

## OLLI 497: Ancient DNA

### Session 3: October 12th

### Summary and Observations

#### Chapter 2: Encounters With Neanderthals

##### *The Meeting of Neanderthals and Modern Humans*

Reich begins this chapter on Neanderthals and modern humans with a thumbnail sketch of what we know about Neanderthals. I won't attempt to summarize this, but urge you to read it. However, one statement caught my eye: "... studies revealed that Neanderthals had **evolved in Europe** from even more archaic humans." Until now, I thought the "out of Africa" origin of Neanderthals still held.

Reich quickly gets to the heart of this section, namely the evidence we have for direct encounters between Neanderthals and modern humans. "There is hard scientific evidence that modern humans and Neanderthals met. The most direct is from western Europe, where Neanderthals disappeared around thirty-nine thousand years ago. The arrival of modern humans in western Europe was at least a few thousand years earlier, as is evident at Fumane in southern Italy where around forty-four thousand years ago, Neanderthal-type stone tools gave way to tools typical of modern humans."

"Meetings between Neanderthals and modern humans took place not only in Europe but almost certainly in the Near East as well. After around seventy thousand years ago, a strong and successful Neanderthal population expanded from Europe into central Asia as far as the Altai Mountains, and into the Near East. The Near East had already been inhabited by modern humans... [with their remains] dating to between about 130,000 and 100,000 years ago. Later, Neanderthals moved into the region... [with their remains] dating to between sixty and forty-eight thousand years ago. Reversing the expectation we might have that modern humans displaced Neanderthals at every encounter, Neanderthals were advancing from their homeland (Europe) even as modern humans retreated. Sometime after sixty thousand years ago, though, modern humans began to predominate in the Near East. Now the Neanderthals were the losers in the encounter, and they went extinct not only in the Near East but eventually elsewhere in Eurasia as well. So it was that in the Near East there were **at least two opportunities** for encounters between Neanderthals and modern humans: when early modern humans first peopled the region before around one hundred thousand years ago and established a population that met the expanding Neanderthals, and when modern humans returned and displaced the Neanderthals there sometime around sixty or fifty thousand years ago."

Given the extent of the encounters between Neanderthals and modern humans, Reich poses two questions: "Did the two populations **interbreed**? Are the Neanderthals among the direct ancestors of any present-day humans?" He notes that there "... is some skeletal evidence for hybridization." But "... shared skeletal features sometimes reflect adaptation to the same environmental pressures, not shared ancestry. This is why archaeological and skeletal records cannot determine the relatedness of Neanderthals to us. Studies of the genome can."

##### *Neanderthal DNA*

In an attempt to answer those two questions, Reich turns to the sequencing of Neanderthal DNA. He recounts that early researchers focused on mitochondrial DNA; lacking the

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sophisticated sequencers in use today, this tactic made it easier and more reliable to gain knowledge about ancient DNA. “Mitochondrial data analysis confirmed that Neanderthals shared maternal-line ancestors with modern humans more recently than previously thought—the best current estimate is 470,000 to 360,000 years ago. Mitochondrial DNA analysis also confirmed that the Neanderthals were highly distinctive. Their DNA type was outside the range of present-day variation in humans, sharing a common ancestor with us at a date several times more ancient than the time when “Mitochondrial Eve” lived.”

Reich notes that analysis of mitochondrial DNA could not support the conclusion that Neanderthals and modern humans interbred. “... the mitochondrial data were not conclusive, but nevertheless the view that Neanderthals and modern humans did not mix remained the scientific orthodoxy until Svante Pääbo’s team extracted DNA from the whole genome of a Neanderthal, making it possible to examine the history of all its ancestors, not just the exclusively maternal line.”

“The advance to sequencing the whole Neanderthal genome was made possible by a huge leap in the efficiency of the technology for studying ancient DNA in the decade after the sequencing of Neanderthal mitochondrial DNA.”

Prior to the new sequencing techniques, researchers relied on PCR: “The mainstay of ancient DNA research prior to 2010 was a technique called polymerase chain reaction (PCR).” After giving us a brief description of this technique, he concludes: “The effect is to take a tiny fraction of all the DNA in the sample and make it the dominant sequence. This method throws away the vast majority of DNA (the part that is not targeted). Nevertheless, it can extract at least some DNA that is of interest.”

“The new approach for extracting ancient DNA was radically different. It relied on sequencing all of the DNA in the sample, regardless of the part of the genome it comes from, and without preselecting the DNA based on targeting sequences.”

He next recounts the hurdles that stood in the way of implementing this new technique: “To make the new approach work, Pääbo’s team needed to overcome several challenges. First, they needed to find a bone from which they could extract enough DNA. Anthropologists often work with fossils—bones completely mineralized into rocks. But it is impossible to get any DNA from a true fossil. Pääbo was therefore looking for bones that were not completely mineralized but contained organic material, including stretches of well-preserved DNA. Second, supposing the team could find a “golden sample” with well-preserved DNA, they still had to overcome the problem of **contamination** of the sample by microbial DNA, which comes from the bacteria and fungi that embed themselves in bone after an individual’s death. These contribute the overwhelming majority of DNA in most ancient samples. Finally, the team had to consider the likelihood of contamination by the researchers—archaeologists or molecular biologists—who handled the samples and chemicals and may have left traces of their own DNA on them.”

Reich reiterates: “Contamination is a huge danger for studies of ancient human DNA.... Contamination has bedeviled ancient DNA researchers time and again. For example, in 2006 Pääbo’s group sequenced about a million letters of DNA from Neanderthals as a trial run prior to whole-genome sequencing. A high fraction of the sequences were modern human contaminants, compromising interpretation of the data.”

He describes some of the countermeasures that were developed to fight contamination: “Modern measures to minimize the possibility of contamination in ancient DNA analysis, which had already begun to be implemented in the 2006 study and which became even more elaborate afterward, involve an obsessive set of precautions. ...they took each of the bones

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they screened into a “clean room,” which they adapted from the blueprints of the clean spaces used in microchip fabrication facilities in the computer industry.... they placed the bones chosen for sampling into a chamber where they were exposed to high-energy UV radiation, again with the goal of converting the contaminating DNA that might be lying on the surface into a form that cannot be sequenced.... The air was ultra-filtered to remove tiny dust particles—anything more than one thousand times smaller than the width of a human hair—that might contain DNA. The suite was pressurized so that air flowed from inside to outside, to protect the samples from any contaminating DNA wafting in from outside the lab.”

Reich continues with a detailed description of the three step process of extracting and purifying ancient DNA from Neanderthal bones. I won’t attempt to summarize it here, but urge you to read it. But I do want to note the last step of the process: “In an extra measure to remove contamination beyond what had been done in the 2006 study, Pääbo and his team attached an artificially synthesized sequence of letters, **a chemical “barcode,”** to the ends of the DNA fragments. Any contaminating sequences that entered the experiment after the attachment of the barcode could thus be distinguished from the DNA of the ancient sample.”

Finally, he sums up the results: “The best-preserved Neanderthal samples turned out to be three approximately forty-thousand-year-old arm and leg bones from Vindija Cave in the highlands of Croatia. After sequencing from these bones, Pääbo’s team found that the great majority of DNA fragments they obtained were from bacteria and fungi that had colonized the bones. But by comparing the millions of fragments to the present-day human and chimpanzee genome sequences, they found gold amidst the dross. These reference genomes were like the picture on a jigsaw puzzle box, providing the key to aligning the tiny fragments of DNA they had sequenced. The bones contained as much as 4 percent archaic human DNA.”

### *Affinities Between Neanderthals and Non-Africans*

“The Neanderthal genome sequences we were working with were unfortunately full of errors. We could see as much because the data suggested that several times more mutations had occurred on the Neanderthal lineage than on the modern human lineage after the two sequences separated from their common ancestors.” He describes the reasoning that led to this conclusion, and concludes that “... most of the differences we found between the Neanderthal sequence and present-day human sequences were **errors created by the measurement process** and not genuine differences between the Neanderthal and present-day human genomes. To deal with the problem, we restricted our study to positions in the genome that are known to be variable among present-day humans. At these positions, an error rate of about 0.5 percent was too low to confuse the interpretation. Based on these positions, we designed a mathematical test for measuring whether Neanderthals were more closely related to some present-day humans than to others.”

The test they devised is now known as the “Four Population Test.” [I will go into the details of this test in our next meeting.] Reich describes the test as follows: “The test takes as its input the DNA letters seen at the same position in four genomes: for example, two modern human genomes, the Neanderthal, and a chimpanzee. It examines whether, at positions where there is a mutation distinguishing the two modern human genomes that is also observed in the Neanderthal genome—which must reflect a mutation that occurred prior to the final separation of Neanderthals and modern humans—the Neanderthal matches the second human population at a different rate from the first. If the two modern humans descend from a common ancestral population that separated earlier from the ancestors of Neanderthals, there is no reason why the mutation is more likely to have been passed down one modern human line than another,

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and thus the rate of matching of each of the two modern human genomes to Neanderthal is expected to be equal. In contrast, if Neanderthals and some modern humans interbred, the modern human population descended from the interbreeding will share more mutations with Neanderthals.”

Their conclusion: “When we tested diverse present-day human populations, we found Neanderthals to be about equally close to Europeans, East Asians, and New Guineans, but closer to all non-Africans than to all sub-Saharan Africans, including populations as different as West Africans and San hunter-gatherers from southern Africa. The difference was slight, but the probability of these findings happening by chance was less than one in a quadrillion. We reached this conclusion however we analyzed the data. This was the pattern that would be expected if Neanderthals had interbred with the ancestors of non-Africans but not Africans.”

### *Trying to Make the Evidence Go Away*

This finding led to a great deal of skepticism among Pääbo’s team: “We were skeptical about this conclusion because it went against the scientific consensus of the time—a consensus that had been strongly impressed on many members of our team.” This skepticism extended to Reich as well.

This skepticism led to several intense rounds of second guessing the results, and redoing the analysis with different techniques; Reich describes all this effort. But to no avail - the results stood the test.

“We remained skeptical, wondering if something we had not thought of could explain the patterns. Then, in June 2009, I attended a conference at the University of Michigan where I met Rasmus Nielsen, who had been scanning through the genomes of diverse humans from around the world. In most parts of the genome, Africans are more genetically diverse than non-Africans and carry the most deeply diverging lineages, as is the case with mitochondrial DNA. But Nielsen was identifying **rare places in the genome where the genetic diversity among non-Africans was greater than in Africans** because of lineages that split off the tree of present-day human sequences early and were present only in non-Africans. These sequences just might be derived from archaic humans who had interbred with non-Africans. Nielsen joined our collaboration and compared the regions he and his colleagues identified to the data. When he compared twelve of his special regions to the Neanderthal genome sequence, he found that in ten of them there was **a close match to the Neanderthal**. This was far too high a fraction to happen by chance. Most of Nielsen’s highly divergent bits of DNA had to be Neanderthal in origin.”

Reassured by this finding, the team continued to press ahead: “... we obtained a date for when the Neanderthal-related genetic material entered the ancestors of non-Africans. To do this, we took advantage of recombination—the process that occurs during the production of a person’s sperm or eggs that swaps large segments of parental DNA to produce novel spliced chromosomes that are passed to the offspring. For example, consider a woman who is a first-generation mixture of a Neanderthal mother and a modern human father. In her cells, each pair of her chromosomes consists of one unbroken Neanderthal chromosome and one unbroken modern human chromosome. However, her eggs contain twenty-three mixed chromosomes. One chromosome in an egg of hers might have its first half of Neanderthal origin and its other half of modern human origin. Suppose she mates with a modern human, and mixture continues down the generations with more modern humans. Over the generations, the segments of Neanderthal DNA get chopped into smaller and smaller bits, with recombination operating like

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the whirring blade of a food processor, splicing the parental DNA at random positions along the chromosome in each generation. By measuring the typical sizes of the stretches of Neanderthal-related DNA in present humans, evident from the size of sequences that match the Neanderthal genome more than they do sub-Saharan African genomes, we can learn how many generations have passed since the Neanderthal DNA entered a modern person's ancestors."

"With this approach, we found that at least some Neanderthal-related genetic material came into the ancestors of present-day non-Africans eighty-six thousand to thirty-seven thousand years ago. We have since refined this date by analyzing ancient DNA from a modern human from Siberia who, radiocarbon dating studies show, lived around forty-five thousand years ago. The stretches of Neanderthal-derived DNA in this individual are on average seven times larger than the stretches of Neanderthal-derived DNA in modern humans today, confirming that he lived much closer to the time of Neanderthal mixture. His proximity in time to the mixing event makes it possible to obtain a more accurate date of fifty-four thousand to forty-nine thousand years ago."

One objection to his conclusion pointed out that all the team had proven was that interbreeding between modern humans and archaic humans had occurred; these archaic humans may not have been Neanderthals. This objection was put to rest after the team was able to get a high-quality sequence of an at least 50,000 year old Neanderthal bone found in Siberia. "The high-quality sequence allowed us to determine how closely related modern humans and Neanderthals are to each other based on the number of mutations that have occurred on the lineages since they separated. We found few or no segments where the Siberian Neanderthal shared common ancestors with present-day sub-Saharan Africans within the last half million years. However, there were **shared segments with non-Africans** roughly within the past one hundred thousand years. These dates fell within the time frame when Neanderthals were fully established in West Eurasia. This meant that **the interbreeding was with true Neanderthals**, not some distantly related groups."

### *Mixing in the Near East*

"So how much Neanderthal ancestry do people outside of Africa carry today? We found that non-African genomes today are around 1.5 to 2.1 percent Neanderthal in origin, with the higher numbers in East Asians and the lower numbers in Europeans, despite the fact that Europe was the homeland of the Neanderthals. We now know that at least part of the explanation is dilution. Ancient DNA from Europeans who lived before nine thousand years ago shows that pre-farming Europeans had just as much Neanderthal ancestry as East Asians do today. The reduction in Neanderthal ancestry in present-day Europeans is due to the fact that they harbor some of their ancestry from **a group of people who separated from all other non-Africans prior to the mixture with Neanderthals** (the story of this early-splitting group revealed by ancient DNA is told in part II of this book). The spread of farmers with this inheritance diluted the Neanderthal ancestry in Europe, but not in East Asia."

Reich notes that Europe is "... the place where Neanderthals originated." So he asks "... is that the place where the main interbreeding that left its mark in people today occurred?" But he also notes that: "Evidence of interbreeding is detected today not just in Europeans but also in East Asians and New Guineans." Which leads to a further question: "So where could Neanderthals and modern humans have met and mixed to give rise to a population that expanded not only to Europe but also to East Asia and New Guinea? Archaeologists have shown how in the Near East, Neanderthals and modern humans traded places as the dominant

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human population at least twice between 130,000 and 50,000 years ago, and it is reasonable to guess that they might have met during this period. So **interbreeding in the Near East** provides a plausible explanation for the Neanderthal ancestry that is shared by Europeans and East Asians.”

But did they interbreed in Europe? Evidence came from the “... DNA from a skeleton from Oase Cave in Romania.... analysis of the data showed that the Oase individual, who radiocarbon dating studies had shown lived about forty thousand years ago, had around 6 to 9 percent Neanderthal ancestry, far more than the approximately 2 percent that we measure in present-day non-Africans. Some stretches of Neanderthal DNA extend a third of the length of his chromosomes—a span so large and unbroken by recombination that we can be sure that the Oase individual had an actual Neanderthal no more than six generations back in his family tree.”

Reich’s conclusion: “The discoveries about the interbreeding in the recent family tree of the Oase individual suggested that modern humans and Neanderthals also hybridized in Europe, the homeland of the Neanderthals. But the population of which Oase was a part—and which carried this clear imprint of interbreeding with European Neanderthals—may not have left any descendants among people living today. When we analyzed the genome of Oase, we found no evidence that he was more closely related to Europeans than to East Asians. This means that he had to have been part of a population that was **an evolutionary dead end**—a pioneer modern human population that arrived early in Europe, flourished there briefly and interbred with local Neanderthals, and then went extinct. Thus, while the Oase individual provides powerful evidence that interbreeding between Neanderthals and modern humans occurred in Europe, he does not provide any evidence that Neanderthal ancestry in non-Africans today is derived from European Neanderthals. **It remains the case that the most likely source of Neanderthal ancestry in non-Africans is Near Eastern Neanderthals.**”

### *Two Groups at the Edge of Compatibility*

Reich adds a further wrinkle to the low presence of Neanderthal DNA in contemporary humans “The **low fertility of hybrids** may also have reduced Neanderthal ancestry in the DNA of people living today.” Reich points to the work of Laurent Excoffier, “... who knew from studies of animals and plants that when one population moves into a region occupied by another population with which it can interbreed, even a small rate of interbreeding is enough to produce high proportions of mixture in the descendants—far more than the approximately 2 percent Neanderthal ancestry seen in non-Africans today. Excoffier argued that the only way that the modern human genome could have ended up with so little Neanderthal ancestry was if expanding modern humans had offspring with other modern humans **at least fifty times more often** than they did with the Neanderthals living in their midst. He thought that the most likely explanation for this was that Neanderthals and modern human offspring were **much less fertile** than the offspring of matings between pairs of modern humans.”

Reich originally held a different position: “Rather than low hybrid fertility, I favored the explanation that there simply wasn’t much interbreeding for social reasons. Even today, many groups of modern humans keep largely to themselves because of cultural, religious, or caste barriers. Why should it have been any different for modern humans and Neanderthals when they encountered one another?”

What changed his mind was a closer look at where in the genome Neanderthal DNA remained “Looking at where in the genome these Neanderthal ancestry fragments occurred, it became

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clear that the impact of Neanderthal interbreeding varied dramatically across the genome of non-African people today. The average proportion of Neanderthal ancestry in non-African populations is around 2 percent, but it is not spread evenly. In more than half the genome, no Neanderthal ancestry has been detected in anyone. **But in some unusual places in the genome, more than 50 percent of DNA sequences are from Neanderthals.**"

Looking at places in non-African genomes where Neanderthal ancestry is rare, he realized that natural selection "... systematically worked to remove it." And "... evidence of systematic removal of Neanderthal ancestry is exactly what we found—and, remarkably, we found a particularly intense depletion of Neanderthal ancestry by natural selection in two parts of the genome known to be relevant to the **fertility of hybrids.**"

Reich expands on this finding. "The first place of reduced Neanderthal ancestry was on chromosome X, one of the two sex chromosomes." Citing an earlier study of his on the separation of humans and chimpanzees, he notes a contradiction between what was expected and what was observed. "There are only three copies of chromosome X in any population for every four other chromosomes (because females carry two copies and males only one, in contrast to two copies in each sex for most of the rest of the chromosomes). This means that in any one generation, the probability that any two X chromosomes share a common parent is four-thirds the probability that any two of one of the other chromosomes share a common parent. It follows that the expected time since any pair of X chromosome sequences descend from a common ancestral sequence is about four-thirds of that in the rest of the genome. In fact, though, **the real data suggest a number that is around half or even less.**" He concluded that "... the patterns could be explained by a history in which the ancestors of humans and chimpanzees initially separated, then came together to form either human or chimpanzee ancestors before the final separation of the two lineages."

He applies this insight to the human/Neanderthal interbreeding: "How is it that hybridization can lead to so much less genetic variation on chromosome X than on the rest of the genome? From studies of a variety of species across the animal kingdom, it is known that when two populations are separated for long enough, hybrid offspring have reduced fertility. In mammals like us, reduced fertility is much more common in males, and the genetic factors contributing to this reduced fertility are concentrated on chromosome X. So when two populations are so separated that their offspring have reduced fertility, but nevertheless mix together to produce hybrids, it is expected that there will be intense natural selection to remove the factors contributing to reduced fertility. This process will be especially evident on chromosome X because of the concentration of genes contributing to infertility on it. As a result, there tends to be natural selection on chromosome X for stretches of DNA from the population that contributed most of the hybrid population's ancestry. This causes the hybrid population to derive its chromosome X almost entirely from the majority population, leading to an anomalously low genetic divergence on chromosome X between the hybrid population and one of the hybridizing populations, consistent with the pattern seen in humans and chimpanzees."

Reich next cites studies which support both the human/chimpanzee, and the Neanderthal/human findings. One study also showed that "... the regions that are denuded of Neanderthal mixture on chromosome X in non-Africans are to a large extent the same regions that are driving the low genetic divergence between humans and chimpanzees. This is what would be expected if mutations that contribute to reduced fertility when they occur in a hybrid individual tend to be concentrated not just on chromosome X, but in particular regions along chromosome X, causing the minority ancestry to be removed from the population by natural selection against the male hybrids who carry it. The evidence of selection to remove Neanderthal DNA from chromosome X was a tell-tale sign that **male hybrids had reduced fertility.**"

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Lastly, Reich points to a larger problem with Neanderthal DNA: “The problems faced by modern humans with Neanderthal ancestry went beyond reduced fertility, as it turns out that Neanderthal ancestry is not just reduced on the X chromosome and around genes important in male reproduction, but is also **reduced around the great majority of genes** (there is far more Neanderthal ancestry in “junk” parts of the genome with few biological functions). The clearest evidence for this came from a study in 2016, in which we published a genome-wide ancient DNA dataset from more than fifty Eurasians spread over the last forty-five thousand years. We showed that Neanderthal ancestry decreased continually from 3 to 6 percent in most of the samples we analyzed from earlier times to its present-day value of around 2 percent at later times and that this was **driven by widespread natural selection against Neanderthal DNA.**”

### *Thesis, Antithesis, Synthesis*

Reich ends this chapter with a comparison of the two main theories on human origin: multiregionalism and “out of Africa.” The latter superseded the former. But he believes the “out of Africa” theory is not quite the whole story. “We now have a synthesis, driven by the finding of gene flow between Neanderthals and modern humans based on ancient DNA. This affirms a “mostly out-of-Africa” theory, and also reveals something profound about the culture of those modern humans who must have known Neanderthals intimately. While it is clear from the genetic data that modern humans outside of Africa descend from the expansion of an African-origin group that swept around the world, we now know that some interbreeding occurred. This must make us think differently about our ancestors and the archaic humans they encountered. The Neanderthals were more like us than we had imagined, perhaps capable of many behaviors that we typically associate with modern humans. There must have been cultural exchange that accompanied the mixture.... We also know that there has been a biological legacy bequeathed by Neanderthals to non-Africans, including genes for adapting to different Eurasian environments....”

This leaves Reich with a question: “... the genetic record has forced our hand. Instead of confirming scientists’ expectations, it has produced surprises. We now know that Neanderthal/modern human hybrid populations were living in Europe and across Eurasia, and that while many hybrid populations eventually died out, some survived and gave rise to large numbers of people today. We now know approximately when the modern human and Neanderthal lineages separated. We now also know that when these lineages reencountered each other, they had evolved to such an extent that they were at the very limit of biological compatibility. This raises a question: Were the Neanderthals the **only** archaic humans who interbred with our ancestors? Or were there other major hybridizations in our past?”