PNAS PROFILE

Profile of Svante Pääbo: 2022 Nobel laureate in physiology or medicine

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Svante Pääbo, a Swedish geneticist who directs the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, was awarded the 2022 Nobel Prize in Physiology or Medicine for pioneering the extraction and analysis of DNA from ancient bones, above all those of the Neanderthals. Pääbo was born in 1955 and educated through the PhD in Sweden. As a graduate student in medicine and molecular biology at the University of Uppsala, he was intrigued by the idea that ancient tissues might preserve DNA. In a frank and engaging autobiographical memoir (1), he describes his graduate-student attempts to detect DNA in Egyptian mummies. At first, he thought that he was successful (2), but he later realized that what he observed could have been recent contaminant, not authentic ancient, DNA (3). Like Pääbo's initial effort, other early searches for ancient DNA did not consider the possibility; even the likelihood that contamination from handling, packaging, or the lab environment would overwhelm any genuine ancient DNA and produce a seemingly positive but misleading result. Possible contamination remains a major concern in ancient DNA research, and when Pääbo's team searched for authentic Neanderthal DNA, they took great pains to limit the chances.

Pääbo received his PhD in 1986, and in 1987, he became a postdoctoral researcher in the laboratory of the distinguished Berkeley biochemist Allan Wilson. Pääbo and others revered Wilson for his use of molecules from extant species to illuminate their evolutionary history. Wilson and his student Vincent Sarich were famous for showing that human and chimpanzee albumin proteins were about equally different from those of cercopithecoid monkeys, which implied that humans and chimpanzees had accumulated their differences at roughly the same rate. Assuming a constant rate of albumin change and accepting from fossils that the last shared ancestor of cercopithecoids and apes lived about 30 million years ago, the albumin difference between people and chimpanzees implied that they last shared a common ancestor 6 to 4 million years ago (4). This conflicted with paleontological estimates that placed the common ancestor closer to 12 million years ago, but subsequent analysis of hemoglobin diversity returned the 6-to-4-million-year estimate (5), and so far, no fossil findings unequivocally contradict it.

With his students Rebeca Cann and Mark Stoneking, Wilson further undertook a landmark analysis of variation in mitochondrial DNA (mtDNA) among 147 individuals, characterized as sub-Saharan Africans (6), Asians (7), Caucasians (Europeans, north Africans, and Near Easterners) (46), Aboriginal Australians (8), and New Guineans (26) (9). mtDNA resides in energy-producing organelles in the cytoplasm outside the nucleus of each cell and Cann et al. focused on it because of its simple mode of inheritance, exclusively through females. mtDNA variants had not yet been described by their particular

Image credit: Max Planck Institute for Evolutionary Anthropology.

sequences of the four nucleotides or bases (abbreviated A, C, T, and G) from which all DNA is built, so the authors used a method called restriction-fragment mapping to characterize the observed mtDNA variants. They found that the 147 subjects possessed 133 different variants, all presumed to originate ultimately from a single variant by mutation or, more precisely, by a series of mutations. They assumed that the smallest number of possible mutations approximated the actual number and computed a genealogical tree linking the

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observed variants by their degree of similarity. The tree turned out to have two main branches—one that included only Africans and the other that had some Africans and everyone else. Since the individual branches that had experienced the most mutations (and that had therefore probably existed the longest) were African, the investigators concluded that the woman whose mtDNA diversified into all later types lived in Africa, and science writer Roger Lewin (10) dubbed her "Mitochondrial Eve." The observation that every living person's mtDNA derives from the mtDNA of a single woman does not mean that she was the only female ancestor of all living people but only that the mtDNA variants of her female contemporaries have been lost by chance over time. In fact, variants of all genes, not just those of mtDNA, must coalesce back to an original version in a single individual, and mtDNA occupies a special place in evolutionary studies mainly because of its strictly maternal inheritance and its relatively rapid, broadly clocklike rate of diversification.

The tree provided strong support for the "out-of-Africa" hypothesis strongly advocated by Cavalli-Sforza (11), according to which modern humans evolved in Africa and expanded to replace other kinds of people elsewhere. A team including Pääbo subsequently confirmed the tree when they examined variation in the mtDNA nucleotide sequences of 53 geographically dispersed individuals, and they demonstrated conclusively that it was rooted in Africa (12). The antiquity of the last shared mtDNA ancestor remains debatable because it depends on varying estimates of the mtDNA mutation rate, generation lengths, and past population size and structure (13–15), but all studies accept an age of 200,000 years or less.

In 1991, Pääbo became Professor of General Biology at the University of Munich and began the search for DNA in Neanderthal bones. The Neanderthals seemed an ideal target since in their west Eurasian homeland, they were the immediate predecessors of fully modern people, and from the time of their discovery in the second half of the 19th century and the early part of the 20th, specialists vigorously debated whether they were ancestral to modern humans, perhaps particularly to modern Europeans, or whether they were an extinct human variety that fully modern humans replaced (16). Neanderthal and early modern Eurasian human bones and archeological residues could be argued to support either position. Neanderthal DNA was likely to support only one.

Aided by archeology graduate student Ralf Schmitz, Pääbo sought permission to search for DNA in the bone of the type Neanderthal recovered by limestone quarry workers from Feldhofer Cave near Dusseldorf in 1856. The Feldhofer bones included a skullcap with a large double-arched brow-ridge, a low, receding forehead, and bulging sidewalls (vs. the relatively parallel-sided sidewalls of modern skulls). It was accompanied by thick, powerfully built limb bones. Early on, some authorities thought that the bones came from a diseased historic human, but in the 1860s, specialist opinion settled on the idea that they represented a vanished human type labeled Neanderthal after the location of Feldhofer Cave in the Neander Valley (=*Neanderthal* in German when the bones were discovered, *Neandertal* today). In 1864, the Irish geologist William King (17) proposed assigning the Feldhofer bones to *Homo neanderthalensis*, a Linnaean species name that is still widely applied to fossil hominins across western Eurasia whose bones resembled those from Feldhofer Cave and who probably existed at the same time. This is now often estimated to be between about 300,000 and 40,000 years ago.

Permission to sample Feldhofer bone came slowly and grudgingly because curators are understandably reluctant to approve research that would irreparably damage precious specimens. Eventually, Pääbo was allowed to remove a 3.5-g section of bone from the shaft of a right humerus. Previous research had shown that DNA was particularly likely to survive in bones that retained proteins, so as a first step, Pääbo extracted various amino acids to determine whether their proportions implied protein survival and whether the proteins had been strongly altered during burial. When both indicators suggested a promising degree of protein retention, the investigators turned to retrieving mtDNA, chosen because it is 500 to 1,000 times more abundant than nuclear DNA in live individuals and thus more likely to be found in fossil bone.

In live humans, the mtDNA genome comprises about 16,500 nucleotides; however, Pääbo's team never expected to recover a complete mtDNA sequence, and they were delighted when the Feldhofer bone provided small fragments. For analysis, they amplified the fragments using the famous PCR, and they sought to determine whether the fragments might originate from shed skin cells or ill-timed sneezes by some of the people who had handled the Feldhofer humerus since its discovery 140 years earlier. Ten percent of the fragments exhibited sequences that suggested that they were modern contaminants, but the remaining 90% were readily distinguished from their counterparts in living humans. Pääbo's team used overlap among the endogenous fragments to reconstruct a 379-nucleotide-long stretch of a hypervariable part of the mitochondrial control region. They sequenced this and compared the result to sequences at the same position in the mtDNA of 994 living humans from across the globe. On average, the modern sequences differed from each other at eight nucleotide positions, while the Neanderthal sequence differed from the modem ones at 27 positions. This implied that Neanderthal mtDNA was outside the range of modern human mtDNA variation and that Neanderthals had contributed no mtDNA to modern human populations.

To maximize credibility, Pääbo sent a 0.4-g subsample of the Feldhofer humerus to the Anthropological Genetics Laboratory at Pennsylvania State University, and when the second lab independently extracted mtDNA with the same sequence, the two labs published the result jointly (18). An accompanying commentary (19) called their effort "a tour-de-force investigation of ancient DNA."

When Pääbo's team assumed that the human and chimpanzee lines diverged 5 to 4 million years ago, their observed mtDNA variation indicated that the Neanderthal and modern human lines separated 600 to 550,000 years ago and that the last shared mtDNA ancestor of modern humans existed 150 to 120,000 years ago. These remain reasonable ballpark estimates. By my count, 30 additional Neanderthal bones from at least 14 additional sites have confirmed that Neanderthal mtDNA falls outside the range of modern human mtDNA variation. The sites extend across southern Eurasia, from Spain on the west to south-central Siberia on the east. The 30 include 13 bones recently reported from two caves in southern Siberia (20).

In 1997, Pääbo relocated to Leipzig to become a principal founder of the Max Planck Institute for Evolutionary Anthropology, which he presently directs. With colleagues and students in his new lab, he turned to retrieving and analyzing the far-larger genome housed in the nuclei of Neanderthal cells. The publications I cite below and many others I could have cited demonstrate their collective success in this and related efforts, all tied to Pääbo's leadership and to the unique facility that he developed in Leipzig.

In living humans, the nuclear genome comprises about 3.2 billion nucleotides, and Neanderthals probably had a similar number. Three Neanderthal bones from Vindjja Cave, Croatia, provided particularly abundant nuclear DNA. One of the bones yielded a sequence of 65,250 nucleotides to one group (21) and a sequence of more than 1 million to a second (22). The difference in length reflected different retrieval procedures, but subsequent analysis indicated that the longer sequence was contaminated by at least 11% of living human DNA. Three Vindija bones, including the one that had provided the shorter sequence, then produced more than 4 billion nucleotides in small fragments, some of which could be stitched together to cover about 60% of the Neanderthal nuclear genome (6). Contamination probably did not significantly affect the outcome since the accompanying mtDNA appeared less than 1% detectably contaminated.

The nuclear sequence in a Neanderthal bone from Mezmajskaya Cave, south European Russia, and the especially high-quality sequence in a bone from Denisova Cave, south-central Siberia, supported the Vindija result (8). A comparison of the Neanderthal and modern genomes indicated that Neanderthals and living Eurasians shared a small number of genes with each other that neither shared with living Africans (23). The most economic explanation was that Neanderthals introduced these genes to the modern Africans who colonized Eurasia. Genetic observations place the introgression of Neanderthal genes into the earliest modern Eurasians between 65,000 and 47,000 years ago and perhaps as recently as 37,000 years ago (24). Establishing the time when fully modern humans arrived in Eurasia depends mostly on radiocarbon dating of their distinctive archeological residues, and it is difficult to fix because it was near the 50,000-year lower bound of the radiocarbon method when even a small amount of more recent carbon contamination can make a sample appear younger than it is. Still, careful sample selection and preparation indicate that modern humans were well established in Europe by 40,000 years ago (25). They were present by 45,000 years ago at Bacho Kiro Cave, Bulgaria (26), and at Ust'-Ishim, western Siberia (27). The evidence from Ust'-Ishim is a directly dated morphologically modern human femur shaft with DNA that was essentially modern but that included a small Neanderthal component. Over time, recombination in the formation of the sex cells chops an original Neanderthal contribution into ever-smaller stretches in the genome. The length of unbroken stretches in the Ust'-Ishim DNA indicates that Neanderthals contributed DNA to the ancestors of the individual 7,000 to 13,000 years earlier. A modern skull recovered from Koněprusy cave in the Czech Republic also provided unusually long stretches of Neanderthal nuclear DNA, though its radiocarbon age is imprecisely estimated at ≥ 34,000 years ago. The average length of its Neanderthal DNA stretches suggests that it is likely to be close to the 45,000-year age of the Ust'-Ishim femur (28).

A Neanderthal contribution today is more obvious in east Asians (roughly 1.7 to 2.1%) than in Europeans (1.6 to 1.8%) (27, 29–31). This was unexpected since Neanderthal fossils are well known in Europe but totally unknown in eastern Asia. The higher frequency on the east is unexplained, but the lower frequency on the west could mean that Europeans trace some of their ancestry to a group of early modern humans who separated from other non-Africans before interbreeding occurred (23).

Introgressed Neanderthal genes that affected skin and hair color and the immune system seem to have been selectively favored in modern human populations (32), probably because they enhanced the fitness of expanding Africans in Eurasian environments. Introgressed Neanderthal genes that bore on cognition were selectively disfavored.

In 2010, a finger bone from Denisova Cave, south-central Siberia, provided an mtDNA sequence in Leipzig that was neither Neanderthal nor modern human (33, 34). This was the same cave from which another bone had provided the nearly complete Neanderthal nuclear sequence noted above. The layer that contained both bones also contained artifacts that were almost certainly left by fully modern humans. Stratigraphic mixing is implied since it is unlikely that three different kinds of people occupied the cave simultaneously.

The finger bone produced abundant nuclear DNA, which has been characterized in exquisite detail, thanks to an innovative methodology that isolates single strands of DNA separated from their initial double-stranded state (30). Ancient DNA is particularly likely to have been damaged and thus unanalyzable, but the likelihood is reduced when analysis can focus on just one of a pair of strands. The result for the nuclear DNA in the finger bone was that more than 99% of the nucleotides could be observed at least ten times. This allowed the full genome to be characterized about as precisely as the genomes of living humans. Taxonomic rules require that a type specimen must be diagnosed before a new species can be proposed. Since the finger bone was inadequate for the purpose, the Leipzig investigators decided to use the vernacular term Denisovans for the putative new species. Genomic comparisons indicate that the Denisovans were more closely related to the Neanderthals than to living humans.

A large, morphologically distinct upper molar from the same layer as the finger bone produced the same distinctive mtDNA sequence, and a partial mandible from Baishiya Karst Cave, Tibet, provided a protein sequence that has been interpreted as Denisovan (7). The cave sediments contained Denisovan mtDNA (35), likely originating from human waste. Two skulls from Xuchang, China, that have not been referred to the Denisovans may yet prove to be so since they differ from Neanderthal skulls to about the same degree as Neanderthal and Denisovan nuclear DNA differ from each other. The sum could mean that the Neanderthals and the Denisovans were members of a Eurasian meta-population that comprised Neanderthals in Europe, western Asia, and central Asia, the Denisovans in eastern Asia, and a zone of overlap in central Siberia.

Collagen peptide mass fingerprinting (PMF) can identify morphologically undiagnostic human bone fragments among

a much larger number from nonhuman species. A small bone fragment from Denisova Cave isolated by PMF provided DNA of a woman who appears to have had a Neanderthal mother and a Denisovan father (36), demonstrating Denisovan/Neanderthal interbreeding. Denisovan DNA is rare in modern east Asians, but it makes up 4 to 6% of the genome of Aboriginal Australians, Papua New Guineans, and nearby Melanesians. The interbreeding probably occurred in southeast Asia before the initial peopling of Australia and New Guinea 50 to 45,000 years ago.

DNA survives best in cool, dry conditions, and even then, it probably rarely persists for more than 100,000 years. The Neanderthals and Denisovans who provided DNA to Pääbo and his colleagues probably mostly lived more recently than this. However, the Leipzig lab found both mitochondrial and nuclear DNA in roughly 430,000-year-old bones from the Sima de los Huesos site in northern Spain (37, 38). The nuclear DNA resembled Neanderthal nuclear DNA, but the mtDNA was more Denisovan-like. Morphologically, the Sima people anticipated the Neanderthals, and the nuclear result confirms their assignment to the Neanderthal line. Distinctive Neanderthal mtDNA may derive from an African lineage that arrived in western Eurasia after 430,000 years ago (23).

Only 25 years ago, few specialists believed that it would ever be possible to retrieve DNA from Neanderthal bones. Svante Pääbo and his colleagues in Leipzig have now proven not only that retrieval is practical but also their DNA analysis has demonstrated that Neanderthals were not ancestral to modern humans, although they occasionally interbred with modern Africans expanding to Eurasia beginning about 50,000 years ago. This closed out a 150-year-long polemic about the place of the Neanderthals in human evolution that could have gone on indefinitely. Based entirely on DNA extracted from ancient bones, Pääbo and colleagues also discovered the Denisovans, a sister group of the Neanderthals, that existed mainly to the east during roughly the same interval, between perhaps 300,000 and 40,000 years ago. Along the way, under Pääbo's leadership, his Leipzig lab also substantially refined the methodology for extracting and analyzing ancient DNA.

When Pääbo began his quest for Neanderthal DNA, he hoped that comparison to the modern human genome would reveal the genetic basis of the uniquely modern human behavioral capabilities that likely explain why we are still here and the Neanderthals are not (39). This goal may long remain elusive (23), but modern human observers would mostly agree that it is worth pursuing.

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