

DNA and Early Human History Neandertals and Early Humans: But Did They Mate?

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Studies in Molecular Evolution, especially those with forensic, ancient DNA or human history implications, provide a compelling framework for teaching. Since such research topics tend to capture the attention and imaginations of students, they provide excellent opportunities for instruction in basic biological disciplines such as evolutionary biology, genetics, molecular biology, physiology and anatomy, biochemistry and cell biology. In addition, they illustrate the capacity of biological studies to reinforce and contribute to knowledge in history, archaeology and anthropology, geography and geology, linguistics, law, and social studies. We are also provided with the opportunity to emphasize critical and recurrent themes in scientific research such as the importance of sample sizes and experimental design, the linkage between methodology and conclusions, and the differences between results, interpretation, and extrapolation. In this example we consider the nature of the relationship between Neandertals and the ancestors of modern humans.

Neandertals, named after the German valley (Neander Tal) where their fossil remains were first discovered in 1856, are extinct hominids that lived in Europe and Western Asia. Living from approximately 30,000 to 300,000 years ago, they were the prototype for the "robust" classic "caveman", in contrast to the "gracile" modern human. Neandertal skeletons suggest they were a larger and more muscular version of modern man with low foreheads, protruding brows, poorly defined chins, and large noses with broad nostrils. Portrayed at various times in modern history as brutish, clumsy, ape-like creatures, the Neandertals were, in fact, large-brained hominids who survived for hundreds of thousands of years with distinctive culture (including burial of their dead) and techniques for making tools, spears, and objects of art.

Archaeological evidence reveals that the Neandertals disappeared somewhat abruptly 25,000 to 30,000 years ago. Their demise was preceded by the arrival in their geography of our direct ancestor, the anatomically modern Cro-Magnons. There is ample archaeological evidence that the two groups likely co-existed and even sequentially inhabited the same sites over perhaps thousands of years. Were the two groups completely unaware of each other, and thus could not interact at all? Were the two aware of each other only through distant observation? Or as some scientists believe, did they directly interact with each other, leading to stimulations in cultural achievements for both groups? Or, in the intriguing question that inquiring minds want to know, "Did they mate?" Of course, this is the way the question is posed to capture our attention. What the scientist really wishes to know is "Did Neandertals contribute DNA to modern humans?" Whether or not our ancestors had sex with Neandertals, if there were no fertile offspring, there cannot be any historical record in DNA.

Another way of stating this is if there were widespread, successful matings among Neandertals and the ancestors of modern humans, then there would be contribution of Neandertal genetic sequences to the genomes of modern humans.

Three hypotheses about the nature of the genetic relationship between Neandertals and modern humans have been offered. At one extreme is a replacement hypothesis, proposing that the Neandertals were a fundamentally different type of human (perhaps even a different species) that represent an evolutionary dead-end, with no genetic contributions to present-day humans. At the other extreme is a linear evolution hypothesis that modern humans in Europe evolved directly from Neandertals, providing major genetic contributions to present-day humans. In an intermediate model, it is possible that Neandertals made limited genetic contributions of some genes to modern humans.

Testing these hypotheses requires a comparison of DNA from Neandertal and modern human samples - a formidable task at best. Technical challenges abound in the analysis of ancient DNA. Accurately dated, well-preserved fossils are rare and extremely valuable. Even when such exist, the DNA will be subjected to normal degradative processes that occur over time, resulting in both very short segments of DNA and alterations in chemical structure. The extremely sensitive polymerase chain reaction (PCR) that is instrumental in retrieving DNA segments from ancient DNA is particularly prone to problems with damaged DNA. Further complicating the problem is the likelihood that handling of the fossils by modern humans may have resulted in contamination. For these reasons, a number of criteria for acceptance of ancient DNA sequences have been widely adopted, including sequencing from multiple, cloned segments and reproduction of results in independent laboratories.

Nuclear genes occur in two copies per cell. The combination of their low abundance and the aforementioned technical problems make it highly unlikely that nuclear DNA sequences will be amenable to analysis in ancient samples. Scientists have concentrated instead on the use of mitochondrial DNA (mt DNA). The mitochondrial genome is present in hundreds to thousands of copies in

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each cell, providing an important technical advantage. The control region of the mitochondrial genome does not encode proteins, and contains hypervariable regions that are known to reveal differences between populations of humans. MtDNA has an additional unique feature in that it traces maternal lines of inheritance only.

In what has been widely hailed as a milestone in genetic studies of early human history, Svante Paabo and his collaborators (Krings et al., 1997) successfully isolated and analyzed a segment of Neandertal DNA. They used PCR to amplify sequences from the control region in mitochondrial DNA from samples isolated from a piece of right humerus from the Neandertal type specimen. They obtained the Neandertal sequence of one of the hypervariable regions and compared it to the reference sequence for modern humans. The Neandertal sequence was closer to that of humans than to chimpanzees, although there were dramatic differences from modern humans. Within the small region analyzed, there were 24 transition mutations, 2 transversion mutations, and one single nucleotide insertion when compared to modern humans. By comparison, modern humans differ, on average, by 8 substitutions in this same region; interestingly, the sites that vary between modern humans are not identical to those that vary between them and the Neandertal DNA. Krings et al. (1997) find that the Neandertal sequences lie outside of the variation present in modern humans, and suggest that Neandertals became extinct without contributing to the mtDNA pool present in extant human populations.

While acknowledging this research as a pioneering study in Neandertal scholarship, the scientific community (including the authors) raised the problems inherent in generalizing from a single sample. What if this individual were at an extreme in the genetic variation present in the Neandertal population? Could there be other, more prevalent, Neandertal mtDNA types that would be represented in the mtDNA spectrum of modern man? The critical corroboration of Neandertal mtDNA sequences came shortly after the Krings' study with the publication of two additional Neandertal sequences: one from an individual from Mezmaiskaya Cave in Russia (Ovchinnikov et al., 2000) and the other from Vindija Cave in Croatia (Krings et al., 2000). The three Neandertal sequences form a clade distinct from modern humans. Knowledge of mtDNA types from three different individuals who are geographically and temporally isolated reinforces the fact that Neandertals are genetically distinct from modern humans. It also strongly corroborates the interpretation that Neandertal mtDNA types are not present in extant human pools.

For the conclusion that Neandertals do not contribute to modern gene pools to be correct, it is necessary for the known human sequences to be an accurate representation of the event in question. The unique inheritance patterns and evolutionary pressures on mtDNA make it reasonable to question whether it accurately represents all possible genetic pathways. Since we do not yet have (and may never get) other DNA sequences from ancient DNA, this is something of a moot objection. Assuming that mtDNA is the only option, it is important to note that the current human database includes over one thousand individuals representing a variety of different populations. While there may be additional variants not yet discovered, it is unlikely that the current view of existing mtDNA types is fundamentally unrepresentative of existing humans.

A more thorny issue is whether the mtDNA variation present in modern people is an accurate representation of our full genetic history. What if the existing variation is but a subset of that which has occurred in the direct lineage of modern man? Is it possible that Neandertals mated extensively with our ancestors and that the Neandertal type mtDNA contribution has been lost? In other words, could an absence of Neandertal mtDNA types in modern humans be due to forces of evolution other than reproductive isolation? The fact that much evidence points to modern humans as arising from a small number of progenitors in a bottle neck event makes this an important consideration.

Recently, Adcock et al. (2001) provided evidence that mtDNA sequences can "go extinct". Among the remains of ten ancient Australian individuals typed for mtDNA sequences was an anatomically modern man from Lake Mungo. The remains of the Lake Mungo man had been dated by three separate methods to be older than 60,000 years. The Lake Mungo man mtDNA contains a sequence that is different from the other fossil remains and different from all modern humans, meaning that he must have possessed a now extinct lineage of mtDNA. Interestingly, a remnant of this particular mtDNA sequence does survive in modern people as an inserted segment on chromosome 11 in the nucleus! If a mtDNA sequence found in an early modern human can so easily "go extinct", the possibility exists that the same thing could have happened with a mtDNA from Neandertals.

While it remains to be seen whether the interactions between Neandertals and our ancestors were G, R, or X-rated, it is certain that the Neandertals and Cro-Magnons did not interact as portrayed in "B" movies. Neandertals were hardly the simple-minded, big and bumbling brutes who fell prey to the invading, intelligent and gracile Cro-Magnons. Both types of "humans" walked the earth together for thousands of years in and around Europe. Like many of the most interesting evolutionary questions, the issues surrounding the co-existence of Neandertal (and other ancient hominids) with our early ancestors will be investigated for years to come as new specimens and new approaches come to light. The final chapter in the relationship between Neandertal and modern man remains to be written.....

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