

Scientists recover an ancient woman's DNA from a 20,000-year-old pendant

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Pierced deer tooth discovered from Denisova Cave in southern Siberia that yielded ancient human DNA. Credit: Max Planck Institute for Evolutionary Anthropology



Artifacts made of stone, bones or teeth provide important insights into the subsistence strategies of early humans, their behavior and culture. However, until now it has been difficult to attribute these artifacts to specific individuals, since burials and grave goods were very rare in the Paleolithic. This has limited the possibilities of drawing conclusions about, for example, division of labor or the social roles of individuals during this period.

In order to directly link cultural objects to specific individuals and thus gain deeper insights into Paleolithic societies, an international, interdisciplinary research team, led by the Max Planck Institute for Evolutionary Anthropology in Leipzig, has developed a novel, non-destructive method for DNA isolation from bones and teeth. Although they are generally rarer than <u>stone tools</u>, the scientists focused specifically on artifacts made from skeletal elements, because these are more porous and are therefore more likely to retain DNA present in skin cells, sweat and other body fluids.

A new DNA extraction method

Before the team could work with real artifacts, they first had to ensure that the precious objects would not be damaged. "The surface structure of Paleolithic bone and tooth artifacts provides important information about their production and use. Therefore, preserving the integrity of the artifacts, including microstructures on their surface, was a top priority," says Marie Soressi, an archaeologist from the University of Leiden who supervised the work together with Matthias Meyer, a Max Planck geneticist.





Elena Essel working in the clean laboratory on the pierced deer tooth discovered from Denisova Cave. Credit: Max Planck Institute for Evolutionary Anthropology

The team tested the influence of various chemicals on the surface structure of archaeological bone and tooth pieces and developed a nondestructive phosphate-based method for DNA extraction. "One could say we have created a washing machine for <u>ancient artifacts</u> within our clean laboratory," explains Elena Essel, the lead author of the study who developed the method. "By washing the artifacts at temperatures of up to 90°C, we are able to extract DNA from the wash waters, while keeping



the artifacts intact."

Early setbacks

The team first applied the method to a set of artifacts from the French cave Quinçay excavated back in the 1970s to 1990s. Although in some cases it was possible to identify DNA from the animals from which the artifacts were made, the vast majority of the DNA obtained came from the people who had handled the artifacts during or after excavation. This made it difficult to identify ancient human DNA.



The pierced deer tooth discovered from Denisova Cave before DNA extraction.



Credit: Max Planck Institute for Evolutionary Anthropology

To overcome the problem of modern human contamination, the researchers then focused on material that had been freshly excavated using gloves and face masks and put into clean plastic bags with sediment still attached. Three tooth pendants from Bacho Kiro Cave in Bulgaria, home to the oldest securely dated <u>modern humans</u> in Europe, showed significantly lower levels of modern DNA contamination; however, no ancient human DNA could be identified in these samples.

A pendant from Denisova Cave

The breakthrough was finally enabled by Maxim Kozlikin and Michael Shunkov, archaeologists excavating the famous Denisova Cave in Russia. In 2019, unaware of the new method being developed in Leipzig, they cleanly excavated and set aside an Upper Paleolithic deer tooth pendant. From this, the geneticists in Leipzig isolated not only the DNA from the animal itself, a wapiti deer, but also large quantities of ancient human DNA. "The amount of human DNA we recovered from the pendant was extraordinary," says Elena Essel, "almost as if we had sampled a human tooth." The findings are published in the journal *Nature*.





The pierced deer tooth discovered from Denisova Cave after DNA extraction. Credit: Max Planck Institute for Evolutionary Anthropology





Excavation works in the South Chamber of Denisova Cave in 2019. Credit: Sergey Zelensky





The entrance to Denisova Cave. Credit: Richard G. Roberts





Top view of the pierced deer tooth discovered from Denisova Cave in southern Siberia. Credit: Max Planck Institute for Evolutionary Anthropology

Based on the analysis of mitochondrial DNA, the small part of the genome that is exclusively inherited from the mother to their children, the researchers concluded that most of the DNA likely originated from a single human individual. Using the wapiti and human mitochondrial genomes they were able to estimate the age of the pendant at 19,000 to 25,000 years, without sampling the precious object for C14 dating.

In addition to mitochondrial DNA, the researchers also recovered a substantial fraction of the nuclear genome of its human owner. Based on



the number of X chromosomes they determined that the pendant was made, used or worn by a woman. They also found that this woman was genetically closely related to contemporaneous ancient individuals from further east in Siberia, the so called "Ancient North Eurasians" for whom skeletal remains have previously been analyzed. "Forensic scientists will not be surprised that human DNA can be isolated from an object that has been handled a lot," says Matthias Meyer, "but it is amazing that this is still possible after 20,000 years."

The scientists now hope to apply their method to many other objects made from bone and teeth in the Stone Age to learn more about the genetic ancestry and sex of the individuals who made, used, or wore them.

More information: Elena Essel, Ancient human DNA recovered from a Palaeolithic pendant, *Nature* (2023). DOI: <u>10.1038/s41586-023-06035-2</u>. www.nature.com/articles/s41586-023-06035-2

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