

OLLI 497: Ancient DNA

Session 4: October 19th

Summary and Observations

Chapter 3: Ancient DNA Opens the Floodgates

There are several themes that run through this chapter. One is the greater insight into our human past gained by the “**whole genome**” approach as opposed to the analysis of mitochondrial DNA. Both yield interesting results, but are often in conflict. Reich continually emphasizes the value of the whole genome analysis. A second is the constant and continuous interbreeding between modern humans and archaic populations, and between archaic populations as well. **Successful interbreeding**, in spite of the designation of some of these archaic populations as **separate species** of *Homo*. And the third is the power of the whole genome approach to identify previously unknown archaic populations, ghost populations. Reich is able to infer the existence of Australo-Denisovans, a superarchaic ancestral population, the likelihood that Eurasia was a hot bed of human evolution, and that there may have been a backward migration into Africa of the ancestral population of modern humans. Whew!

A few quibbles... Reich does lead us down several blind alleys in his quest for an explanation of some phenomena. Going down these blind alleys does provide some insight into the problem, and does point to the ultimate solution. But I found myself wanting to say “Just get to the solution.” The other is the incredibly small sample of ancient DNA he is working with - 7 from Neanderthals, 1 (maybe 2?) from Denisovans. Stay tuned for some revisions.

A Surprise From the East

The ancient DNA “floodgates” began to open with the discovery of a small piece of human finger bone in the Denisova Cave in southern Siberia. A small piece of the bone went to Pääbo’s lab for sequencing: “Pääbo’s team, led by Johannes Krause, was successful in extracting **mitochondrial DNA** from the Denisova Cave bone. Its sequence was of a type that had never before been observed in more than ten thousand modern human and **seven Neanderthal sequences**. There are around two hundred mutational differences separating the mitochondrial DNA of people living today from that of Neanderthals. The new mitochondrial DNA from the Denisova finger bone featured nearly four hundred differences from the mitochondrial DNA of both present-day humans and Neanderthals. Based on the rate at which mutations accumulate, mitochondrial DNA sequences from present-day humans and Neanderthals are estimated to have separated from each other 470,000 to 360,000 years ago. The number of mutational differences found in the mitochondrial DNA from the Denisova finger bone suggested a separation time of roughly eight hundred thousand to one million years ago. This suggested that the finger bone might belong to **a member of a never-before-sampled group of archaic humans.**”

There was no archaeological data to help identify the population; genetic data came first.

A Genome in Search of a Fossil

The quality of the DNA extracted from the Denisovan finger bone was very high: “...the Denisova finger bone had provided one of the best-preserved samples of ancient DNA ever

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found. While Pääbo had screened dozens of Neanderthal samples to find a few with up to 4 percent primate DNA, this finger bone had about **70 percent**. Pääbo and his team had already been able to obtain more data on the whole genome (not just mitochondrial DNA) from this small bone than they had previously obtained from Neanderthals.”

Given the wealth of genetic information harvested from the Denisovan bone, Pääbo’s lab was able to reconstruct the whole genome. And once again: “For the Denisova finger bone, the whole genome painted a very different picture from what was recorded in the mitochondrial DNA.”

“The first revelation from the whole genome was that **Neanderthals and the new humans from Denisova Cave were more closely related to each other than either was to modern humans**—a different pattern from what was observed in mitochondrial DNA. We eventually estimated the separation between the Neanderthal and Denisovan ancestral populations to have occurred 470,000 to 380,000 years ago, and the separation between the common ancestral populations of both of these archaic groups and modern humans to have occurred 770,000 to 550,000 years ago.... Our findings meant that the Denisovans were cousins of Neanderthals, but were also very different, having separated from Neanderthal ancestors before many Neanderthal traits appeared in the fossil record.”

A debate ensued among the lab members on what to name this new population. They decided to use a generic name, “Denisovans”, after the cave where the bone was found. “This decision distressed some of our colleagues, who lobbied for a new species name—perhaps *Homo altaiensis*, after the mountains where Denisova Cave is located. *Homo altaiensis* is now used in a museum exhibit in Novosibirsk in Russia that describes the discovery at Denisova. We geneticists, however, were reluctant to use a species name. There has long been contention as to whether Neanderthals constitute a species separate from modern humans, with some experts designating Neanderthals as a distinct species of the genus *Homo* (*Homo neanderthalensis*), and others as a subgroup of modern humans (*Homo sapiens neanderthalensis*). The designation of two living groups as distinct species is often based on the supposition that the two do not in practice **interbreed**. But we now know Neanderthals interbred successfully with modern humans and in fact did so on multiple occasions seems to undermine the argument that they are distinct species. Our data showed that Denisovans were cousins of Neanderthals, and thus if we are uncertain about whether Neanderthals are a species, we need to be uncertain about whether Denisovans are a species as well.”

The Hybridization Principle

Reich expands the scope of the analysis: “Armed with a whole-genome sequence, we tested whether the Denisovans were more closely related to some present-day populations than others. This led to a huge surprise.”

“Denisovans were genetically a little closer to **New Guineans** than they were to any population from mainland Eurasia, suggesting that New Guinean ancestors had interbred with Denisovans. Yet the distance from Denisova Cave to New Guinea is around nine thousand kilometers, and New Guinea is, of course, separated by sea from the Asian mainland. The climate in New Guinea is also largely tropical, which could not be more different from Siberia’s bitter winters, and this makes it unlikely that archaic humans adapted to one environment would have flourished in the other.”

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The team searched for alternative explanations, one of which implied interbreeding between ancestral modern humans and Denisovans hundreds of thousand years ago. The whole genome analysis belied that scenario: “As soon as archaic populations mix with modern ones, the DNA segments contributed by archaic humans are chopped up by the process of **recombination**, spliced together with modern human segments at the rate of one or two splices per chromosome per generation. ...the length of Neanderthal ancestry segments corresponds to mixture between fifty-four and forty-nine thousand years ago. Based on how much longer the Denisovan segments were than the Neanderthal segments in New Guineans, we could conclude that the interbreeding between Denisovan and New Guinean ancestors occurred **fifty-nine to forty-four thousand years ago.**”

Reich goes on: “What percentage of New Guinean genomes today derives from Denisovans? By measuring how much stronger the genetic evidence of archaic ancestry is in New Guineans compared to other non-Africans, we estimated that about 3 to 6 percent of New Guinean ancestry derives from Denisovans. That is above and beyond the approximately 2 percent from Neanderthals. Thus in total, **5 to 8 percent of New Guinean ancestry comes from archaic humans.** This is the largest known contribution of archaic humans to any present-day human population.”

“The Denisova discovery proved that interbreeding between archaic and modern humans during the migration of modern humans from Africa and the Near East was not a freak event. So far, DNA from two archaic human populations—Neanderthals and Denisovans—has been sequenced, and in both cases, the data made it possible to detect hybridization between modern and archaic humans that had been previously unknown. I would not be surprised if DNA sequenced from the next newly discovered archaic population will also point to a previously unknown hybridization event.”

But this still leaves the question of where the interbreeding occurred that led to the high percentage of archaic human DNA in present-day New Guineans.

Breaching Huxley's Line

“Where, given the vast distance between Siberia and New Guinea, did interbreeding between Denisovans and the ancestors of New Guineans occur?”

“Our first guess was mainland Asia, perhaps India or central Asia, on a plausible human migratory path from Africa to New Guinea.”

In support of this hypothesis... “We collected DNA from present-day humans from the islands of the Southwest Pacific and from East Asia, South Asia, and Australia, and estimated how much Denisovan-related ancestry each of them had. We found the largest amounts of ancestry in indigenous populations in the islands off Southeast Asia and especially in the Philippines and the very large islands of New Guinea and Australia (by the word “indigenous” I refer to people who were established prior to the population movements associated with the spread of farming). The populations in question are largely east of Huxley's Line, a natural boundary that separates New Guinea, Australia, and the Philippines from the western parts of Indonesia and the Asian mainland. This line was described by the nineteenth-century British naturalist Alfred Russel Wallace, and adapted by his contemporary the biologist Thomas Henry Huxley to highlight differences in the animals living on either side, for example, it roughly forms the boundary between placental mammals to the west and marsupials to the east. It corresponds to deep ocean trenches that have formed geographical barriers to the crossing of animals and

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plants, even in ice ages when sea levels were up to one hundred meters lower. It is remarkable that modern humans after fifty thousand years ago made it across this barrier. These pioneers did manage to cross, but it must have been difficult. Modern humans with Denisovan-related ancestry living east of Huxley's Line—the ancestors of New Guineans, Australians, and Philippine populations who we found—are the groups with the largest proportions of Denisovan ancestry today—are likely to have been protected by the same barrier from further migrations from Asia, just like the animals with whom they share their landscape.”

“But a deeper look suggests that population mixture in the heart of Asia is not as easy an explanation as it might at first seem. Although some populations east of Huxley's Line have large amounts of Denisovan-related ancestry, the situation is very different to the west. Most notably, the indigenous hunter-gatherers of the Andaman Island chain off the coasts of India and Sumatra, and also the indigenous hunter-gatherers of the Malay Peninsula of mainland Southeast Asia, descend from lineages just as divergent as those in indigenous New Guineans and Australians, and yet they do not have much Denisovan-related ancestry. There is also no evidence of elevated Denisovan-related ancestry in genome-wide data from the approximately forty-thousand-year-old human of Tianyuan Cave near Beijing in China, which was sequenced several years later by Pääbo and his laboratory. Had the interbreeding occurred in mainland Asia, and modern humans carrying Denisovan-related ancestry then spread all over, multiple populations of the region as well as ancient humans from East Asia would be expected to carry Denisovan-related ancestry in amounts comparable to what is seen in New Guineans. But this is not what we observe.”

Reich suggests... “The simplest explanation for the large fractions of Denisovan-related ancestry on the islands off the southeastern tip of Asia and in New Guinea and Australia would be the occurrence of **interbreeding near the islands**—on the islands themselves or in mainland Southeast Asia—but in either case in a tropical region very far from Denisova Cave.” But he goes on to point out that there is no archaeological evidence to support this claim... so far. So it appears unlikely that this occurred.

But other archaeological evidence may point to a solution: “... it is more likely that interbreeding occurred in southern China or mainland Southeast Asia. There are archaic human remains from Dali in Shaanxi province in north-central China, from Jinniushan in Liaoning in northeastern China, and from Maba in Guangdong in southeastern China, all dating to around two hundred thousand years ago, all of which are more plausible skeletal matches for the Denisovans. An archaic human from Narmada in central India may date to around seventy-five thousand years ago.”

Meet the Australo-Denisovans

Reich offers a further twist to this story: “While the interbreeding Neanderthals were close relatives of those we obtained samples from and sequenced, the archaic people who interbred with the ancestors of New Guineans were not close relatives of the Siberian Denisovans. When we examined the genomes of present-day New Guineans and Australians, and counted the number of DNA letter differences between them and the Siberian Denisovans to estimate when their ancestors separated from a common parent population, we discovered that everywhere in the genome, the number of differences was at least what would be expected for a population split that occurred 400,000 to 280,000 years ago. This meant that **the ancestors of the Siberian Denisovans separated from the Denisovan lineage that contributed ancestry to New Guineans** two-thirds of the way back to the separation of the ancestors of Denisovans from Neanderthals.”

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“In light of the remote relationship, the two groups probably had different adaptations, which would explain how they were able to thrive in such different climates. Given the extraordinary diversity of Denisovans—with much more time separation among their populations than exists among present-day groups—it makes sense to think of them as **a broad category of humans**, one branch of which became the ancestors of the archaic population that interbred with New Guineans and another that became Siberian Denisovans. Most likely there are other Denisovan populations as well that we haven’t sampled at all. **Maybe we should even consider Neanderthals as part of this broad Denisovan family.**”

Reich likes to call the Denisovans who contributed DNA to the modern humans east of Huxley’s line as “Australo-Denisovans.”

But where did these archaic humans come from? “... It is tempting to think that the Australo-Denisovans, Denisovans, and Neanderthals descend from the first *Homo erectus* populations that expanded out of Africa, and that modern humans descend from the *Homo erectus* populations that stayed in Africa, but that would be wrong.... If *Homo erectus* from the first radiation out of Africa was ancestral to the Denisovans and Neanderthals, then the split of these populations from modern humans would be at least as old as the dispersal to Eurasia—far too old to be consistent with the genetic observations. The genetic data give a split date of 770,000 to 550,000 years ago, too recent to be consistent with a 1.8-million-year-old population separation.”

“There is, however, a candidate in the fossil record for an ancestor in the right period, dating to long after the *Homo erectus* out-of-Africa migration but **after** [did he mean before?] the *Homo sapiens* one. A big-skulled skeleton found near Heidelberg in Germany in 1907 and dated to around six hundred thousand years ago was plausibly from a species that was ancestral to modern humans and Neanderthals, and by implication, Denisovans too. *Homo heidelbergensis* is often viewed as both a West Eurasian and an African species, but not an East Eurasian species. However, the genetic evidence from the Australo-Denisovans shows that the *Homo heidelbergensis* lineage may have been established very anciently in East Eurasia too.”

“So we now have access to genome-wide data from four highly divergent human populations that all likely had big brains, and that were all still living more recently than seventy thousand years ago. These populations are modern humans, Neanderthals, Siberian Denisovans, and Australo-Denisovans.... Seventy thousand years ago, the world was populated by very diverse human forms, and we have genomes from an increasing number of them, allowing us to peer back to a time when humanity was much more variable than it is today.”

How Archaic Encounters Helped Modern Humans

Reich next asks: “What is the biological legacy of the interbreeding between modern humans and Denisovans?” Data from mainland Asia provides an answer.

Reich notes that we have not yet been able to determine the full range of Denisovan ancestry in modern humans. But: “Denisovan interbreeding was biologically significant.”

“One of the most striking genomic discoveries of the past few years is a mutation in a gene that is active in red blood cells and that allows people who live in high-altitude Tibet to thrive in their oxygen-poor environment. Rasmus Nielsen and colleagues have shown that the segment

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of DNA on which this mutation occurs matches much more closely to the Siberian Denisovan genome than to DNA from Neanderthals or present-day Africans. This suggests that some Denisovan relatives in mainland Asia may have harbored an adaptation to high altitude, which the ancestors of Tibetans inherited through Denisovan interbreeding.”

Turning his attention to Neanderthal interbreeding, Reich notes: “... at genes associated with the biology of keratin proteins, present-day Europeans and East Asians have inherited much more Neanderthal ancestry on average than is the case for most other groups of genes. This suggests that versions of keratin biology genes carried by Neanderthals were preserved in non-Africans by the pressures of natural selection, perhaps because keratin is an essential ingredient of skin and hair, which are important for providing protection from the elements in cold environments such as the ones that modern humans were moving into and to which Neanderthals were already adapted.”

Superarchaic Humans

Reich next offers another example of the inferences he can make from the whole genome samples he has been able to analyze from both modern human and archaic populations.

“Given that Denisovans and Neanderthals are genetically closer to each other than either is to modern humans, it would be reasonable to expect them to be equidistantly related to present-day populations that have not received genetic input from either of these archaic populations—that is, to sub-Saharan Africans. Yet we found sub-Saharan Africans to be slightly **more closely related to Neanderthals than to Denisovans**. This must reflect another example of interbreeding we didn’t know about. The pattern we observed could only be explained by Denisovan interbreeding with a **deeply divergent, still unknown archaic population**—one from which Africans and Neanderthals have little or no DNA, and which separated from the common ancestors of modern humans, Neanderthals, and Denisovans well before their separation from each other.”

“The evidence for an unknown archaic contribution to Denisovans is that at locations in the genome where all Africans share a mutation, the mutation is more often seen in Neanderthals than in Denisovans. Because these are mutations that all Africans carry, we know that they occurred long ago, as it typically takes around a million years or more in humans for a new mutation not under natural selection to spread throughout a population and achieve 100 percent frequency. The only way to explain the fact that Denisovans do not also share these mutations is if the ancestors of the Denisovans interbred with a population that diverged from Denisovans, Neanderthals, and modern humans so long ago that nearly all modern humans carry the new mutation.”

“... we estimated that the **unknown archaic population** that interbred into Denisovans first split off from the lineage leading to modern humans 1.4 to 0.9 million years ago and that this unknown archaic population contributed at least 3 to 6 percent of Denisovan-related ancestry. The date is shaky, as knowledge of the human mutation rate is poor. However, even with the uncertainty about the mutation rate, we can estimate relative dates reasonably well, and we can be confident that this previously unsampled human population split off at about twice the separation time of Denisovans, Neanderthals, and modern humans. I think of this group as **“superarchaic” humans**, as they represent a more deeply splitting lineage than Denisovans. They are what I call a **“ghost” population**, a population we do not have data from in unmixed form, but whose past existence can be detected from its genetic contributions to later people.”

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Eurasia As a Hothouse of Human Evolution

Reich now challenges another assumption about the history of modern humans. The narrative he provides needs no comment or explanation from me:

“From a combination of archaeological and genetic data, we can be confident of at least four major population separations involving modern and archaic human lineages over the last two million years.”

“The skeletal evidence shows that the first important spread of humans to Eurasia occurred at least 1.8 million years ago, bringing *Homo erectus* from Africa. The genetic evidence suggests that a second lineage split from the one leading to modern humans around 1.4 to 0.9 million years ago, giving rise to the superarchaic group that we have evidence of through its mixture with the ancestors of Denisovans and that plausibly contributed the highly divergent Denisovan mitochondrial DNA sequence that shares a common ancestor with both Neanderthals and modern humans in this time frame. Genetics also suggests a third major split 770,000 to 550,000 years ago when the ancestors of modern humans separated from Denisovans and Neanderthals, followed by Denisovans and Neanderthals from each other 470,000 to 380,000 years ago.”

“The usual assumption is that all four of these splits correspond to ancestral populations in Africa expanding into Eurasia. But does this really have to be the case?”

“With ancient DNA data in hand, we are confronted with the observation that of the four deepest human lineages from which we have DNA data, the three most deeply branching ones are represented **only in human specimens excavated from Eurasia**: the Neanderthals, the Denisovans, and the “superarchaic” population that left traces among the Siberian Denisovans.”

“But another possibility suggests itself, which is that the ancestral population of modern humans, Neanderthals, and Denisovans **actually lived in Eurasia**, descending from the original *Homo erectus* spread out of Africa. In this scenario, there was **later migration back from Eurasia to Africa**, providing the primary founders of the population that later evolved into modern humans. The attraction of this theory is its economy: it requires one less major population movement between Africa and Eurasia to explain the data. The superarchaic population and the ancestral population of modern humans, Denisovans, and Neanderthals could both have arisen within Eurasia, without requiring two further out-of-Africa migrations, as long as there was just one later migration back into Africa to establish shared ancestry with modern humans there.”

“Based on the skeletal record, it is certain that Africa played a central role in the evolution of our lineage **prior** to two million years ago, as we have known ever since the discovery of the upright walking apes who lived in Africa millions of years before *Homo*. We know too that Africa has played a central role in the origin of anatomically modern humans, based on the skeletons of humans with anatomically modern features there up to around three hundred thousand years ago, and the genetic evidence for a dispersal in the last fifty thousand years out of Africa and the Near East. But what of the intervening period between two million years ago and about three hundred thousand years ago? In a large part of this time, the human skeletons we have from Africa are not obviously more closely related to modern humans than are the human skeletons of Eurasia. Over the last couple of decades, there has been a pendulum swing

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toward the view that because our lineage was in Africa before two million years ago and after three hundred thousand years ago, our ancestors must always have been there. But Eurasia is a rich and varied supercontinent, and there is no fundamental reason that the lineage leading to modern humans cannot have sojourned there for an important period before **returning to Africa.**”

“The genetic evidence that the ancestors of modern humans may have spent a substantial part of their evolutionary history in Eurasia is in fact consistent with a theory advanced by María Martín-Torres and Robin Dennell.... They argue that humans they call *Homo antecessor*, found in Atapuerca, Spain, and dating to around one million years ago, show a mix of traits indicating that they are from a population ancestral to modern humans and Neanderthals. This is a very ancient date for a modern human/Neanderthal ancestral population to exist in Eurasia. Many who think that Neanderthals in Europe descend from an out-of-Africa radiation of an ancestral population would assume that the ancestors of both populations were still in Africa at that time. Combining this evidence with archaeological analysis of stone tool types, Martín-Torres and Dennell argue for the possibility of continuous Eurasian habitation from at least 1.4 million years ago until the most recent common ancestor of humans and Neanderthals after eight hundred thousand years ago, at which point **one lineage migrated back to Africa to become the lineage that evolved into modern humans.** The Martín-Torres and Dennell theory becomes more plausible in light of the new genetic evidence.”

“The genetic data show that **many groups of archaic humans populated Eurasia** and that some of these interbred with modern humans. This forces us to question why the direction of migration would have always been out of Africa and into Eurasia, and whether it could sometimes have been the other way around.”

The Most Ancient DNA Yet

The “superarchaic” population returns.

“At the beginning of 2014, Matthias Meyer, Svante Pääbo, and their colleagues in Leipzig extended by a factor of around four the record for the oldest human DNA obtained, sequencing mitochondrial DNA from a more than **four-hundred-thousand-year-old** *Homo heidelbergensis* individual from the Sima de los Huesos cave system in Spain where twenty-eight ancient humans were found at the bottom of a thirteen-meter shaft. The Sima skeletons have early Neanderthal-like traits, and the archaeologists who excavated them have interpreted them as being on the lineage leading to Neanderthals after the separation from the ancestors of modern humans. Two years after Meyer and Pääbo published mitochondrial DNA data from Sima de los Huesos, they published genome-wide data. Their analysis not only confirmed that the Sima humans were on the Neanderthal lineage, but went further in showing that the Sima humans were more closely related to Neanderthals than they are to Denisovans. These results provided direct evidence that Neanderthal ancestors were already evolving in Europe at least four hundred thousand years ago, and that the separation of the Neanderthal and Denisovan lineages had already begun by that time.”

“But the Sima data were also perplexing: Sima’s **mitochondrial genome** was more closely related to Denisovans than to Neanderthals, at odds with the genome-wide pattern of it being most closely related to Neanderthals.” This was no statistical fluke: “... there are two discrepancies in the genetic relationships: the fact that the Sima de los Huesos individual has Denisovan-type mitochondrial DNA despite being closer to Neanderthals in the rest of the genome, and the fact that the Siberian Denisovan individual has mitochondrial DNA twice as

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divergent from modern humans and Neanderthals as they were from each other despite being closer to Neanderthals in the rest of the genome. The coincidence of these two observations is so improbable that it seems more likely that there is a deeper story to unravel.”

Reich offers a number of “perhaps” and “may have” scenarios to explain the observations, but concludes: “... the patterns suggest that Denisovans and Neanderthals both had ancestry from the same superarchaic population, with just a larger proportion present in the Denisovans.”

Reich sums up: “Whatever explains these patterns, it is clear that we have much more to learn. The period before fifty thousand years ago was a busy time in Eurasia, with multiple human populations arriving from Africa beginning at least 1.8 million years ago. These populations split into sister groups, diverged, and mixed again with each other and with new arrivals. Most of those groups have since gone extinct, at least in their “pure” forms. We have known for a while, from skeletons and archaeology, that there was some impressive human diversity prior to the migration of modern humans out of Africa. However, we did not know before ancient DNA was extracted and studied that Eurasia was a locus of human evolution that rivaled Africa. Against this background, the fierce debates about whether modern humans and Neanderthals interbred when they met in western Eurasia—which have been definitively resolved in favor of interbreeding events that made a contribution to billions of people living today—seem merely anticipatory. Europe is a peninsula, a modest-sized tip of Eurasia. Given the wide diversity of Denisovans and Neanderthals—already represented in DNA sequences from at least three populations separated from each other by hundreds of thousands of years, namely Siberian Denisovans, Australo-Denisovans, and Neanderthals—the right way to view these populations is as members of a loosely related family of highly evolved archaic humans who inhabited a vast region of Eurasia.”