [2015].

Ancient DNA analyses. Ancient DNA was analyzed in altogether 27 Eneolithic Bronze Age human skeletons. From all individuals teeth and/or petrous bones were collected. DNA studies were performed in sterile ancient DNA laboratory at the Faculty of Biology, Adam Mickiewicz University in Poznań (UAM), Poland. Cleaning of the samples, drilling the roots of teeth and/or inner parts of petrous bones (regions of semicircular canals), as well as DNA extraction were conducted following the methods previously described in Litvinova *et al.* [2015] and Juras *et al.* [2017]. For each individual one blunt-end genomic library was build according to Mayer and Kircher [2010], omitting the first nebulization step due to degraded character of aDNA. Amplification of the genomic libraries and their purification was performed following Günther *et al.* [2015], with slight modifications. Concentration measurements and DNA fragment length distribution were estimated with the use of 2200 Tape Station System (Agilent). Shotgun sequencing of DNA libraries on Illumina HiSeq followed by bioinformatic and statistical analyses were carried out as previously described in Litvinova *et al.* [2015].

2. RESULTS

The proportion of human DNA generated through shotgun sequencing of one particular DNA library for each individual, ranged from 0.02% to 13% (Tab. 1). The highest proportion of human DNA (>5%) was found in two individuals from Pidlisivka 1/13, 1/1B site and one individual from Prydnistryanske I/4 (ind. 1).

Table 1

Description of analyzed individuals

Individual	DNA	Archaeological Culture	Dating	Bone material	Proportion of human DNA* (%)	No. of mtDNA fragments	Deposition depth [m]	Collagen Extraction Efficiency (%)	Type of soil	pH (in H_2O)	Ground water depth	Permeability of water (calcium deposits)
Pidlisivka 1 site												
1B	90	Eneolithic	n.a.	teeth	5	1059	1,4	n.a.				
11	91	Yamnaya	2836-2575 BC	teeth	0.1	7	2,4	7,3				
13	94	Babino	n.a.	teeth	10	9063	0,35	n.a.	*Chernozem*	8.4-8.6	n.d. (>2 m)	medium ($CaCO_3$ 5.7-10.3%)
				petrous bone	13	1742		n.a.				
1A	95	Yamnaya	2886-2701 BC	teeth	0.02	37	1	n.a.				
Klembivka 1 site												
5	211	Eneolithic?	2898-2761 BC	teeth	2	162	1,45	7,6				
				petrous bone	3	397		n.a.				
11	212	Noua	3022-2918 BC	teeth	2	299	1,1	0,6				
3	356	Babino	1880-1771 BC	petrous bone	0.3	45	1,3	3,3	*Chernozem*	7.0*-8.5	n.d. (>2 m)	medium ($CaCO_3$ 0.2-1.3%)
12	213	Babino	2117-1950 BC	teeth	2	1942	1,2	5,0				
14	214	Eneolithic	2863-2630 BC	teeth	0.8	81	3	1,9				
Prydnistryanske 1 site												
IV/ 3	219	Yamnaya	2847-2574 BC	teeth	0.08	0	0,3	4,6				
I/4, ind. 1	220	Catacomb	2834-2499 BC	teeth	5	793	1,3	13,6				

I/4, ind. 2	221	Catacomb	2548-2348 BC	teeth	1.1	806	1,3	11,0	*Chernozem*	7.9-8.8	n.d. (>2 m)	medium ($CaCO_3$ 0.8-6.8%)
IV/4	222	Yamnaya	3023-2911 BC	teeth	2.2	737	3,1	1,5				
IV/6	223	Yamnaya	2850-2573 BC	teeth	0.09	28	1,5	7,0				
IV/8	224	Yamnaya	2847-2574 BC	teeth	0.05	675	2,2	9,0				
IV/9	225	Yamnaya	2858-2621 BC	teeth	10	1050	2,25	8,0				
Porohy 3A site												
1	199	Yamnaya	2275-2064 BC	teeth	0.02	0	2,15	8,2	n.a.	n.a.	n.a.	n.a.
3	200	Noua	n.a.	teeth	0.04	1	0,6	n.a.				
5	201	Noua	n.a.	teeth	0.02	1	0,85	n.a.				
7	202	Eneolithic/ Yamnaya	2856-2601 BC	teeth	0.03	2	0,65	2,1				
10	203	Yamnaya	2619-2490 BC	teeth	0.05	13	1,3	4,8; 4,0				
11	204	Yamnaya	2836-2500 BC	teeth	0.1	1	1,45	1,1				
12, ind. I	205	Yamnaya	2566-2471 BC	teeth	0.1	44	1,15	0,9				
15	206	Yamnaya	n.a.	teeth	0.04	16	1,35	n.a.				
19	208	Yamnaya	2882-2698 BC	teeth	0.1	55	1,35	2,5				
20	209	Yamnaya	2884-2700 BC	teeth	0.1	17	2,6	2,5; 1,4				
22	210	Noua	1734-1630 BC	teeth	0.02	1	1,05	1,3				
14	355	Eneolithic	2134-1982 BC	petrous bone	0.05	5	2,2	1,5				

*data from the screening of one genomic library on Illumina HiSeq
n.a. – not analyzed

The lowest levels of human DNA content (< 0.05%) were identified in individuals from Porohy archaeological site and one individual from Pidlisivka (sample from grave no. 1A) (Tab. 1). The number of mitochondrial DNA (mtDNA) fragments which are present in living human cells in a high number of copies, varied between ancient individuals and was the highest (>1000 mtDNA fragments) in Pidlisivka 1/13, 1/1B, Klembivka 1/12 and Prydnistryanske IV/9 (Tab. 1). The lowest amount of mtDNA fragments (<100) were found in all individuals from Porohy archaeological site, two individuals from Pidlisivka (samples 11 and 1A), two individuals from Klembivka 1/3, 1/14 and two individuals from Prydnistryanske IV/3, IV/6. The success rate of recovering DNA was the highest in Klembivka site where statistically 66% of individuals possessed well preserved DNA. Prydnistryanske and Pidlisivka archaeological sites had 57% and 50% of individuals with well recovered DNA, respectively. We did not find any individual with the amount of DNA suitable for genetic analyses (>1%) from Porohy archaeological site.

The deposition depth of human skeletons varied from min. 0.3 to max. 2.6 m. (Tab. 1). Collagen extraction efficiency (%) differed between samples and archaeological sites. The lowest levels were observed in Porohy archaeological site, with the exception of individual from grave 3A/1 (Tab. 1). Higher levels of collagen extraction efficiencies were found in samples from Pidlisivka, Klembivka and Prydnistryanske, with the highest identified in five individuals from Prydnistryanske (>7%) (Tab. 1).

The soil environment of the studied barrows at Pidlisivka, Klembivka and Prydnistryanske described in greater detail in Jankowski *et al.* [2017] had an alkaline reaction with slight differences between particular archaeological sites (Tab. 1). In some cases, only surface layers had a neutral reaction. All studied samples contained calcium carbonates: in barrow material, it was up to about 10 per cent (Tab. 1) but in the reference soil profile it was even 19 per cent, which did not form an impregnated and impermeable layer. The loess of which the soils (and barrow mounds) were built had the character of silty formations with a substantial share of a clayey fraction. They were characterized by high porosity and an ability to hold moisture in capillaries. Their considerable thickness and the deep downcutting of the valleys surrounding the region suggest that the water table was very low (at least several metres under the ground level). No impact of it could be seen in barrow samples or in the soil profile down to a depth of 1.5 m. On account of the climate, it can be presumed that the soils could have been moist for a greater part of the year, but rather not wet. On the other hand, during a hot dry summer, they could have dried. For more information on the soil environment of the studied barrows [*see* Jankowski *et al.* 2017].

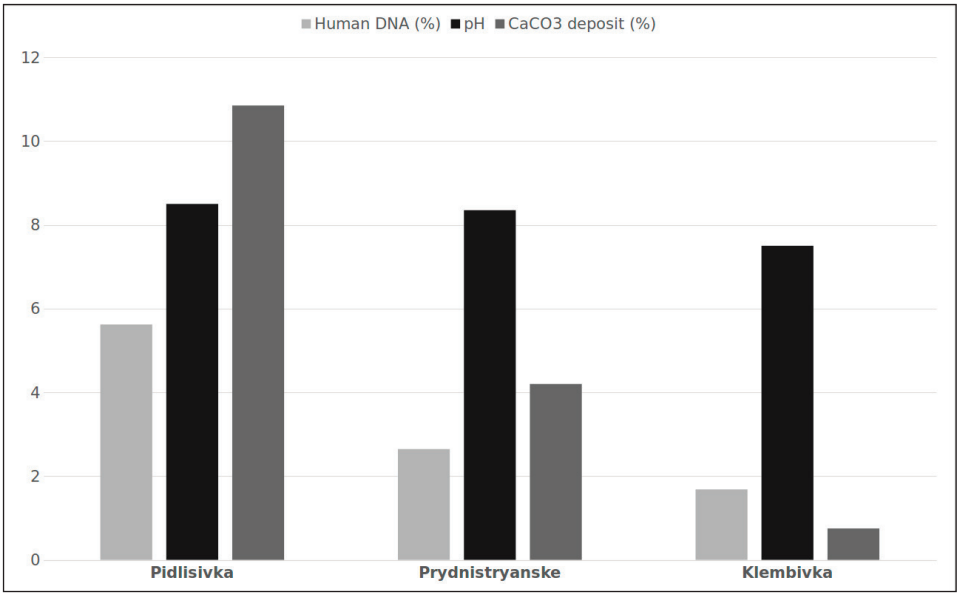

Fig. 1. The correlations between the average proportion of human DNA and soil properties (pH and $CaCO_3$ concentration) for three out of four analyzed archaeological sites

3. DISCUSSION

The results of our analyses provide evidence that recovery of DNA molecules suitable for genetic analyses is more dependent on the specificity of the archaeological site and is not strongly correlated with particular environmental factors. In all analyzed archaeological sites we didn't find correlation between proportion of human DNA in teeth or petrous bones and the depth of deposition of the skeletons. Due to high temperatures on the steppes during the summer one could assume that the deeper the human remains were deposited, the better DNA would have been preserved. Unfortunately, even a deeper deposition, protecting DNA against the effects of high temperatures, did not increase the proportion of genetic material, especially in individuals from Porohy archaeological site. The human remains were deposited there at a depth of 0.6 – 2.6 meters and a small number of mtDNA fragments were obtained only from individuals deposited at a depth of around 1.15 – 1.3 m. The best preserved samples from our sample set including individual 1/3 from Pidlisivka and individual IV/9 from Prydnistryanske were deposited at a depth of 0.35 and 2.25 metres, respectively. Results obtained for these samples confirmed no significant correlation between deposition depth and preservation of human DNA in analyzed archaeological sites in Yampil Region.

Rough correlation can be seen between the average DNA preservation (seen as an average proportion of human DNA in all of the samples from each site) and the pH of the layer the individuals were deposited in (Fig. 1). Interestingly similar concentration can be seen between the amount of $CaCO_3$ (indicative of permeability of water). That last result could suggest that the presence of water has negative effect on DNA preservation only when coupled with other factors. However, in both cases only the average data for each site was available. In order to more definitively discuss the idea, the information from the burial infills should be acquired and the samples should be compared individually.

We did not find direct correlation between collagen extraction efficiency and preservation of DNA. However, individuals with the best preserved DNA from Klembivka archaeological site retained the highest percentages of collagen extraction efficiency (1.5 to 13.6) among all analyzed individuals. In the same time, samples from Porohy were characterized by the lowest percentages of collagen extraction efficiency (0.9-4.8), with the exception of individual from grave 3A/1. Result obtained for sample from grave 3A/1 in Porohy is in accordance with the statement that the presence of collagen not necessarily confirms adequate DNA preservation [Schotsmans *et al.* (Eds) 2017].

In the case of three individuals DNA was extracted from petrous part of temporal bones (Tab. 1). Dense bone parts of the petrous bone were proved to provide high endogenous aDNA yields from most of ancient individuals [Pinhasi 2015; Hansen *et al.* 2017]. However, petrous bones from individual 1/3 from Klembivka and individual 3A/14 from Porohy used for DNA extractions did not produce sufficient amount of DNA. This stays in agreement with Hansen *et al.* [2017] who pointed that using petrous bone not always lead to better results as whole array of factors plays role in DNA preservation. On the other hand, we extracted DNA from both teeth and petrous bone belonging to the individual 1/13 from Pidlisivka and retrieved slightly higher amount of human DNA from petrous bone than from the teeth.

A comprehensive treatment of *Yampil population* fossil DNA analyses from the transitions periods between the Eneolithic and the Early and Late Bronze Age, and their relation to the Corded Ware culture in the Polish lands is to be found in a separate publication [Juras *et al.*, forthcoming].

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