Profile of Svante Pääbo

The Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany was abuzz in the summer of 2006, and not just because the city was one of the hosts of the World Cup soccer tournament. On July 20, 2006, the institute announced the start of one of the most ambitious research projects in recent years: sequencing the complete genome of a Neanderthal. These ancient hominids, who shared the Earth with modern humans before dying out 30,000 years ago, represent humans' closest relative. If their genome can be deciphered, then combined with the recently completed genome of chimpanzees, humans' closest living relative, the road may finally be paved for understanding the origins of humans and what makes us unique.

If that happens, the scientist Svante Pääbo, elected to the National Academy of Sciences as a foreign associate in 2004 and the Director of the Max Planck Institute's Department of Evolutionary Biology, would be very pleased. Since his days in graduate school in Sweden, Pääbo, who once dreamed of becoming an Egyptologist, has uncovered ancient secrets perhaps even more valuable than King Tut's treasures. Pääbo has been instrumental in creating the field of molecular paleontology, having developed and refined the techniques used to isolate and sequence ancient DNA. In addition, he has worked extensively with modern DNA samples in his studies of genetic variation and human evolution.

A recent advance in high-throughput DNA sequencing, which has enabled Pääbo to coax DNA out of the remains of mammoths, ground sloths, marsupial wolves, and ancient corn, may help in obtaining enough nuclear DNA from 40,000-year-old bones to complete the Neanderthal genome. To prepare for this endeavor, Pääbo, in his Inaugural Article in this issue of PNAS (1), discusses how damaged bases in DNA can cause sequence errors and what general patterns they may display. These findings should help researchers detect and filter nucleotide misincorporations, a potential hazard of this new sequencing technique. "There are some special technical issues with this," he says, "but I think within 2 years we will have a rough draft version of the Neanderthal genome."

Working with Mummy DNA

Mummies, not cavemen, first drew Pääbo to the ancient world. Born in Stockholm, Sweden in 1955, Pääbo,

Svante Pääbo

at 13, traveled to Egypt with his mother for vacation, and he became spellbound by the country's archaeological wonders. From that point on, Pääbo wanted to become an Egyptologist. However, when he entered the Uppsala University (Uppsala, Sweden) in 1975 to pursue a degree in Egyptology, he became disenchanted with some of the realities of the field. "I think I had a far too romantic idea of what Egyptology was. I thought it would be all about discovering mummies and pyramids, but, in Uppsala at least, it was quite linguistically oriented," he says. Instead of combing the Egyptian desert for lost tombs, Pääbo spent much of his time combing the library for books on the grammatical construction of hieroglyphics and Coptic language.

After 2 years, Pääbo switched his studies to medicine. Ostensibly, he had made the move so he could have a job once he finished, but he soon found the everyday work of seeing patients much more rewarding than anticipated. Nevertheless, he interrupted his medical studies in 1980 to pursue a Ph.D. in molecular genetics and returned to clinical practice. While studying adenoviruses and their interaction with the immune system with Per A. Peterson, Pääbo came up with a tantalizing idea. "I started realizing that we had all these technologies to clone DNA, but no one seemed to have applied it to archaeological remains, in particular Egyptian mummies," he says. Pääbo began a side project, one that would provide some of the Egyptian adventure he had once dreamed about. With the help of his Egyptology professor, Rostislav Holthoer, Pääbo gathered soft tissue samples from various mummy specimens. Working secretly on nights and weekends—"I feared that my Ph.D. advisor would not have approved," Pääbo says—he attempted to isolate DNA from the mummy samples.

In 1984, Pääbo's efforts met with success: he had created a DNA library of his tissue samples in bacteria and screened them with human repeat sequences, revealing some human DNA among the clones (2). But while he was writing about his work for a *Nature* article, the journal published an article by Allan Wilson's group at the University of California (Berkeley, CA) on the isolation of old DNA from a quagga, a zebra-like animal that became extinct in the 19th century (3). "So, I was a little sad that I had just been scooped," he says. Still, Pääbo thought that Wilson, perhaps the preeminent molecular geneticist of the time, would be interested in his work, so he sent proofs of his manuscript to Wilson. Not long after, Pääbo received a letter from Wilson with a most surprising request. "He suggested that he would do a sabbatical in my laboratory," says Pääbo, who politely wrote back and corrected the misunderstanding. "I said, 'Well, that's not really in the cards, but perhaps I might do a postdoc with you?'" he says.

Of Cave Bears and Cave Men

In 1987, Pääbo moved to California to begin working with Wilson. It was truly a "right place, right time" event, for not only had Pääbo joined the only laboratory that had done work on ancient DNA, but he did so right on the heels of the discovery of PCR. "Allan's lab was the first where PCR was applied outside of Cetus, the company where it was invented," says Pääbo. PCR was ideally suited for retrieving ancient DNA because it could specifically locate and amplify the desired DNA from among the bacterial and fungal DNA that compromise the majority of ancient remains. Pääbo and Wilson began applying PCR to the extraction of ancient DNA and amplified mitochondrial DNA from a well-preserved 7,000-year-old human brain (4). Still, Pääbo faced numerous technical obstacles with the technique. "There is all sorts of damage in the DNA that can cause you to determine incorrect sequences, especially when you start from very few molecules, and there is also contamination from human DNA that is almost everywhere" (5), he says. He notes that his groundbreaking mummy DNA sequences likely had some modern contamination among them.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 13578.

Considering the contamination risks, Pääbo decided to shy away from working with human remains and focused instead on animals. Over the next several years, first with Wilson and then in his own laboratory at the University of Munich (Munich, Germany), Pääbo amplified DNA from a variety of ancient creatures, including giant sloths, mammoths, cave bears, and the marsupial wolf (6–9). His work helped answer many questions about the phylogeny of these extinct species and their relationship to modern ones. For example, Pääbo showed that kiwis were more closely related to Australian flightless birds like emus than to moas, extinct flightless birds native to New Zealand, indicating that ancestor species likely colonized New Zealand twice (10). In addition to tissue and fossil samples, Pääbo also extracted DNA from coprolites, or fossilized animal feces, which provided additional information such as an animal's diet (11).

Working with Wilson, who had redefined the human–chimpanzee split with his research on molecular clocks and later proposed that modern humans originated in Africa 200,000 years ago, gave Pääbo a strong desire to study human evolution. This interest allowed Pääbo to continue the work of his mentor, who had passed away from leukemia in 1991, just 1 year after Pääbo left the laboratory. "Allan Wilson clearly influenced my thinking about evolutionary biology very, very much," says Pääbo, "and he died far too early."

Pääbo decided to focus his research on Neanderthals, a hominid species that lived in Europe ≈30,000 years ago. Much debate existed as to how closely Neanderthals were related to ancient humans, whether the two species interbred, and whether Neanderthals were the ancestors of modern Europeans. To begin to answer these questions, Pääbo sought useful specimens. "It was important that we start with a specimen that was for sure a Neanderthal, because there was so much discussion at the time about other fossils. Are they typical Neanderthals, or are they not?" he says. In 1996, Pääbo persuaded the curators of the state museum in Bonn, Germany, which held the remains of a Neanderthal-type specimen, to allow him to remove a small piece of its humerus for study. "And if that Neanderthal is not a Neanderthal, then there are no Neanderthals," says Pääbo. Under extremely sterile conditions, his group extracted and amplified mitochondrial DNA from the ancient fossil, which was impressive considering its age and condition.

After seeing the sequencing results, Pääbo experienced what he refers to as "one of those really cool moments in life." He says, "We immediately saw that it looked very unusual. It was clear that Neanderthals have not contributed mitochondrial DNA to modern humans" (12). His findings indicated that Neanderthals split off from humans a little over 550,000 years ago and strengthened the view that all modern humans originated from Africa. Of course, using only mitochondrial DNA would not rule out all genetic contribution. Pääbo recently used population models based on several specimens to suggest that Neanderthals could have contributed up to 25% of their genetic makeup to modern humans, although the true percentage is likely much smaller (13).

Chimpanzee Connection

After having made a name for himself with his groundbreaking Neanderthal study, Pääbo in 1997 was invited to take part in the founding of a new research institute in Leipzig, one that would symbolize a fresh start for anthropology research in Germany. The idea of the new institute was to bring together experts from a range of scientific disciplines, including linguistics, primatology, paleontology, and comparative psychology, under one roof to answer the question: What makes humans human? Pääbo was selected to head the Department of Evolutionary Genetics, which was charged with uncovering the genetic differences that lay between humans and their closest relatives as well as the forces shaping those differences. Although Pääbo was enthusiastic about the overall concept of the institute, he harbored some doubts as to whether it would work on a practical level. "Would we talk to each other and would we get along among so many different scientific cultures?" he asked.

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Nearly a decade later, Pääbo believes the institute has performed extremely well, providing a stimulating environment that has helped him pursue his latest challenge: comparing humans and their closest living relative, the chimpanzee. Humans and chimpanzees share almost 99% genetic identity yet are highly different phenotypically. Pääbo has investigated one possible key to this difference: gene expression versus gene sequence. "We've been very interested in how the transcriptome activity evolves in apes and humans," he says. Indeed, he found that ≈10% of genes differed in their expression levels between the two species, although much like sequence mutations, these expression changes can fit under the neutral theory of evolution and are thus likely to be inconsequential. "There are two tissues that seem to stand out, though. Quite clearly the male germ line has been the target of lots of positive selection, and there are subtle indications that something may have changed in the brain also, on the human lineage" (14), says Pääbo.

One particularly interesting gene of study has been *FOXP2*, which is important for brain and lung development but is also implicated in language articulation. Humans with a mutant *FOXP2* display speech difficulties. Pääbo and his colleagues found that the human *FOXP2* gene had changes that altered two amino acids of the protein (15). These changes were influenced by positive selection and occurred <200,000 years ago. This finding suggests that aspects of language are exclusive to humans. Characterizing such positively selected genes has been a bit of a struggle for Pääbo, but the completion of the chimpanzee genome in August 2005, a project that Pääbo was a part of, and the current rhesus macaque genome project may facilitate the study of the few but precious differences between humans and apes.

Future of the Past

Whereas the complete genomes of living animals will continue to roll off sequencing machines in an almost assembly line-like fashion, ancient samples, for the most part, still only reveal their secrets through mitochondrial DNA. "Under special circumstances, when something is extremely well preserved, one can retrieve nuclear DNA," says Pääbo, noting his recent analysis of nuclear DNA from 4,000-year-old Mexican maize (16). This study showed that selection for desired agricultural traits had already taken place by that time. Even in these cases, however, nuclear DNA can only be recovered in short fragments, effectively prohibiting comprehensive studies.

However, a recent high-throughput technological breakthrough, known as pyrosequencing, may soon alleviate this

difficulty. "In a way, it's going back to the first technology with the mummy. One makes a plasmid library in bacteria or just sequences directly from the fossils and looks at millions of molecules," says Pääbo. This massive approach will presumably make it feasible to separate desired DNA from the bacterial and fungal chaff in a sample. Of course, with any newly developed technique, one must try to work out the technical bugs, which Pääbo and his colleagues address in his PNAS Inaugural Article (1). Using pyrosequencing, Pääbo and his team analyzed samples of ancient wolf and mammoth DNA, as well as synthetic templates with predesigned modifications, to determine the mechanics and patterning of nucleotide misincorporation. Working out such technical details has always been highly important to Pääbo. "You have this little baby that you let out in the world, and you try and educate it and tell it what it should do, but it doesn't always live as you have tried to tell it. Sometimes people publish work where you don't feel so secure about the results, and that can be frustrating," he says.

Fortunately, Pääbo does not feel any such frustration right now. "At the moment, I have this great expectation that things will really take off in the ancient DNA field with these new technologies," he says. The first launch will be the sequencing of the Neanderthal genome. The sequencing consortium has already identified Neanderthal fossils that are either completely or almost completely free of human contamination, making them ideal specimens. "There will be other Pleistocene mammal genomes, such as the mammoth, that will be done too," he says, "but the Neanderthal genome is, to my mind, one of the most exciting ones."

Pääbo is careful to temper enthusiasts who dream about cloning extinct organisms or sequencing dinosaur DNA. "One doesn't really know what may come in the future, but cloning an organism from a genome fragmented into small pieces of DNA will probably always be impossible," he says, "and from what we know about the chemical stability of DNA, sequence retrieval will always be on this side of a million years ago, so dinosaur DNA is beyond our reach." The problem, he says, is that DNA is extremely hydrophilic, and exposure to water molecules as well as atmospheric oxygen and background radiation breaks it down. Even under highly favorable preservation conditions, such as mummies in a dry and cold desert, DNA disappears from a specimen within a few hundred thousand years. However, even given such limitations, many interesting discoveries still lie in ancient DNA. Someday soon, Pääbo may uncover exactly what separated human beings genetically from the Neanderthals.

Nick Zagorski, *Science Writer*

1. Stiller M, Green RE, Ronan M, Simons JF, Du L, He W, Egholm M, Rothberg JM, Keats SG, Ovodov ND, *et al.* (2006) *Proc Natl Acad Sci USA* 103:13578–13584.
2. Pääbo S (1985) *Nature* 314:644–645.
3. Higuchi RG, Bowman B, Freiberger BM, Ryder OA, Wilson AC (1984) *Nature* 312:282–284.
4. Pääbo S, Gifford JA, Wilson AC (1988) *Nucleic Acids Res* 16:9775–9787.
5. Pääbo S (1989) *Proc Natl Acad Sci USA* 86:1939–1943.
6. Thomas RH, Schaffner W, Wilson AC, Pääbo S (1989) *Nature* 340:465–467.
7. Höss M, Pääbo S, Vereshchagin NK (1994) *Nature* 370:333.
8. Höss M, Dilling A, Currant A, Pääbo S (1996) *Proc Natl Acad Sci USA* 93:181–185.
9. Hofreiter M, Capelli C, Krings M, Waits L, Conard N, Münzel S, Rabeder G, Nagel D, Paunovic M, Jambresic G, *et al.* (2002) *Mol Biol Evol* 19:1244–1250.
10. Cooper A, Mourer-Chauviré C, Chambers GK, von Haeseler A, Wilson AC, Pääbo S (1992) *Proc Natl Acad Sci USA* 89:8741–8744.
11. Höss M, Kohn M, Pääbo S, Knauer F, Schröder W (1992) *Nature* 359:199.
12. Krings M, Stone A, Schmitz RW, Krainitzki H, Stoneking M, Pääbo S (1997) *Cell* 90:19–30.
13. Serre D, Langaney A, Chech M, Teschler-Nicola M, Paunovic M, Mennecier P, Hofreiter M, Possnert G, Pääbo S (2004) *PLoS Biol* 2: 313–317.
14. Enard W, Khaitovich P, Klose J, Zöllner S, Heissig F, Giavalisco P, Nieselt-Struwe K, Muchmore E, Varki A, Ravid R, *et al.* (2002) *Science* 296:340–343.
15. Enard W, Przeworski M, Fisher SE, Lai CSL, Wiebe V, Kitano T, Monaco AP, Pääbo S (2002) *Nature* 418:869–872.
16. Jaenicke-Després V, Buckler E, Smith BD, Gilbert MTP, Cooper A, Doebley J, Pääbo S (2003) *Science* 302:1206–1208.