Abstract

Archaeologists digging in Bacho Kiro Cave in Bulgaria found bones from people who lived 45,000 years ago. This period is known as the early Upper Paleolithic. It was an exciting time for human evolution as humans took a big jump forward in art and technology. It’s also a time when Neanderthals were still around.

We wanted to know what these people’s DNA could tell us about where their ancestors came from and where their descendants ended up. Surprisingly, even though the bones were found in Europe, their DNA had more in common with people from East Asia and Native Americans. We also found that all the individuals whose remains were found in Bacho Kiro Cave had Neanderthal ancestors only a few generations back. That means that not only did Stone Age humans and Neanderthals know about each other, they sometimes had children together.

Introduction

Today, there’s only one species of human: Homo sapiens. But step back in time 45,000 years to the beginning of the Upper Paleolithic (the Late Stone Age), and you’d find that Homo sapiens weren’t the only people on the planet. Archaic humans, including Neanderthals in Europe and Denisovans in Asia, interacted with humans for thousands of years before going extinct.

We don’t know a whole lot about how humans and Neanderthals interacted. From DNA evidence, though, we know that they did! Many people today have traces of Neanderthal genes in their DNA. This means that humans and Neanderthals must sometimes have had children together.

Neanderthals looked very similar to humans. Like humans, they made stone tools, hunted with spears, used fire, and buried their dead. However, archaeologists can tell humans and Neanderthals apart by the differences in their skeletons. Neanderthals had deeper chests, shorter limbs, and thicker brows.

Around 50,000 years ago, something changed. Humans started making fancier tools, including artwork, jewelry, and even musical instruments! In 2015, a team of archaeologists started digging in Bacho Kiro Cave in Bulgaria (in Eastern Europe). They found thousands of specimens, including animal bones, stone tools, and jewelry. They also found fragments of ancient human bones and teeth. Using radiocarbon dating, they found out that these people lived more than 40,000 years ago. These fragments are the oldest remains of Upper Paleolithic people found in Europe!

We wanted to know whether these people were
What Can Ancient DNA Tell Us about Stone Age People?

**Methods**

We analyzed four bone fragments and a lower molar found near each other in Bacho Kiro Cave (Figure 1). These five specimens are all between 46,000 and 43,000 years old, from the initial Upper Paleolithic era. We also analyzed a few specimens that were a little younger, including:

1. A bone fragment from a different section of Bacho Kiro Cave, found around the same time as the first five specimens (about 35,000 years old).
2. A bone fragment from a different section of Bacho Kiro Cave, found in the 1970s (also about 35,000 years old).
3. A mandible (jaw bone) found in Peștera, Romania (between 42,000 and 37,000 years old).

To find the DNA, we scraped a tiny piece off of each specimen, then ground that piece into powder. We had to be careful not to mix up ancient DNA with newer DNA. In a similar way to how old paper looks and feels different than new paper, there are differences between ancient DNA and modern DNA. We only used the DNA that had signs of being ancient in our analysis.

Among the ancestors of people living today. We also wanted to know where these people came from. If we could find any of their DNA in the specimens, we would have an opportunity to answer those questions!

Figure 1:

We analyzed specimens found at Bacho Kiro Cave, Bulgaria and Peștera cu Oase, Romania. These locations are within the area where Neanderthals are thought to have lived (blue region). Most of the region north of the edge of the blue line was ice and polar desert 50,000 years ago.
Results

From the five initial Upper Paleolithic specimens, two were from the same person, and one was too contaminated for us to analyze. That left us with three individuals from Bacho Kiro Cave whose DNA we could study.

All three of these individuals:

1. Had Neanderthal ancestors between five and seven generations back in their family trees (Figure 2).
2. Were more closely related to each other than to individuals found at other ancient sites.
3. Were more closely related to people from East Asia, Oceania, and the Americas than to people from western Eurasia (Europe and West Asia).

Of the two more recent specimens from Bacho Kiro Cave, only one had enough DNA in the sample for us to use. That person’s DNA had more in common with people from present-day western Eurasia than the DNA from the first three people.

The individual whose mandible was found in Romania also had a recent Neanderthal ancestor. But their DNA did not have much in common with any present-day people.

Figure 2:
This chart shows the DNA from one of the Bacho Kiro Cave individuals. This person lived 43,000 years ago. About 3.4% of this person’s DNA came from their Neanderthal ancestor. That’s almost twice as much Neanderthal DNA as you would find in a person today! Since there were still some long sections of Neanderthal DNA, we knew that this person’s Neanderthal ancestor was only about 6 steps back in their family tree.

Which chromosome helped us identify this person as having a recent Neanderthal ancestor?
Discussion

Ancient DNA is very fragile, and we were worried that there wouldn’t be any usable DNA. We were surprised and excited that we were able to extract ancient DNA from almost all the specimens! Even though we only found a tiny bit of ancient DNA, it was enough for us to discover some interesting things. Before we did this research, only one specimen had ever been found from a person with recent Neanderthal ancestors. All three of the initial Upper Paleolithic people we studied had recent Neanderthal ancestors. We think that means that it was pretty common for there to be Neanderthals in people’s family trees.

It was surprising that the three initial Upper Paleolithic people weren’t related to the person who lived 10,000 years later in the same location. This means that the descendants of the initial Upper Paleolithic people migrated east to Asia, Oceania, and the Americas. In the meantime, a different group took their place in Europe.

Conclusion

The beginning of the Upper Paleolithic is an important time in human history. A lot of modern human behaviors started to appear, like making art, music, and sophisticated tools. But there are not very many specimens of people from that time. There are even fewer specimens whose DNA has been analyzed. We now know that humans and Neanderthals interacted with each other during the early Upper Paleolithic.

There is still so much to learn about prehistoric people, and our research continues!

Have a think about your own family history. It is highly likely that you do not know everything about your ancestors, even with the increasing availability of modern records and technology. How far back can you trace your family tree?

Glossary of Key Terms

- **Archaic human**: an extinct species of primates (the group of mammals that includes humans, apes, and monkeys) that are in the same family as humans.
- **DNA**: the material that carries information about how every living thing will look and function. It is in every cell of all living things and can be found in structures of the cell called chromosomes.
- **Migration**: traveling from one place to another. Migration can be fast, like when birds travel from their nesting area and back every year, or it can happen over long periods of time and even over many generations.
- **Radiocarbon dating**: a method to estimate how old a specimen is based on counting the number of carbon atoms with extra neutrons compared to the number of normal carbon atoms. Radiocarbon dating only works for specimens that came from living things (e.g. pieces of bone) and only works on things that are less than 60,000 years old.
- **Upper Paleolithic**: the period between 50,000 years to 12,000 years ago, beginning with advances in human art and technology and ending with the melting of glaciers in northern Europe and America.
Check your understanding

1. What did Neanderthals look like?
2. What makes the initial Upper Paleolithic an interesting time for archaeologists?
3. What happened to the descendants of the initial Upper Paleolithic people found in Bacho Kiro Cave? How do we know?
4. What were some things that surprised us?
5. What are the differences between you and your ancestors from 5 generations ago? (A generation is 15-30 years.) How were music, art, and tools different then?

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