REVISTA CICSA

serie nouă

IX/2023

ISSN 2457 – 3809 ISSN – L 2457 – 3809
https://cicsaunibuc.wordpress.com/revista/revista-online

In memoriam

LIGIAE BÂRZU

(1930-2003)

archaeologist and professor

Cuprins/ Content

Articole și Studii/Articles and Studies

Recenzii și prezentări de carte/ Reviews and Book Presentations

Exchanging genes and goods: How ancient DNA can potentially illuminate complex mobility patterns in the Black Sea region

Sterling WRIGHT,
Department of Anthropology, Penn State University,
Franceska-Cristina ȘTIRBU,
Faculty of Biology, University of Bucharest.

https://www.doi.org/10.31178/cicsa.2023.9.2

Abstract: Ancient DNA (aDNA) has emerged as a groundbreaking tool in the field of archaeology, providing insights into the movement of individuals and their goods. The field combines genetics, anthropology, and archaeology to reconstruct the intricate complexities of human history. Here, we discuss how ancient DNA analysis has the potential to enhance our understanding of historical mobility patterns in the Black Sea region, specifically in Dobrogea. We also offer broad guidelines and optimal practices that researchers should consider following when working with skeletal collections if they are interested in performing aDNA analyses. As aDNA methods advance and become more refined, we anticipate that our ability to unravel the intricate interplay between human mobility and cultural exchange in the past will also improve.

Keywords: ancient DNA, human mobility, Dobrogea, Romanian archaeology, Black Sea history

Introduction

Ancient DNA (aDNA) has made significant contributions to the field of archaeology on a broad range of topics. For instance, aDNA extracted from human teeth and petrous bones have elucidated the genetic relationship between humans, Neanderthals, and Denisovans.[1] Empirical observations in the field have also afforded a chronological window of how the genetic profiles of regions have continued to be reshaped and re-established over time.[2] In several instances, aDNA has brought forth evidence that challenged our previous understandings. As an example, a study analyzing hundreds of ancient genomes from various locations within the Southern Arc, spanning more than 11,000 years, has shown the remarkable diversity within this region.[3] While these remarkable strides by the aDNA field are notable, one region has remained relatively underexplored: Dobrogea. Although Dobrogea was situated in a historical junction where

---

[1] Krause *et al.* 2010; Reich *et al.* 2010; Meyer *et al.* 2012.

[2] Nielsen *et al.* 2017; Antonio *et al.* 2019; Llamas *et al.* 2016.

[3] Lazaridis *et al.* 2022; Lazaridis *et al.* 2022.

Mediterranean and steppe cultures converged, our understanding of the extent of the interactions between these cultures remains limited. As such, the benefits of performing aDNA research in Dobrogea are compelling, but it is equally important to undertake certain precautions before proceeding.

We outline the several precautions that should be implemented when carrying out an aDNA project involving human remains. We also describe how such techniques can have the potential to elucidate the reasons underlying the need for heightened attention to populations in the Dobrogea region. We argue that studying the genetic landscape of this region can provide novel insights into its unique migration patterns, cultural exchanges, and genetic diversification. In the following sections, we provide an overview of the methodology of aDNA, current hypotheses and research objectives, and guidelines for future researchers to consider if they too wish to perform aDNA analyses.

Overview of aDNA methods and history

All aDNA studies require careful scientific and ethical planning when being carried out. During this process, formal agreements between collaborators are generally established. This process may encompass, though not exclusively, researchers reaching a consensus on factors such as the quantity of samples to be collected, the allocation of authorship, and the overall project scope. We recommend that all researchers reach a consensus on each of these considerations before the sampling process.

Because all aDNA research involves the destructive sampling of biomaterial, especially human teeth and dental calculus, samples should be photographed with a high-resolution camera and a millimeter-scale ruler. This step is important as it provides a record of the visual aspects of a sample, which can be important in downstream analyses. Notes pertaining to the oral health status, age, and sex should also be documented before sampling begins. In the case of collecting dental calculus, researchers should document the location of where the sample was collected (e.g., the type of tooth, tooth surface, and gingival margin).[4] Furthermore, it is recommended to decontaminate the sampling area and equipment with NaCl solution before proceeding. After each sample, researchers should wipe down the area and tools with a cleaning solution (ideally bleach),

---

[4] Moore and Weyrich 2021; Farrer *et al.* 2018; Welch *et al.* 2020.

which will minimize the effects of cross contamination. If possible, controls should also be collected during this process. This may involve taking a cotton swab of the sampling area or collecting soil samples around the human burial. Such measures can help monitor the amount of contamination present in the samples and improve the robusticity of results if included.

Following sample collection, samples are generally shipped to a facility dedicated for aDNA research. The initial first step in the laboratory involves decontamination. Various protocols for decontamination exist, lacking an unanimous consensus.[5] However, they often involve UV irradiation and NaCl treatment. DNA purification and concentration are the next steps, which are then used to construct a Next Generation Sequencing (NGS) library.

Applying NGS techniques to archaeological biomaterials represent a pivotal breakthrough in aDNA research.[6] Unlike the previous PCR-based approaches, NGS methods can recover millions of DNA fragments from a sample in parallel. Furthermore, only NGS-based methods provide the means for researchers to determine whether the DNA they recover from archaeological biomaterial are ancient or modern contaminants.[7] A key characteristic of aDNA is the increased occurrence of Cytosine (C) to Thymine (T) substitutions in proximity to the 5’ ends.[8] Conversely, this implies that aDNA also exhibits a heightened frequency of Guanine (G) to Adenine (A) substitutions nearer to the 3’ ends. These distinctive traits of aDNA led to the development of MapDamage,[9] which employs a Bayesian algorithm to gauge whether the DNA of an archaeological sample exhibits post-mortem damage. This assessment, in turn, aids in determining whether the DNA fragments in a sample are authentically ancient or derived from modern contaminants. Numerous studies utilizing this tool have demonstrated correlations between the age of the sample and the burial environment with the frequency of misincorporations,[10] specifically older samples and samples from warmer climates having higher rates of post-mortem damage. The advent of other computational methods that were designed for NGS datasets over the past decade have continued to improve the reliability of results.[11] For instance, PMDtools separates DNA fragments with post-mortem damage (PMD) from ones without, ensuring that only aDNA

---

[5] Farrer *et al.* 2021; Warinner *et al.* 2017.
[6] Orlando *et al.* 2021; Wright *et al.* 2021.
[7] Liu *et al.* 2022; Jónsson *et al.* 2013; Ginolhac *et al.* 2011.
[8] Hofreiter *et al.* 2001; Hansen *et al.* 2001; Brotherton *et al.* 2007.
[9] Jónsson *et al.* 2013.
[10] Sawyer *et al.* 2012; Garcia-Garcera *et al.* 2011.
[11] Knights *et al.* 2011; Renaud *et al.* 2015; Huang and Ringbauer 2022.

sequences are included in downstream analyses. The tools discussed here, along with additional ones, have continued to enhance the reliability of aDNA analyses, especially when investigating the mobility of past peoples.

In addition to improving the overall quality of data generated, NGS methods have also made it possible to generate genome-wide data of ancient individuals.[12] Such high-resolution has provided a greater deal of confidence in assessing the degree of admixture within populations, estimating the biological sex of individuals, and understanding kinship relationships. Although the number of tools to understand population structures continues to grow, Principal components analysis (PCA) and ADMIXTURE remain two popular choices.

In population genetics, PCA serves as a powerful tool to understand the genetic interconnectedness among individuals. PCA enables the visualization of genetic relatedness of individuals through the calculation of eigenvalues and eigenvectors derived from the covariance matrix of allele frequencies across all pairs of individuals. This approach reduces the multidimensionality of the data and indicates which allele-frequency space accounts for most of the variation in a dataset. Throughout its extensive application, PCA has consistently demonstrated a robust connection between geographic origins of individuals and their placements within the principal components space.[13] The efficacy of PCA to investigate the genetic relatedness of individuals to address questions pertaining to cultural exchange and migration has additionally been validated through follow-up investigations that use this technique, consistently revealing analogous patterns.[14]

ADMIXTURE is a widely used tool in population genetics to infer the ancestral proportions of individuals or populations based on their genome-wide SNPs (Single Nucleotide Polymorphisms).[15] In aDNA research, ADMIXTURE assists in discerning the population structure and identifying the potential genetic ancestral groups for a given ancient population, elucidating their migration patterns, cultural exchanges, and their interactions with other genetically distinct groups. As such, the program offers insights into where individuals or populations intermix genetically due to migration or cultural contacts. However, a major limitation of ADMIXTURE is

---

[12] Haak *et al.* 2015; Allentoft *et al.* 2015.

[13] Novembre *et al.* 2008.

[14] Lazaridis *et al.* 2022; Haak *et al.* 2015; Allentoft *et al.* 2015; Margaryan *et al.* 2020; Mathieson *et al* 2015; Mathieson *et al.* 2018.

[15] Alexander *et al.* 2009.

that it assumes a simple model of population admixture, where the genes of ancestral populations can be mapped to fixed cultural identities.[16] It also relies on the availability of appropriate reference populations for ancestral inference. Therefore, if the reference population(s) is not included in the analysis, the results that are generated would be misleading. As such, researchers must be aware of the assumptions and limitations of this tool.

In summary, aDNA has furthered our understanding of the genetic landscapes of past populations. The advancements in the wide array of techniques, ranging from sample collection and DNA extraction to authentication and population structure analyses, have enabled researchers to uncover the complex population dynamics of the past in unprecedented ways. These aDNA techniques will continue to offer a multidimensional perspective that archaeology can continue to use to shed light on ancient migrations and interactions.

Exploring the Untapped Potential: Rationale for Ancient DNA Research in Dobrogea

Dobrogea represents an historical region that is situated in southeastern Europe, between the Danube and the Black Sea. The archaeological record in Dobrogea is rich with archaeological material, from prehistoric settlements to Roman forts and medieval trading centers. While the geographic positioning of this region along with its wealth of archaeological material makes it an ideal study area to investigate complex interactions between cultural and genetic exchange, Dobrogea remains an understudied region in aDNA research.

Extensive aDNA research has been conducted on populations from the southeastern European region. Genome-wide data from hundreds of individuals in southeastern Europe, dating from 12,000 to 500 BCE, indicate that the region was a place of genetic admixture and cultural exchange for millennia.[17] Subsequent investigations have filled in geographical and chronological gaps in southeastern Europe, but yet Dobrogea and populations from its many historical periods remain overlooked. A few studies have conducted aDNA studies on archaeological populations from Dobrogea,[18] but they employed only low-resolution techniques (i.e., mtDNA) that only give a small glimpse into the complex genetic history of the region. Generating genome-wide data for these populations could have huge implications in transforming our understanding about the

---

[16] Lawson *et al.* 2018.

[17] Mathieson *et al.* 2018.

[18] Rusu *et al.* 2018; Rusu *et al.* 2019; Gînguță *et al.* 2021.

population dynamics of this region. For instance, generating aDNA for the Babadag culture could provide insights into the genetic origins of this enigmatic group, which is currently poorly understood. The Babadag culture emerged in Dobrogea during the 11th century BCE, when a demographic boom in the region occurred.[19] More than a hundred settlements associated with this culture have been excavated in Dobrogea, eastern Wallachia, and south-eastern Moldavia.[20] This culture has several defining characteristics, but it is mostly known for its pottery style, pits, and oval-shaped dwellings. While the material record for this culture is rich, many researchers are still puzzled on whether the Babadag culture was an autochthonous or allochthonous group. Furthermore, it still remains an open question whether this group left any genetic traces in the region after the arrival of the Greek colonists. Potentially, comparing the aDNA of the Babadag culture with their predecessors, contemporaries, and successors counterparts may offer the evidence needed to answer these questions.

Exploring the aDNA of individuals who adhered to Roman burial customs may also provide critical insights into the ramifications of Roman annexation on populations in Dobrogea. Genetic studies on Roman era populations remain limited, but have already been fruitful. For instance, Rome, during the Imperial era, had a much greater diversity of genetic ancestries from across the Mediterranean and Near East than inhabitants who lived in the area during the previous historic periods.[21] The extent to which Roman imperialism impacted the genetic diversity of its *limes*, or provinces, in a similar fashion is poorly understood, especially in Dobrogea. Characterizing the genetic ancestry of individuals from Roman settlements in Dobrogea, such as Jijila and Histria, will help determine whether individuals practicing Roman burial customs were locals or non-locals, especially when the aDNA data is integrated with other lines of evidence, such as isotopic data. These potential findings would elucidate the social dynamics of Roman imperialism on its Danubian frontiers.

Summary

In summary, aDNA research will help unravel the complex social dynamics of Dobrogea that are currently unattainable with current approaches. We anticipate that aDNA research in Dobrogea

---

[19] Ailincăi 2013.
[20] Ailincăi and Constantinescu 2015.
[21] Antonio *et al.* 2019.

will reshape our understanding about the population dynamics in this remarkable region. Furthermore, we encourage researchers who work with archaeological biomaterials, whether in the field or institutional setting, to consider following the precautions described in this article as such adherence will, hopefully, minimize the amount of contamination introduced samples, and therefore, will improve overall results. In doing so, aDNA analyses will have the power to help disentangle the complex social interactions, migrations, and cultural exchanges that are unique to the history of Dobrogea.

Bibliography

Ailincăi, S. and M. Constantinescu, 2015, *Trăind cu morții: Înmormântări în așezările de la începutul epocii fierului între Balcani, Tisa și Nistru*, Cluj-Napoca: Editura Academia Românǎ.

Ailincăi, S. 2013, „Începuturile epocii fierului în Dobrogea”, in *SCIVA*, 64, 3-4, 223-292.

Alexander, D., J. Novembre, and K. Lange, 2009, „Fast model-based estimation of ancestry in unrelated individuals”, in *Genome Research*, 19, 9, 1655-1664.

Allentoft, M., M. Sikora, K. Sjögren, S. Rasmussen, M. Rasmussen, J. Stenderup, P. Damgaard, H. Schroeder, T. Ahlström, L. Vinner, A. Malaspinas, A. Margaryan, T. Higham, D. Chivall, N. Lynnerup, L. Harvig, J. Baron, P. Della Casa, P. Dbrowski, P. Duffy, A. Ebel, A. Epimakhov, K. Frei, M. Furmanek, T. Gralak, A. Gromov, S. Gronkiewicz, G. Grupe, T. Hajdu, R. Jarysz, V. Khartanovich, A. Khokhlov, V. Kiss, J. Kolár, A. Kriiska, I. Lasak, C. Longhi, G. McGlynn, A. Merkevicius, I. Merkyte, M. Metspalu, R. Mkrtchyan, V. Moiseyev, L. Paja, G. Pálfi, D. Pokutta, Ł. Pospieszny, T. Price, L. Saag, M. Sablin, N. Shishlina, V. Smrčka, V. Soenov, V. Szeverényi, G. Tóth, S. Trifanova, L. Varul, M. Vicze, L. Yepiskoposyan, V. Zhitenev, L. Orlando, T. Sicheritz-Pontén, S. Brunak, R. Nielsen, K. Kristiansen, and E. Willerslev, 2015, „Population genomics of Bronze Age Eurasia” in *Nature* 522, 167-172.

Antonio, M., Z. Gao, H. Moots, M. Lucci, F. Candilio, S. Sawyer8, V. Oberreiter, D. Calderon, K. Devitofranceschi, R. Aikens, S. Aneli, F. Bartoli, A. Bedini, O. Cheronet, D. Cotter, D. Fernandes, G. Gasperetti, R. Grifoni, A. Guidi, F. La Pastina, E. Loreti, D. Manacorda, G. Matullo, S. Morretta, A. Nava, V. Fiocchi Nicolai, F. Nomi, C. Pavolini, M. Pentiricci, P. Pergola, M. Piranomonte, R. Schmidt, G. Spinola, A. Sperduti, M. Rubini, L. Bondioli, A. Coppa, R. Pinhasi, and J. Pritchard, 2019, „Ancient Rome: A genetic crossroads of Europe and the Mediterranean”, in *Science*, 366, 6466, 708-714.

Brotherton, P., P. Endicott, J. Sanchez, M. Beaumont, R. Barnett, J. Austin, and A. Cooper, 2007, „Novel high-resolution characterization of ancient DNA reveals C>U–type base modification events as the sole cause of post mortem miscoding lesions”, *Nucleic Acids Research*, 35, 17, 5717-5728.

Farrer, A., J. Bekvalac, R. Redfern, N. Gully, K. Dobney, A. Cooper, and L. Weyrich, 2018, „Biological and cultural drivers of oral microbiota in Medieval and Post-Medieval London, UK”, in *bioRxiv*, 343889.

Farrer, A., S. Wright, E. Skelly, R. Eisenhofer, K. Dobney, and L. Weyrich, 2021, „Effectiveness of decontamination protocols When analyzing ancient DNA preserved in dental calculus", in *Scientific Reports*, 11, 7456.

Garcia-Garcera, M., E. Gigli, F. Sanchez-Quinto, O. Ramirez, F. Calafell, S. Civit, and C. Lalueza-Fox, 2011, „Fragmentation of Contaminant and Endogenous DNA in Ancient Samples Determined by Shotgun Sequencing; Prospects for Human Palaeogenomics", *PLoS One*, 6, 8, e24161.

Ginolhac, A., M. Rasmussen, M. Gilbert, E. Willerslev, and L. Orlando, 2011, „mapDamage: testing for damage patterns in ancient DNA sequences", *Bioinformatics*, 27, 15, 2153-2155.

Gînguță, A., I. Rusu, C. Mircea, A. Ioniță, H. Banciu, B. Kelemen, 2021, „Mitochondrial DNA Profiles of Individuals from a 12th Century Necropolis in Feldioara (Transylvania)", in *Genes*, 12, 3, 436.

Haak, W., I. Lazaridis, N. Patterson, N. Rohland, S. Mallick, B. Llamas, G. Brandt, S. Nordenfelt, E. Harney, K. Stewardson, Q. Fu, A. Mittnik, E. Bánffy, C. Economou, M. Francken, S. Friederich, R. Garrido Pena, F. Hallgren, V. Khartanovich, A. Khokhlov, M. Kunst, P. Kuznetsov, H. Meller, O. Mochalov, V. Moiseyev, N. Nicklisch, S. Pichler, R. Risch, M. Rojo Guerra, C. Roth, A. Szécsényi-Nagy, J. Wahl, M. Meyer, J. Krause, D. Brown, D. Anthony, A. Cooper, K. Werner Alt, and D. Reich, 2015, „Massive migration from the steppe was a source for Indo-European languages in Europe", *Nature*, 522, 7555, 207-211.

Hansen, A., E. Willerslev, C. Wiuf, T. Mourier, and P. Arctander, 2001, „Statistical Evidence for Miscoding Lesions in Ancient DNA Templates", *Molecular Biology and Evolution*, 18, 2, 262-265.

Hofreiter, M., V. Jaenicke, D. Serre, A. von Haeseler, and S. Pääbo, 2001, „DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA", *Nucleic Acids Research*, 29, 23, 4793-4799.

Huang, Y., and H. Ringbauer, 2022, „hapCon: estimating contamination of ancient genomes by copying from reference haplotypes", *Bioinformatics*, 38, 15, 3768-3777.

Jónsson, H., A. Ginolhac, M. Schubert, P. Johnson, and L. Orlando, 2013, „mapDamage2. 0: fast approximate Bayesian estimates of ancient DNA damage parameters", *Bioinformatics*, 29, 13, 1682-1684.

Knights, D., J. Kuczynski, E. Charlson, J. Zaneveld, M. Mozer, R. Collman, F. Bushman, R. Knight, and S. Kelley, 2011, „Bayesian community-wide culture-independent microbial source tracking", *Nature Methods*, 8, 9, 761-763.

Krause, J., Q. Fu, J. Good, B. Viola, M. Shunkov, A. Derevianko, and S. Pääbo, 2010, „The complete mitochondrial DNA genome of an unknown hominin from southern Siberia", *Nature*, 464, 7290, 894-897.

Lawson, D., L. van Dorp, and D. Falush, 2018, „A tutorial on how not to over-interpret STRUCTURE and ADMIXTURE bar plots", *Nature Communications*, 9, 1, 3258.

Lazaridis, I., S. Alpaslan-Roodenberg, A. Acar, A. Açıkkol, A. Agelarakis, L. Aghikyan, U. Akyüz, D. Andreeva, G. Andrijašević, D. Antonović, I. Armit, A. Atmaca, P. Avetisyan, A. Aytek, K. Bacvarov, R. Badalyan, S. Bakardzhiev, J. Balen, L. Bejko, R. Bernardos, A. Bertsatos, H. Biber, A. Bilir, M. Bodružić, M. Bonogofsky, C. Bonsall, D. Borić, N. Borovinić, G. Bravo Morante, K. Buttinger, K. Callan, F. Candilio, M. Carić, O. Cheronet, S. Chohadzhiev, M. Chovalopoulou, S. Chryssoulaki, I. Ciobanu, N. Čondić, M. Constantinescu, E. Cristiani, B. Culleton, E. Curtis, J. Davis, R. Davtyan, T. Demcenco, V. Dergachev, Z.

Derin, S. Deskaj, S. Devejyan, V. Djordjević, K. Duffett Carlson, L. Eccles, N. Elenski, A. Engin, N. Erdoğan, S. Erir-Pazarcı, D. Fernandes, M. Ferry, S. Freilich, A. Frînculeasa, M. Galaty, B. Gamarra, B. Gasparyan, B. Gaydarska, E. Genç, T. Gültekin, S. Gündüz, T. Hajdu, V. Heyd, S. Hobosyan, N. Hovhannisyan, I. Iliev, L. Iliev, S. Iliev, İ. İvgin, I. Janković, L. Jovanova, P. Karkanas, B. Kavaz-Kındığılı, E. Kaya, D. Keating, D. Kennett, S. Deniz Kesici, A. Khudaverdyan, K. Kiss, S. Kılıç, P. Klostermann, S. Kostak Boca Negra Valdes, S. Kovačević, M. Krenz-Niedbała, M. Krznarić Škrivanko, R. Kurti, P. Kuzman, A. Lawson, C. Lazar, K. Leshtakov, T. Levy, I. Liritzis, K. Lorentz, S. Łukasik, M. Mah, S. Mallick, K. Mandl, K. Martirosyan-Olshansky, R. Matthews, W. Matthews, K. McSweeney, V. Melikyan, A. Micco, M. Michel, L. Milašinović, A. Mittnik, J. Monge, G. Nekhrizov, R. Nicholls, A. Nikitin, V. Nikolov, M. Novak, I. Olalde, J. Oppenheimer, A. Osterholtz, C. Özdemir, K. Özdoğan, N. Öztürk, N. Papadimitriou, N. Papakonstantinou, A. Papathanasiou, L. Paraman, E. Paskary, N. Patterson, I. Petrakiev, L. Petrosyan, V. Petrova, A. Philippa-Touchais, A. Piliposyan, N. Pocuca Kuzman, H. Potrebica, B. Preda-Bălănică, Z. Premužić, T. Douglas Price, L. Qiu, S. Radović, K. Raeuf Aziz, P. Rajić Šikanjić, K. Rasheed Raheem, S. Razumov, A. Richardson, J. Roodenberg, R. Ruka, V. Russeva, M. Şahin, A. Şarbak, E. Savaş, C. Schattke, L. Schepartz, T. Selçuk, A. Sevim-Erol, M. Shamoon-Pour, H. Shephard, A. Sideris, A. Simalcsik, H. Simonyan, V. Sinika, K. Sirak, G. Sirbu, M. Šlaus, A. Soficaru, B. Söğüt, A. Sołtysiak, Ç. Sönmez-Sözer, M. Stathi, M. Steskal, K. Stewardson, S. Stocker, F. Suata-Alpaslan, A, Suvorov, A. Szécsényi-Nagy, T. Szeniczey, N. Telnov, S. Temov, N. Todorova, U. Tota, G. Touchais, S. Triantaphyllou, A. Türker, M. Ugarković, T. Valchev, F. Veljanovska, Z. Videvski, C. Virag, A. Wagner, S. Walsh, P. Włodarczak, N. Workman, A. Yardumian, E. Yarovoy, A. Yener Yavuz, H. Yılmaz, F. Zalzala, A, Zettl, Z. Zhang, R. Çavuşoğlu, N. Rohland, R. Pinhasi, and D. Reich, 2022, „The genetic history of the Southern Arc: A bridge between West Asia and Europe", *Science*, 377, 6609, eabm4247.

Lazardis, I., S. Alpaslan-Roodenberg, A. Acar, A. Açıkkol, A. Agelarakis, L. Aghikyan, U. Akyüz, D. Andreeva, G. Andrijašević, D. Antonović, I. Armit, A. Atmaca, P. Avetisyan, A. Aytek, K. Bacvarov, R. Badalyan, S. Bakardzhiev, J. Balen, L. Bejko, R. Bernardos, A. Bertsatos, H. Biber, A. Bilir, M. Bodružić, M. Bonogofsky, C. Bonsall, D. Borić, N. Borovinić, G. Bravo Morante, K. Buttinger, K. Callan, F. Candilio, M. Carić, O. Cheronet, S. Chohadzhiev, M. Chovalopoulou, S. Chryssoulaki, I. Ciobanu, N. Čondić, M. Constantinescu, E. Cristiani, B. Culleton, E. Curtis, J. Davis, R. Davtyan, T. Demcenco, V. Dergachev, Z. Derin, S. Deskaj, S. Devejyan, V. Djordjević, K. Duffett Carlson, L. Eccles, N. Elenski, A. Engin, N. Erdoğan, S. Erir-Pazarcı, D. Fernandes, M. Ferry, S. Freilich, A. Frînculeasa, M. Galaty, B. Gamarra, B. Gasparyan, B. Gaydarska, E. Genç, T. Gültekin, S. Gündüz, T. Hajdu, V. Heyd, S. Hobosyan, N. Hovhannisyan, I. Iliev, L. Iliev, S. Iliev, İ. İvgin, I. Janković, L. Jovanova, P. Karkanas, B. Kavaz-Kındığılı, E. Hilal Kaya, D. Keating, D. Kennett, S. Kesici, A. Khudaverdyan, K. Kiss, S. Kılıç, P. Klostermann, S. Kostak Boca Negra Valdes, S. Kovačević, M. Krenz-Niedbała, M. Krznarić Škrivanko, R. Kurti, P. Kuzman, A. Lawson, C. Lazar, K. Leshtakov, T. Levy, I. Liritzis, K. Lorentz, S. Łukasik, M. Mah, S. Mallick, K. Mandl, K. Martirosyan-Olshansky, R. Matthews, W. Matthews, K. McSweeney, V. Melikyan, A. Micco, M. Michel, L. Milašinović, A. Mittnik, J. Monge, G. Nekhrizov, R. Nicholls, A. Nikitin, V. Nikolov, M. Novak,

I. Olalde, J. Oppenheimer, A. Osterholtz, C. Özdemir, K. Özdoğan, N. Öztürk, N. Papadimitriou, N. Papakonstantinou, A. Papathanasiou, L. Paraman, E. Paskary, N. Patterson, I. Petrakiev, L. Petrosyan, V. Petrova, A. Philippa-Touchais, A. Piliposyan, N. Pocuca Kuzman, H. Potrebica, B. Preda-Bălănică, Z. Premužić, T. Douglas Price, L. Qiu, S. Radović, K. Raeuf Aziz, P. Rajić Šikanjić, K. Rasheed Raheem, S. Razumov, A. Richardson, J. Roodenberg, R. Ruka, V. Russeva, M. Şahin, A. Şarbak, E. Savaş, C. Schattke, L. Schepartz, T. Selçuk, A. Sevim-Erol, M. Shamoon-Pour, H. Shephard, A. Sideris, A. Simalcsik, H. Simonyan, V. Sinika, K. Sirak, G. Sirbu, M. Šlaus, A. Soficaru, B. Söğüt, A. Sołtysiak, Ç. Sönmez-Sözer, M. Stathi, M. Steskal, K. Stewardson, S. Stocker, F. Suata-Alpaslan, A, Suvorov, A. Szécsényi-Nagy, T. Szeniczey, N. Telnov, S. Temov, N. Todorova, U. Tota, G. Touchais, S. Triantaphyllou, A. Türker, M. Ugarković, T. Valchev, F. Veljanovska, Z. Videvski, C. Virag, A. Wagner, S. Walsh, P. Włodarczak, N. Workman, A. Yardumian, E. Yarovoy, A. Yener Yavuz, H. Yılmaz, F. Zalzala, A, Zettl, Z. Zhang, R. Çavuşoğlu, N. Rohland, R. Pinhasi, and D. Reich, 2022, A genetic probe into the ancient and medieval history of Southern Europe and West Asia", *Science*, 377, 6609, 940-951.

Liu, Y., A. Bennett, and Q. Fu, 2022, „Evolving ancient DNA techniques and the future of human history", *Cell*, 185, 15, 2632-2635.

Llamas, B., L. Fehren-Schmitz, G. Valverde, J. Soubrier, S. Mallick, N. Rohland, S. Nordenfelt, C. Valdiosera, S. Richards, A. Rohrlach, M. Barreto Romero, I. Espinoza, E. Tomasto Cagigao, L. Watson Jiménez, K. Makowski, I. Santiago Leboreiro Reyna, J. Mansilla Lory, J. Ballivián Torrez, M. Rivera, G. Politis, R. Burger, M. Constanza Ceruti, J. Reinhard, R. Wells, C. Santoro, V. Standen, C. Smith, D. Reich, S. Ho, A. Cooper, and W. Haak, 2016, „Ancient mitochondrial DNA provides high-resolution time scale of the peopling of the Americas", *Science Advances*, 2, 4, e1501385.

Mark Welch, J., T. Ramírez-Puebla, and G. Borisy, 2020, „Oral Microbiome Geography: Micron-Scale Habitat and Niche", *Cell Host & Microbe*, 28, 2, 160-168.

Margaryan, A., D. Lawson, M. Sikora, F. Racimo, S. Rasmussen, I. Moltke, L. Cassidy, E. Jørsboe, A. Ingason, M. Pedersen, T. Korneliussen, H. Wilhelmson, M. Buś, P. de Barros Damgaard, R. Martiniano, G. Renaud, C. Bhérer, V. Moreno-Mayar, A. Fotakis, M. Allen, M. Molak, E. Cappellini, G. Scorrano, A. Buzhilova, A. Fox, A. Albrechtsen, B. Schütz, B. Skar, C. Arcini, C. Falys, C. Hedenstierna Jonson, D. Błaszczyk, D. Pezhemsky, G. Turner-Walker, H. Gestsdóttir, I. Lundstrøm, I. Gustin, I. Mainland, I. Potekhina, I. Muntoni, J. Cheng, J. Stenderup, J. Ma, J. Gibson, J. Peets, J. Gustafsson, K. Iversen, L. Simpson, L. Strand, L. Loe, M. Sikora, M. Florek, M. Vretemark, M. Redknap, M. Bajka, T. Pushkina, M. Søvsø, N. Grigoreva, T. Christensen, O. Kastholm, O. Uldum, P. Favia, P. Holck, R. Allmäe, S. Sten, S. Arge, S. Ellingvåg, V. Moiseyev, W. Bogdanowicz, Y. Magnusson, L. Orlando, D. Bradley, M. Jørkov, J. Arneborg, N. Lynnerup, N. Price, T. Gilbert, M. Allentoft, J. Bill, S. Sindbæk, Lotte Hedeager, K. Kristiansen, R. Nielsen, T. Werge, and E. Willerslev, 2020, „Population genomics of the Viking world", *Nature*, 585, 7825, 390-396.

Mark Welch, J., T. Ramírez-Puebla, and G. Borisy, 2020, „Oral Microbiome Geography: Micron-Scale Habitat and Niche", *Cell Host & Microbe*, 28, 2, 160-168.

Mathieson, I., S. Alpaslan-Roodenberg, C. Posth, A. Szécsényi-Nagy, N. Rohland, S. Mallick, I. Olalde, N. Broomandkhoshbacht, F. Candilio, O. Cheronet, D. Fernandes, M. Ferry, B. Gamarra, G. González Fortes, W. Haak, E. Harney, E. Jones, D. Keating, B. Krause-Kyora, I. Kucukkalipci, M. Michel, A. Mittnik, K. Nägele, M. Novak, J. Oppenheimer, N. Patterson, S. Pfrengle, K. Sirak, K. Stewardson, S. Vai, S. Alexandrov, K. Alt, R. Andreescu, D. Antonović, A. Ash, N. Atanassova, K. Bacvarov, B. Gusztáv, H. Bocherens, M. Bolus, A. Boroneanț, Y. Boyadzhiev17, A. Budnik, J. Burmaz, S. Chohadzhiev, N. Conard, R. Cottiaux, M. Čuka, C. Cupillard, D. Drucker, N. Elenski, M. Francken, B. Galabova, G. Ganetsovski, B. Gély, T. Hajdu, V. Handzhyiska, K. Harvati, T. Higham, S. Iliev, I. Janković, I. Karavanić, D. Kennett, D. Komšo, A. Kozak, D. Labuda, M. Lari, C. Lazar, M. Leppek, K. Leshtakov, D. Lo Vetro, D. Los, I. Lozanov, M. Malina, F. Martini, K. McSweeney, H. Meller, M. Mendušić, P. Mirea, V. Moiseyev, V. Petrova, D. Price, A. Simalcsik, L. Sineo, M. Šlaus, V. Slavchev, P. Stanev, A. Starović, T. Szeniczey, S. Talamo, M. Teschler-Nicola, C. Thevenet, I. Valchev, F. Valentin, S. Vasilyev, F. Veljanovska, S. Venelinova, E. Veselovskaya, B. Viola, C. Virag, J. Zaninović, S. Zäuner, P. Stockhammer, G. Catalano, R. Krauß, D. Caramelli, G. Zarina, B. Gaydarska, M. Lillie, A. Nikitin, I. Potekhina, A Papathanasiou, D. Borić, C. Bonsall, J. Krause, R. Pinhasi, and D. Reich, 2018, „The genomic history of southeastern Europe”, *Nature*, 555, 7695, 197-203.

Mathieson, I., I. Lazaridis, N. Rohland, S. Mallick, N. Patterson, S. Alpaslan Roodenberg, E. Harney, K. Stewardson, D. Fernandes, M. Novak, K. Sirak, C. Gamba, E. Jones, B. Llamas, S. Dryomov, J. Pickrell, J. Luís Arsuaga, J. María Bermúdez de Castro, E. Carbonell, F. Gerritsen, A. Khokhlov, P. Kuznetsov, M. Lozano, H. Meller, O. Mochalov, V. Moiseyev, M. Rojo Guerra, J. Roodenberg, J. Maria Vergès, J. Krause, A. Cooper, K. Alt, D. Brown, D. Anthony, C. Lalueza-Fox, W. Haak, R. Pinhasi, and D. Reich, 2015, „Genome-wide patterns of selection in 230 ancient Eurasians”, *Nature*, 528, 7583, 499-503.

Meyer, M., M. Kircher, M. Gansauge, H. Li, F. Racimo, S. Mallick, J. Schraiber, F. Jay, K. Prüfer, C. de Filippo, P. Sudmant, C. Alkan, Q. Fu, R. Do, N. Rohland, A. Tandon, M. Siebauer, R. Green, K. Bryc, A. Briggs, U. Stenzel, J. Dabney, J. Shendure, J. Kitzman, M. Hammer, M. Shunkov, A. Derevianko, N. Patterson, A. Andrés, E. Eichler, M Slatkin, D Reich, J Kelso, and S. Pääbo, 2012, „A High-Coverage Genome Sequence from an Archaic Denisovan Individual”, *Science*, 338, 6104, 222-226.

Moore, N., and L. Weyrich, 2021, „A Standardized Approach for Shotgun Metagenomic Analysis of Ancient Dental Calculus”, *The Oral Microbiome*, 2327, 93-118.

Nielsen, R., J. Akey, M. Jakobsson, J. Pritchard, S. Tishkoff, and E. Willerslev, 2017, „Tracing the peopling of the world through genomics”, *Nature*, 541, 7637, 302-310.

Novembre, J., T. Johnson, K. Bryc, Z. Kutalik, A. Boyko, A. Auton, A. Indap, K. King, S. Bergmann, M. Nelson, M. Stephens, and C. Bustamante, 2008, „Genes mirror geography within Europe”, Nature, 456, 7218, 98-101.

Orlando, L., R. Allaby, P. Skoglund, C. Der Sarkissian, P. Stockhammer, M. Ávila-Arcos, Q. Fu, J. Krause, E. Willerslev, A. Stone, C. Warinner, 2021, „Ancient DNA analysis”, *Nature Reviews Methods Primers*, 1, 1, 1-26.

Reich, D., R. Green, M. Kircher, J. Krause, N. Patterson, E. Durand, B. Viola, A. Briggs, U. Stenzel, P.

Johnson, T. Maricic, J. Good, T. Marques-Bonet, C. Alkan, Q. Fu, S. Mallick, H. Li, M. Meyer, E.. Eichler, M. Stoneking, M. Richards, S. Talamo, M. Shunkov, A. Derevianko, J. Hublin, J. Kelso, M. Slatkin, and S. Pääbo, 2010, „Genetic history of an archaic hominin group from Denisova Cave in Siberia”, *Nature*, 468, 7327, 1053-1060.

Renaud, G., V. Slon, A. Duggan, and J. Kelso, 2015, „Schmutzi: estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA”, *Genome Biology*, 16, 1, 224.

Rusu, I., A. Modi, C. Radu, C. Mircea, A. Vulpoi, C. Dobrinescu, V. Bodolică, T. Potârniche, O. Popescu, D. Caramelli, and B. Kelemen, 2019, „Mitochondrial ancestry of medieval individuals carelessly interred in a multiple burial from southeastern Romania”, *Scientific Reports*, 9, 1, 961.

Rusu, I., A. Modi, S. Vai, E. Pilli, C. Mircea, C. Radu, C. Urduzia, Z. Karl Pinter, V Bodolică, C. Dobrinescu, M. Hervella, O. Popescu, M. Lari, D. Caramelli, and B. Kelemen, 2018, „Maternal DNA lineages at the gate of Europe in the 10th century AD”, *PloS One*, 13, 3, e0193578.

Sawyer, S., J. Krause, K. Guschanski, V. Savolainen, and S. Pääbo, 2012, „Temporal Patterns of Nucleotide Misincorporations and DNA Fragmentation in Ancient DNA”, *PloS One*, 7, 3, e34131.

Warinner, C., A. Herbig, A. Mann, J. Fellows Yates, C. Weiß, H. Burbano, L. Orlando, and J. Krause, 2017, „A Robust Framework for Microbial Archaeology”, *Annual Review of Genomics and Human Genetics*, 18, 1, 321-356.

Wright, S., K. Dobney, and L. Weyrich, 2021, „Advancing and refining archaeological dental calculus research using multiomic frameworks”, *STAR: Science & Technology of Archaeological Research*, 7, 1, 13-30.