

## Tracing the Ancestral Roots of Neandertals

*We recovered DNA from two 120,000-year-old Neandertal bones discovered in Europe. All later Neandertals trace at least part of their ancestry to these early European Neandertals. Yet, additional ancestry from an unknown population hints at a mysterious event deeper in Neandertal history.*

Before modern humans started migrating outside Africa, Eurasia was home to Neandertals, a group of humans that parted ways with the ancestors of modern humans half a million years ago. Neandertals lived in Europe for hundreds of thousands of years, from at least 430,000 years ago to about 40,000 years ago. Yet, we know little about their long history. It is only with the recent advances in our ability to retrieve DNA from ancient remains that we can now study the genetic relationship among Neandertals and reconstruct their deep population history.

Retrieving DNA from old specimens is challenging. DNA molecules are fragile and tend to get lost over time. Under favourable conditions, some DNA may preserve until today, but the older the specimen is, the less likely DNA remains. As a result, most of the genetic information retrieved so far comes from the Neandertals that lived most recently (~40,000 years ago). However, DNA from earlier Neandertals is necessary to reconstruct their deep history.

Except for an ancestor of Neandertals found in Northern Spain, the oldest Neandertal DNA retrieved so far comes from a woman who lived 120,000 years ago near Denisova Cave in Southern Siberia. She belonged to a population that disappeared shortly afterwards, as a 90,000-year-old bone found in the same cave comes from an individual who was instead more closely related to Neandertals that lived later in Europe. Thus, we can infer that migrations occurred between Europe and Southern Siberia. But the origin of these migrants is unknown, as we do not have DNA from Neandertals living prior to ~55,000 years ago in Europe.

We therefore set to gather information about early European Neandertals by retrieving DNA from two ~120,000-year-old Neandertal bones found in Europe. The first is a femur from Hohlenstein-Stadel Cave in Germany, and the second is the jawbone of a child from Scladina Cave in Belgium. We compared their DNA to the genomes of a 55,000-year-old Neandertal from Vindija Cave in Croatia and the 120,000-year-old Neandertal from Denisova Cave. As DNA is passed on from generation to generation, mutations occur that change the DNA sequence. By counting how often the Hohlenstein-Stadel or Scladina Neandertals share mutations with the Croatian Neandertal but not the Siberian one, or vice versa, we could identify to which Neandertal they were most closely related and estimate how long ago they shared a common ancestor.

We found that both of the 120,000-year-old European Neandertals from Hohlenstein-Stadel and Scladina were more closely related to the more recent European Neandertal than to the 120,000-year-old Siberian Neandertal. In fact, both the Hohlenstein-Stadel and Scladina Neandertals likely lived among the ancestors of later Neandertals. Thus, the population of late Neandertals that spread across Eurasia, reaching as far East as Southern Siberia by 90,000 years ago, had deep roots in Western Europe, with ancestors going back to at least as far as 120,000 years ago.

Yet, a previous look at the mitochondrial DNA - a tiny fraction of the DNA only transmitted from mother to child - from the Hohlenstein-Stadel Neandertal revealed a twist in the ancestry of this Neandertal. This DNA exhibited many genetic differences to other Neandertals. Using computer simulations, we showed that the mitochondrial DNA of the Hohlenstein-Stadel Neandertal most likely came from a Neandertal population isolated for a long time, perhaps during an ice age. Alternatively, it may also hint at a mixture between Neandertals and a population more related to modern humans. DNA of more Neandertals from an early time in Europe will help us resolve this mystery.