TechNews

STORIES IN ANCIENT DNA

Technology developments are leading to rapid advances in ancient DNA analysis. Nathan Blow talks to researchers peering into the past through ancient DNA.

Entrance to archeological site of Vindija Cave, Croatia. Credit: MPI Evolutionary Anthropology.

It was in the spring of 2015 when an international team of researchers led by Eleftheria Palkopoulou and Love Dalen published the first complete genome sequences from two extinct woolly mammoths (1). From a technical point of view, the sequencing efforts were a tour-de-force. Although woolly mammoth DNA had already been sequenced more than 7 years earlier, with the sequencing of the nuclear genome first reported in 2008, Palkopoulou and Dalen took the genetic analysis of mammoths a step further. By decoding two complete and different mammoth genomes, the scientists were actually able to peer deeper into the lives of these extinct animals.

The two mammoths had not roamed the earth at the same time. One of the mammoth samples dated to around 4300 years ago, while the other was a much older Siberian specimen more than 44,000 years old.

Like detectives working a case, members of the research team used the genetic data they collected from the two samples to learn more about the demographics of ancient mammoth populations. Their data indicated that one of the last surviving mammoth populations actually underwent a severe population bottleneck, which effectively reduced the animals' genetic diversity right before they eventually went extinct.

Such insights into the lives and deaths of extinct animals are a sign of the incredible scientific progress researchers are making using the latest genetics technologies. Credit the development of

high-throughput next-generation sequencing (NGS) platforms possessing the capacity to decipher billions of bases of DNA in short periods of time for this jump in capability. Add to this sequencing technology advances in ancient DNA sample preparation methods and analysis tools, and it becomes easier to understand why there has been such growth in ancient DNA research in recent years—and why scientists are now keenly interested in using ancient DNA to learn more about the past.

Digging in

For many years now, skeletal remains have been the bedrock of ancient DNA studies. In the case of the woolly mammoth, it was soft tissues and teeth that yielded the DNA researchers used in their NGS analyses. These are fairly typical sample types when it comes to ancient DNA studies—bone fragments or samples found in lodged ice often provide the best starting materials for genetic analysis.

But bones or frozen samples are rare finds, and when they are unearthed, they become precious artifacts that need to be carefully preserved. Researchers interested in studying ancient DNA would like to use parts of those samples as starting material for DNA extraction. But that, of course, means destroying some of the rare sample, so the challenge for ancient DNA hunters has been balancing their need for starting material with their colleagues' need for sample preservation.

An example of a clean room used for the analysis of samples in ancient DNA studies. Researchers utilize clean rooms to avoid contamination of samples prior to genetic analyses. Credit: F. Vinken.

Ancient human remains have been found in caves across the world. Because they offer protection from the elements, caves are often where the best-preserved skeletal samples have been recovered. Still, such finds are very rare. More often than not, no human remains are found, even in those caves containing ancient human artifacts such as primitive tools.

Anthropologists can glean significant insights into the world of ancient humans and their interactions by analyzing the tools they used. In a perfect world, skeletal samples and tools the subject used in life would always be found together, enabling side-by-side molecular and anthropological analyses. As it turns out, this might be more possible than previously thought.

Last month, researchers from the Max Planck Institute for Evolutionary Anthropology in Germany provided surprising evidence showing that even when skeletal remains are missing from a cave, the DNA of those that once used the cave could still be recovered.

Matthias Meyer is a member of the Department of Evolutionary Genetics at the Max Planck

Institute. Throughout his research career, Meyer has been keenly interested in applying advanced sequencing technologies to the study of ancient DNA samples. And as NGS technology has advanced over the years, Meyer says his drive to find new sources of ancient human DNA has also grown.

“I have been interested in the possibility of obtaining human DNA from non-human material for a while,” says Meyer. Finding DNA from non-human material would solve the problem of needing to find and process rare bone samples.

Previously, there had been scattered reports describing isolation of ancient human DNA from objects such as stone and bone teeth. The problem was that these studies had not been convincing demonstrations according to Meyer. Yet, they did raise Meyer’s curiosity. He wondered if it would be possible to obtain ancient human DNA for analysis without using skeletal remains, as long as the technology to interrogate the sample was robust enough.

The question, however, was what would be a good source of non-human material? Since many previous

Excavation at a site in El Sidron, Spain. This was one of the sites assessed for ancient human DNA using cave sediments. Credit: El Sidron research team.

studies used bones located in different layers of cave sediments, and there were a number of “empty” caves around the world known to have been used by ancient humans, Meyer decided his best bet would be to look into the soil sediments from caves lacking human remains. But he was not sure exactly what he would find in the soil.

“I thought we would find fauna DNA in the samples,” recalls Meyer, “I was less confident that there would be human DNA.”

With the sampling locations decided, Meyer now had to consider the technical issues they would likely encounter. The biggest of these issues was going to be the fact that the DNA sequences Meyer’s team would obtain from the cave soil would very likely be mixtures of different species’ DNA. The question for the researchers became: If the sequence data obtained was in fact a collection of all the animals that had been using the cave over

thousands of years, how could they focus their experiments specifically on finding human DNA?

Meyer suspected that out of all the possible DNA fragments they might find in the cave soil sample, human DNA would be amongst the rarest. And then they would have to deal with the presence of bacterial DNA, along with the short DNA fragments obtained when working with ancient DNA, which can prove difficult to classify. He did have one thing on his side though.

“We had developed a very robust analysis pipeline for ancient DNA sequencing, so we decided to push forward and see what we would find,” says Meyer.

The team collected 85 cave sediment samples from 7 different locations that early humans are known to have occupied (2). DNA was extracted from the sediment samples, and used to generate single-stranded DNA libraries for sequencing. The initial analysis showed that a majority

of the sequencing reads could not be identified. But there was a bright spot—between 0.05% and 10% of reads from the sequencing runs could be attributed to mammals. And this was the information that Meyer was most interested in. The question then became: How to get specifically at those human DNA sequences? For this, Meyer tried something new for ancient DNA analysis—he decided to directly target human DNA.

Targeted capture is a tech-

Matthias Meyer from the Max Planck Institute for Evolutionary Anthropology in Leipzig led a team of researchers who developed a new method to obtain ancient DNA from cave sediments. Credit: M. Meyer.

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nique that uses specifically designed DNA probes to select for and enrich specific DNA sequences from a larger sample. Targeted capture approaches have been used by many scientists to enrich for exomes or to collect specific genes for sequencing. Meyer's team was going to apply the approach in much the same way, only their probes would exclusively target human mitochondrial DNA so that they could drill down to the human sequences within their sediment samples. The initial numbers were promising—for some sites more than 8000 unique sequences passed the team's filtering workflow. Confident they were on the right track, the scientists then went back and extracted more DNA from the cave sediments to create additional sequencing libraries to obtain even higher numbers of sequences for analysis.

Perhaps somewhat surprisingly, when Meyer's group compared numbers of human DNA fragments obtained from cave sediments with similar starting amounts of bone samples, they found that although yielding slightly lower read numbers, cave sediments actually provided good quantities of DNA for analysis.

With the technical details in place and their data sets in hand, Meyer's interest turned to uncovering long-gone information on our early human relatives. Similar to the mammoth genome sequencing efforts, having a collection of early human sequences is only informative if the data tell a story about how our ancestors lived and moved around the world. Meyer's team has been able to determine whether the DNA they recovered in the caves they studied originated from Neanderthals or Denisovans, and also how many early humans might have occupied those caves. This information is providing new insights into early human population distribution and interactions. Now, Meyer is looking towards a bigger data set.

"It would be good to be able to reconstruct with high resolution the movements of humans through time," says Meyer. This type of analysis could lead to a better understanding of how our human ancestors may have interacted with Neanderthals and others, how communities developed, and how humans evolved over time.

Drilling In

From a technical standpoint, Meyer's cave sediment studies are important for two major reasons. First, they provide researchers with a unique new source for isolating ancient DNA. But secondly, and perhaps in some ways as importantly, obtaining ancient DNA from soil samples means that the fossils traditionally used in ancient DNA analysis studies are not being destroyed or damaged in an effort to collect DNA. Other researchers have also been exploring ways to make collection of ancient DNA samples less destructive.

For skeletal skull samples, the osseous labyrinth located inside the petrous bone has been reported to yield higher amounts of endogenous DNA than any other skeletal element. As such, researchers are interested in using this area in their efforts to recover ancient DNA from skull samples. The problem is that accessing the osseous labyrinth often means inflicting major structural damage on the bone, making it difficult for those seeking to perform morphometric analysis of the skull later on.

Kendra Sirak and her colleagues at Emory University are now reporting a new approach to sampling this region of the skull that might make it possible to get the needed DNA and still preserve the skull's structure. In this issue of *BioTechniques*, Sirak's team details a new drilling technique that enables the extraction of osseous labyrinth samples without destruction of the skull (3).

The protocol developed by Sirak and her colleagues involves drilling into the base of the skull without damaging surrounding structures. When this approach was compared to using whole petrous bone, the data showed that although the new drilling approach yielded lower endogenous DNA amounts, it did provide more DNA than using any other postcranial bone.

The importance of Sirak's work lies in the ability to perform genetic analyses on a very precious sample that would otherwise have been inaccessible, in much the same way Meyer's team has done by using soil samples. The development of tools and methods to preserve rare samples and bones while obtaining DNA is crucial for the furthering of ancient DNA research.

As more researchers develop these tools to interrogate ancient DNA at a finer level, the result will be the emergence of new insights into human and animal evolution. And once these genetic stories can be paired with archeological data and historical knowledge, our understanding and appreciation of human history will only grow deeper.

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