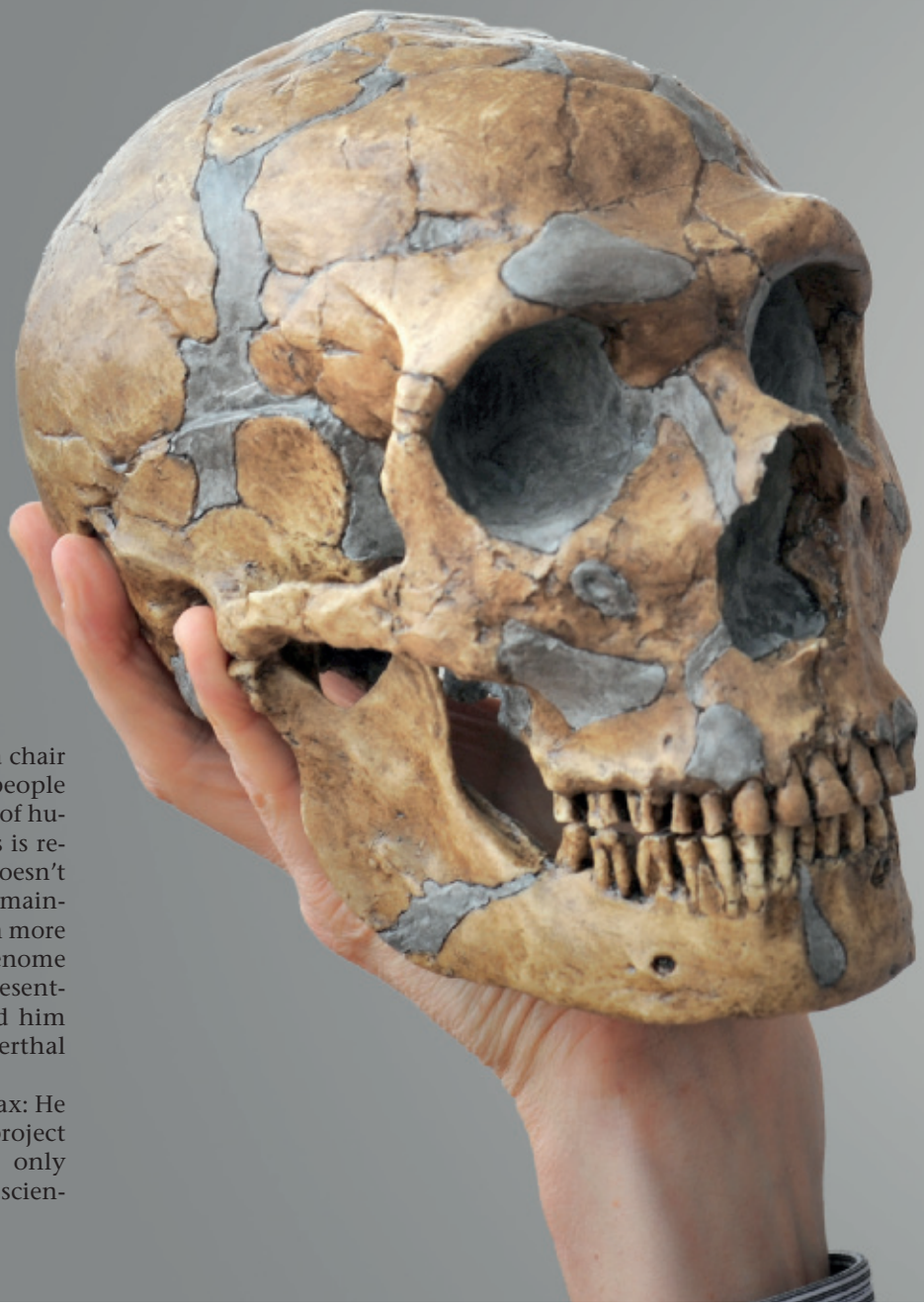
Meet the **Neanderthals**

Well, they actually did it. And the whole world knows about it now, even if the news is tens of thousands of years old. It was, of course, a juicy story for the world's media: Neanderthals mated with modern humans! But for **Svante Pääbo**, Department Director at the **Max Planck Institute for Evolutionary Anthropology** in Leipzig, this is not the most important aspect of his discovery.



TEXT **MARCUS ANHÄUSER**

Svante Pääbo relaxes in a chair in his office. "Many people think the interbreeding of humans and Neanderthals is really cool," he says. He doesn't deny that he thinks so too, but maintains: "In the long run, it is much more important that we now have a genome from the closest relative of all present-day humans." Standing behind him is a life-size model of a Neanderthal skeleton.

Pääbo has every reason to relax: He has completed in five years a project that others in the past could only dream of. For the first time ever, scien-

Face to face: Svante Pääbo holds the skull of one of our closest relatives, the Neanderthal.

Photo: Frank Vinken

» Neanderthal mtDNA was so different from the mtDNA of people today that it was unlikely that Neanderthals had mixed with modern humans.

tists have the genome of an extinct member of the genus *Homo*. The Neanderthals died out some 30,000 years ago. Now that we have their genome sequence, we can delve a little deeper – and not only to find out who or what the Neanderthals were: "Above all, we can describe what changes have occurred in our genome, and some of those must have contributed to making us unique," says Pääbo. This is a point he makes again and again in every interview. It's not primarily about the Neanderthals, it's about us.

SPOTLIGHT ON PALEOGENETICS

The publication of the Neanderthal genome in the journal Science on May 7, 2010 is a high point in the career of the 55-year-old, the son of Nobel laureate and biochemist Sune Bergström. It is also a highlight for his colleagues and the entire research field of paleogenetics, an area in which he played a pioneering role in the 1980s. Pääbo, who studied Egyptology and then medicine, earning a doctorate in immunology, had yet another research interest: he wanted to decode the DNA of mummies. Nobody had ever done that before. Scientists were not even sure that DNA could be extracted from mummies.

Unbeknownst to his dissertation supervisor, he worked nights on the project. "I was a bit afraid of him, as he might have stopped me from doing it," recalls Pääbo. When he actually found the DNA in microscopic sections of mummies and was able to stain it and even extract it, he was ecstatic. "Then I had to tell my supervisor," he remembers with an ironic smile. He submitted his results to Nature and even ended up on the cover of the journal in 1985. It was the perfect start to a career in science.

Following a period in Berkeley, Pääbo was given his own lab at Ludwig Maximilian University in Munich in 1990. It was there, during the mid-1990s, that he studied the DNA of Neanderthals for the first time and found the initial evidence that the "big oaf" was not a direct ancestor of humans. His group had extracted DNA from the mitochondria, the "powerhouses" of the cells. Because many mitochondria are present in each cell, they yield more DNA than the cell nucleus – mitochondrial DNA (mtDNA) can thus be analyzed more easily than nuclear DNA.

Neanderthal mtDNA was so different from human mtDNA that it was unlikely that Neanderthals and *Homo sapiens* had mixed to any large extent.

In 1997, Svante Pääbo moved to Leipzig as one of the five Directors of the new Max Planck Institute for Evolutionary Anthropology in the city. At that time, the focus of the international research community was on sequencing the human genome. The publicly funded human genome project began in the US in 1990 and faced competition from American geneticist Craig Venter's privately owned biotech company, Celera Genomics, which launched a similar project in 1998. The competitive aspect helped to accelerate the research work. In 2001, both the public and private research efforts were in a position to present a draft version of the human genome. At this stage, nobody even dreamed of reconstructing the entire Neanderthal genome.

Compared to the sequencing of the DNA of living individuals, a whole series of obstacles must be overcome before "old DNA" can be decoded. One of the main problems is that a sample from a 10,000-year-old Neanderthal bone may contain a lot of genetic material, but more than 95 percent of it does not belong to our caveman. "This is DNA from bacteria or fungi that have colonized the bone after death," explains Pääbo.

LETTERS SWITCHED IN THE NEANDERTHAL SEQUENCE

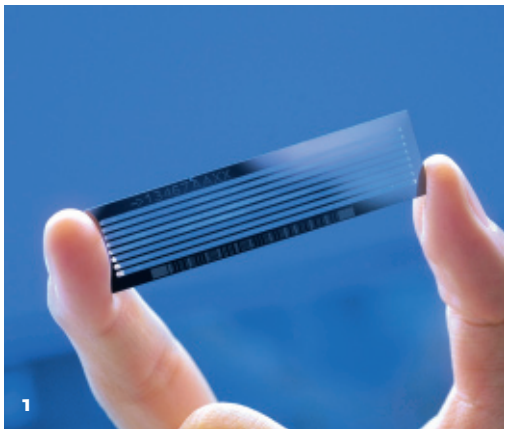
The second problem is that, over time, the DNA strand disintegrates into smaller and smaller fragments, like a jigsaw puzzle whose pieces are scattered in the box. Some parts are still connected, but the overall picture (the sequence) is unrecognizable. The problem is compounded by the fact that some of the building blocks are chemically modified. Pääbo and his team discovered that one of the four bases in the genetic code changes its identity over time. Instead of a C for cytosine, many of the sequence fragments contain a U for uracil, especially at the ends of the fragments, where the cytosines lose their amino groups easily. During sequencing, the U is read as a T for thymine. "So when we find a T in the first position of a sequence, in 40 percent of cases, it is actually a C," explains Pääbo. >

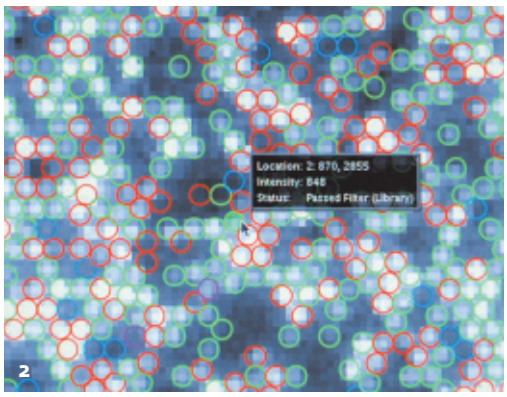
Photos: Frank Vinken (3)

1 | Tomislav Maricic examines the bone fragment of a Neanderthal in the clean room.

2 | The researchers must take extensive precautions before they can enter the clean room.

3 | Only 400 milligrams of bone powder is actually needed for the analysis; the researchers extract this from the bone fragment.





1 | An Illumina GAII flow cell is used to analyze several samples simultaneously in the Illumina Genome Analyzer.
2 | DNA clusters on the screen of the Roche/454 Genome Sequencer.
3 | Johannes Krause and Anne Butthof from Svante Pääbo's team load the Illumina Cluster Station.

A critical problem is caused by the researchers themselves: they can contaminate the samples with their own DNA. Individuals may leave small amounts of DNA behind, whether during the excavation at the archeological site or when preparing the gene fragment in the lab. This is helpful at the scene of a crime, but it can ruin the work of the paleogeneticists.

Pääbo and his team had to overcome all of these obstacles one by one. For example, the researchers succeeded in increasing the yield of Neanderthal DNA by a factor of 300. This was an important achievement as, before these advances, it looked as if the sequencing would take another two decades and cost 60 million euros.

A PAINSTAKING PROCESS WITH OLD DNA

The researchers enrich the Neanderthal DNA by using, for instance, molecular scissors that cut the microbial rather than the human genetic material. "In this way, we enrich the samples by up to 20 percent, which makes the sequencing affordable," says Pääbo. To counteract the changed identity of the cytosine, the researchers wrote bioinformatics programs with which they corrected their computer algorithms for the sequencing to factor in the probability that a T was actually a C. "In a fragment that is 50 base pairs in length, this allows us to decide with confidence whether the DNA is from a Neanderthal or from a bacterium," explains Pääbo.

Contamination by the researchers' own DNA was a problem that the group had to grapple with for a long time. Numerous precautions were taken. This involves handling the samples in a clean room, similar to the type used in the chip industry. The air is constantly filtered and the scientists work in protective clothing. When the room is not in use, UV light destroys any remaining DNA. Yet all of these precautions were not sufficient for an experiment that the team carried out in 2006, details of which were published in Nature. "Given that we had taken these precautions, we thought that we had only 1 percent contamination. In fact, the figure was perhaps 14 percent," says Pääbo. What happened?

A NEW GENERATION OF SEQUENCING MACHINES

The Leipzig team had sent their samples to the US-based manufacturer of the latest generation of high-throughput sequencing machines. However, the manufacturer did not have a clean room there. "We knew that that could be problematic, but we wanted to test the new machines before investing in them," explains the Max Planck Director. And that is where the foreign DNA is likely to have contaminated the samples. "It was annoying, but in retrospect it was a good thing, as it meant that we discovered a problem that we had to solve. This is how progress is made in science – we can't get perfect results right away."

Photos: Frank Vinken (3)

» And once again, a technical advancement led the way for a scientific breakthrough.

In the meantime, the sequencing machines are in Leipzig and each fragment of Neanderthal sequence is marked with a tag of four bases, clearly distinguishing it from contaminating human DNA, which is unmarked. And one improvement followed another. The new generation of high-throughput sequencing machines from the US company 454 Life Science was a technological quantum leap. It really put the reconstruction of the sequence of three billion base pairs within reach: "That was when I realized that we were going to make it," says Svante Pääbo.

Also Adrian Briggs, one of Pääbo's colleagues who recently completed a doctorate in the Leipzig laboratory, believes that the significance of this new generation of sequencing machines cannot be overestimated: "We had to sequence more than one billion fragments. That would have been absolute-

THE THIRD (WO)MAN

Just a few weeks before the Leipzig-based lab made headlines around the world with its revelations about the Neanderthal genome, Svante Pääbo and his colleague Johannes Krause were already dealing with a surge of media interest. In late March, Krause published details in Nature of an exciting discovery in a cave in the Altai Mountains in southern Siberia. They had results that showed that a previously unknown hominin lived there around 40,000 years ago.

If the Max Planck researchers' claims prove to be true, it would be the first time a paleontological human form has been discovered solely through DNA analysis. The DNA sample came from a sliver of finger bone, no bigger than a cherry pit. It was found in 2008 by the Russian researchers from Novosibirsk with whom Pääbo's lab have been collaborating for a number of years.

Johannes Krause sequenced its mtDNA. The advantage of this method is that, with approximately 16,000 base pairs, the sequence is very short and there are several hundred copies in each cell. When Krause examined the preliminary sequencing results, he couldn't believe his eyes. "It was a very exciting moment. Something wasn't quite right," says the 29-year-old. A discovery or an artifact? He was suspicious. Had something gone wrong with the analysis? Had an error occurred in the complex bioinformatics programs and algorithms the paleogeneticist was using to reconstruct the gene fragment?

Several control experiments, however, confirmed the unexpected result. Krause sequenced each position of the sequence more than 150 times. "I even called a meeting with my colleagues at 6:30 on a Friday evening to discuss the results with them," says the scientist. But his colleagues were also unable to explain the phenomenon: this sequence did not come from a modern human or a Neanderthal, but from an unknown third type of human.

The difference is clear: while the Neanderthal mtDNA differs from human mtDNA at an average of 202 positions, and from chimpanzee mtDNA at more than 1,400 positions, the genetic material in the finger bone found in the southern Siberian Denisova Cave differed at 385 positions. Using the mutation rate, the molecular clock, Krause determined that, roughly one million years ago, this hominin's line diverged from the line that subsequently gave rise to Neanderthals and modern humans.

The discovery is exciting also because it adds a new slant to the picture we have of hominin evolution. Modern humans seem to have shared the Earth with other human species for tens of thousands of years. All of these other hominin groups became extinct, while modern humans conquered the world. The discovery of the "hobbit," the small *Homo floresiensis* on the Indonesian island of Flores, is another example of this.

But before the Leipzig-based Max Planck researchers commit themselves definitively, they want to first sequence and analyze the nuclear DNA. "New information is emerging all the time, some of which calls previous results into question. It is all much more complex than we initially thought," says Johannes Krause. Was there perhaps even some interbreeding, as in the case of the Neanderthals? He hopes to have a conclusive answer to this question by the end of the year.

The reconstructed Neanderthal scene shows that the researchers in Pääbo's department also like to have a little fun at work.

ly impossible using the type of machine available in 2004." According to Briggs' calculations, "It would have taken one of those machines 10,000 years, or 10,000 machines one year, to do this – and at a cost of several hundred thousand dollars, it would have been impossible to finance."

DATA ANALYSIS TOOK ALMOST ONE YEAR

The difference between the old and new generation of these otherwise nondescript boxes is perhaps best illustrated by a look at the palm-sized plates that contain wells in which the DNA fragments are sequenced: on the old machines, there were 96 indentations per plate; in the 454 Life Science machine, there are 1.6 million. "This means that we can sequence more than a million DNA fragments per plate, compared to the 96 per plate with the older model," says Briggs.

Thanks to an additional cash injection from the President's Strategic Innovation fund, the researchers were able to afford this state-of-the-art sequencing technology. "It has to be said that people in the Max Planck Society recognized the potential and provided the necessary resources," says Pääbo.

The time, money and hard work paid off. In February 2009, the team announced that it had achieved its objective, but this was all that was made public; the sequence wasn't published as there was still more work to be done. "We had the feeling that we had to tell the world we had done it, as we had questions from journalists all the time," says Pääbo. It took just under a year to perform the analysis of the data.

The results are impressive – a real milestone in the study of human evolution. Pääbo and his colleagues sequenced four billion base pairs, from which they were able to reconstruct more than 60 percent of the Neanderthal sequence by comparing their data with the human genome and the chimpanzee genome.

"We were able to sequence some of the positions in the sequence several times, and others we were not able to sequence at all," says Johannes Krause, who has been working in Pääbo's lab since 2005. To perform the analysis, the Leipzig researchers organized a consortium of groups, mainly in the US, involving more than 50 individuals.

It came as a surprise that there was a small amount of interbreeding between Neanderthals and humans – and the news made headlines around the globe. Between one and four percent of human DNA in people living outside Africa originates from Neanderthals. The researchers were even more surprised by the fact that our burly Ice Age ancestors left their genetic traces not only in Europe, but also in China and Papua New Guinea. "That was something we didn't expect," says Pääbo. Until now, there has been no proof whatsoever that Neanderthals ever lived in these regions. It follows, therefore, that there must have been sexual contact before early modern humans expanded into Eurasia from Africa.

The simplest explanation would be that there was an encounter between the two groups in the Middle East. Paleontological evidence proves that the two groups lived in the same region between 80,000 and 50,000 years ago. However, the possibility that the

Photo: Frank Vinken

Cracking the Neanderthal code. Front row, from left: Hernán Burbano, Anja Buchholz, Svante Pääbo, Janet Kelso, Qiaomei Fu and Martin Kircher; back row, from left: Adrian Briggs, Jesse Dabney, Matthias Meyer, Tomislav Maricic, Johannes Krause, Udo Stenzel and Kay Prüfer.

genetic traces entered the lineage by another route somewhere in Africa about 20,000 to 30,0000 years ago cannot be ruled out completely. It would, however, require a much more complicated scenario. Further work will clarify exactly how the Neanderthal contribution to present-day humans occurred.

WHAT MAKES US UNIQUE?

But Svante Pääbo had several additional priorities: "We can now produce a catalog detailing all of the changes in our genome that make us unique – the changes that distinguish us not only from chimpanzees, but also from our closest relatives. And regions where positive selection seems to have affected modern humans after their separation from Neanderthals." The researchers have already found more than 200 such areas. The 20 most important ones are outlined in the Science article. "These are the areas that fascinate me the most," says Pääbo.

It is still too early to offer any definitive comment; instead, there are vague suggestions. Genes relating to cognitive development have been found. Mutations in these genes are related to schizophrenia and autism in humans. "But this does not mean that Neanderthals suffered from these problems," cautions Svante Pääbo. Another gene of interest is RUNX2. In its mutated form, it leads to *cleidocranial dysplasia* in humans, a condition characterized by skeletal deformities: the rib cage becomes bell-shaped, the shoulder bones change and the brow area protrudes. The result is almost like the skeleton standing behind Svante Pääbo. But the paleogeneticist plays it down: "No, that would be too easy," he laughs.

It is not that easy to identify the genetic differences between humans and Neanderthals. Or sometimes maybe it is? The main thing is that it is possible at all now. We will come closer to understanding the genetic secrets that make us unique by seeing ourselves in our closest relative. The work has just begun. ◀

www.filme.mpg.de
www.youtube.com/user/MaxPlanckSociety

GLOSSARY

Mitochondria
Cell organelles surrounded by a double membrane that contain their own genetic material (mtDNA) whose role is to provide the cell with energy in the form of ATP. New mitochondria are only formed in a process similar to binary fission. They are believed to have originated at an early stage of evolution, by eukaryotic cells (cells containing a nucleus) absorbing bacteria and changing their function (endosymbiosis).

Nucleotides
Nucleotides are the basic building blocks of DNA and RNA. They consist of a phosphate unit, a sugar module and one of the five nucleobases Adenine (A), Guanine (G), Cytosine (C), Thymine (T) or Uracil (U). The latter are the letters of the genetic code and form the DNA sequence.

High-throughput sequencing
This technology is used to determine the sequence of letters from genetic fragments within a very short time. This leads to a huge increase in the generation of sequencing data and reductions in cost. In the foreseeable future, it will be possible to sequence entire genomes for a few thousand euros.

Photo: Frank Vinken