



Neanderthals get in on the action – what DNA analyses tell us about our early history

Who are we? Where do we come from? These are key questions that have occupied us humans for more than a century now – at least since 1856, when workers in Neandertal, around 12 kilometres east of Düsseldorf in Germany, discovered the remains of a skeleton while they were clearing out a small cave in a quarry. The classification of the bone fragments has long been a contentious issue. While a number of anatomists believed that the remains belonged to an early form of the modern human, this assessment was not shared by everyone, especially the influential German pathologist Rudolf Virchow. However, by the end of the 19th century, the prevailing view was that the Neanderthal was a forerunner of the anatomically modern human.

Thanks to more than 300 skeletal finds, the Neanderthal is the most-studied fossil species in the Homo genus. Purely anatomical studies are not enough to answer questions such as how similar Neanderthals were to us, whether they represented an extinct branch in the family tree of early humans and whether some of their genes can still be found in modern humans. Svante Pääbo, Director at the Max Planck Institute for Evolutionary Anthropology in Leipzig, **(Cover photo)**, was convinced that the Neanderthal bones contained an even greater treasure.

In 2005 a scientific consortium involving Pääbo's Research Group had sequenced the chimpanzee genome and proved that there was a difference of just slightly more than 1 percent in the nucleotides in the DNA sequences that the modern human shared with the chimpanzee **(see also BIOMAX 12)**. "The Neanderthals should of course be much closer to us," says Pääbo. According to the molecular biologist: "If we could extract the DNA from their bones and then analyse it, we would definitively establish that the Neanderthal genes are very similar to our own." However, the differences were much more exciting: "Among the tiny deviations that we expected, there should also have been those differences that distinguish us from all our human predecessors and which have been the biological basis for the modern human taking a completely new evolutionary direction – culturally and technologically."

However, the examination of ancient DNA proves to be difficult for two reasons. Firstly, the actual proportion of ancient DNA in a bone fragment can be between 100 to less than 0.1 percent. Secondly, in all cases, the samples were contaminated by the DNA of bacteria. The DNA of modern humans is another source of contamination. This is because it is everywhere – we leave behind our DNA in the smallest flakes of skin, for example, and this also contaminates archaeological finds. When studying early human genetic

Fig. A: Avoiding contamination



In order to avoid contamination of the bones with their own DNA, the researchers need to take extensive safety precautions.

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material, this contamination is difficult to discover due to the similarity of DNA sequences.

To ensure the authenticity of ancient DNA sequences, researchers try to prevent contamination at the excavation site and during their subsequent molecular-biological studies (Fig. A), or – if that is not possible or no longer possible – to identify the contamination when analysing the sequencing data. In doing so, they also avail of the fact that, post mortem, i.e. after death has occurred, letters are shifted in the DNA: cytosine is thus replaced by thymine and guanine is replaced by adenine, if it involves the counterpart of the DNA strand. In addition, the proportion of that cytosine increases at both ends of the DNA molecule where an amino group is lost. The cytosine then turns into uracil, a nucleotide that normally appears in RNA. The DNA polymerase treats this “U” like a “T” – a disproportionate number of Ts in certain regions is therefore a very reliable signal for distinguishing between ancient and new DNA.

Another complication is that ancient DNA is exposed to the chemical degradation processes for longer, leading to considerable fragmentation. The proportion of DNA sequences

with shorter fragment lengths therefore increases in the bone samples. However, the existing DNA sequencing technology does not allow these short fragments to be selected quickly and in large numbers.

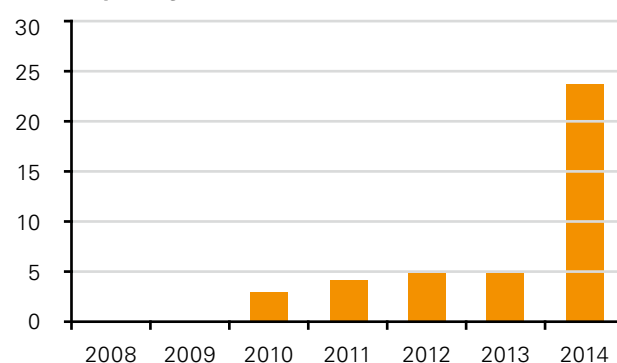
A TECHNOLOGICAL DRIVER FOR PALEOGENETICS

The breakthrough came with the development of a very new **DNA sequencing** technology. The basic principle of sequencing has remained unchanged: a complementary sequence is established along a fragment of DNA that is to be selected. The incorporation of a recognisable nucleotide (marked with dyes in most cases) is registered and the required sequence is determined based on the chronological sequence of the incorporation events. Next-generation sequencing technology (NGS) is also based on this principle. The difference is that in next-generation sequencing, the basic principle of sequencing is applied in an incredibly concentrated and efficient way, with extreme duplication: between several thousand and millions of sequencing steps can take place simultaneously and in a highly automated fashion (see box). This facilitates an extremely high sample throughput with the result that the sequencing of a complete human genome with 3.2 billion letters, which took 10 years and involved hundreds of labs around the world in the Human Genome Project, now can be performed by a single lab within a few days!

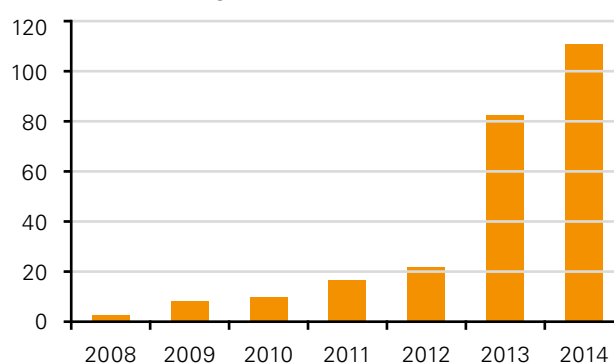
NGS is also a very effective method of sequencing very old, highly fragmented DNA with fragments that are shorter than 60 or 70 base pairs. The result was a positive boom in the sequencing of old DNA (Fig. B). In early 2006 Stephan Schuster, a biochemist at Pennsylvania State University, and his Canadian colleagues presented the nuclear genome, comprising 13 million base pairs, of an extinct woolly mammoth. “We were slightly disappointed that we were not the first to shed some light on the sequencing of ancient DNA with the new sequencing technology,” reports Pääbo. After all, his Research Group had had the data from the mammoth and cave bear bones that they had studied for months. “However, we had carried out other analyses and experiments in order to produce a picture that was as complete as possible, whereas the others simply wanted to be quicker.” The Leipzig-based researchers published their results in September 2006. In

Fig. B: Increase in the total number of complete early-history human genomes published

A Complete genomes



B Mitochondrial genomes



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the same year, they embarked on possibly their riskiest project: sequencing the Neanderthal genome. “I knew that achieving success would not be so easy,” recalls Pääbo. “On the contrary, it depended on three conditions: multiple DNA sequencers, a lot more money and suitable Neanderthal bones. We did not have any of that to start with.”

Four years later, the seemingly impossible became a reality: Pääbo and his colleagues presented an initial draft sequence of the genome of our relations, who have been extinct for around 30,000 years, in the journal Science. The draft was based on an analysis of more than one billion DNA fragments from several Neanderthal bones found in Croatia, Spain, Russia and Germany. In addition, the researchers sequenced five human **genomes** of European, Asian and African origin and compared these with the Neanderthal genome. The comparison revealed some very surprising results: Neanderthal traces were found in all the genomes except those of people who lived in Africa. “Between 1.5 and 2.1 percent of the DNA in the modern-day non-Africans genome originates from Neanderthals,” says Pääbo. “Asians even have a somewhat higher percentage.” This was a clear sign of extensive inter-species sex during the conquest of Eurasia.

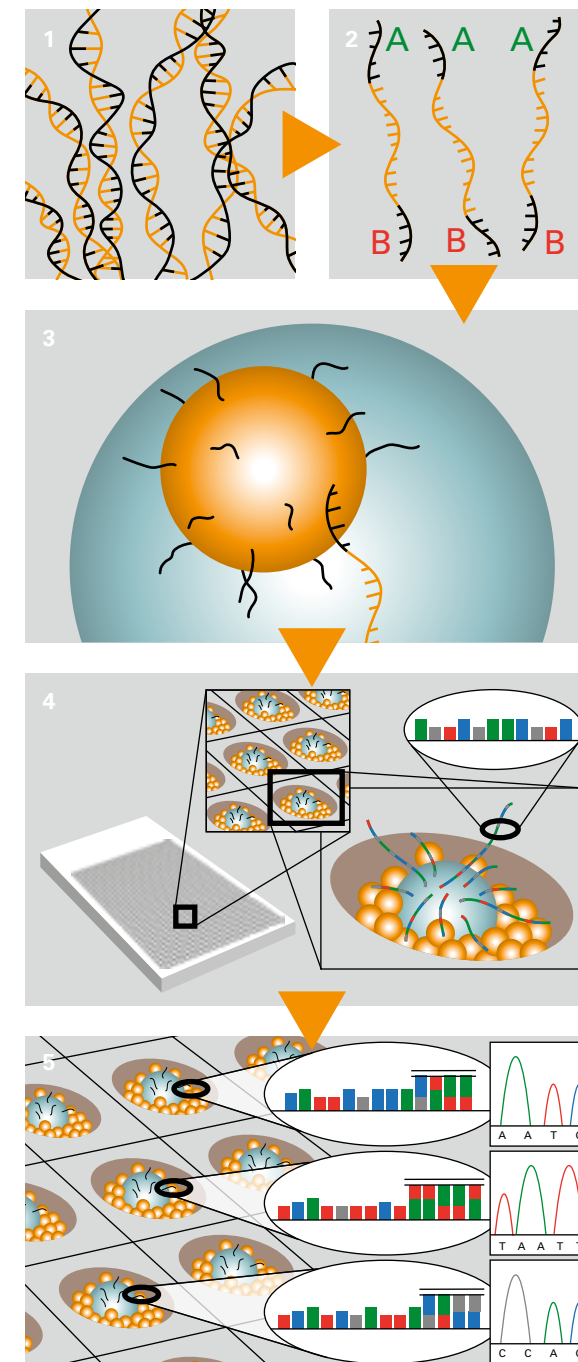
The love affair between Neanderthals and Homo sapiens began around 50,000 to 80,000 years ago, when our ancestors left the African continent and spread out through Europe and Asia, where they encountered Neanderthals. This was a period of successful interbreeding between the closely related species. If all the available snippets are now put together, 20 percent of the former genetic material of Neanderthals can be reconstructed. Our ancestors benefited from this DNA. While most of the harmful Neanderthal genes were purged through **selection**, useful genes settled in the human population. Among them were those associated with the nature of skin and hair. It is quite possible therefore that our forefathers inherited their white skin from the Neanderthals. A light-coloured skin was an advantage particularly at high latitudes as it made the production of vitamin D from sunlight more efficient. “By becoming involved with the original inhabitants of their new home, Homo sapiens were better able to adapt to their new environment,” assumes Pääbo.

ON THE SEARCH FOR CLUES IN THE HOMO SAPIENS GENOME

And what implications do the inherited Neanderthal sequences have for us today? Based on current clinical data, we can see the effects on functions of the skin, the immune system and the metabolism. Some Neanderthal genes that we carry increase the risk of contracting type 2 diabetes or Crohn’s disease. However, in the fight against pathogens, the modern human also benefits from archaic gene sequences: they code for three specific immune receptors and in this way reduce a predisposition to allergies.

While the Neanderthal still has “chimpanzee-like” **gene variants** in certain parts of its genome, most modern humans have gene variants that have already been derived from these at the same location. “It is precisely these areas in our genome that could have been a crucial factor in the evolution of the modern human, because we acquired particularly beneficial mutations early in our evolutionary history,” says Pääbo. The changes in the FOXP2 gene (see also BIOMAX 12), which are presumed to orchestrate

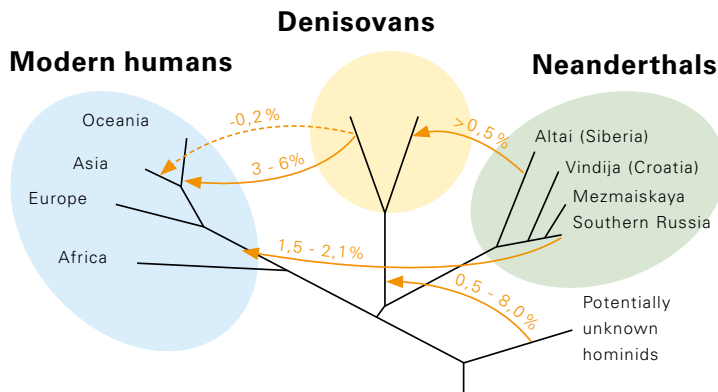
Next-generation sequencing



Svante Pääbo and his colleagues sequenced more than one million base pairs of Neanderthal DNA (1) using an approach known as pyrosequencing. Using this method, the DNA is first converted into individual strands (2) and then joined to the beads, which are populated with oligonucleotides. The DNA-loaded beads are emulsified with the PCR reagents in oil, ideally generating emulsion drops that contain only one bead (3). The DNA strands are now duplicated (emPCR) in this environment and then placed in the wells of a picotiter plate in which an optical fibre under each pore leads to a detector (4). The DNA strands are now duplicated (emPCR) in this environment and then placed in the wells of a picotiter plate in which an optical fibre under each pore leads to a detector (5).

See also <http://goo.gl/8yhC0e>

Fig. C: Gene flow model



The figure shows the direction and the estimated extent of the gene flows between Neanderthals, Denisovans and modern humans. It is doubtful whether there was a direct gene flow from the Denisovan to Asia (dotted line). In three out of five cases, the researchers were able to prove that there was interbreeding between four different hominid populations. The "potentially unknown early human" could have been *Homo erectus*.

the development of speech and language, on the other hand, are shared by *Homo sapiens* and Neanderthals. Is it possible therefore that the Neanderthal had the same cognitive skills in this regard? Overall, the catalogue of genetic differences between early and modern humans totals 87 proteins and a handful of microRNAs (non-coding RNA molecules, which play an important role in gene regulation, particularly when it comes to silencing genes).

And scientists have only just begun to understand the functional consequences of certain genetic modifications. The Max Planck researchers, together with colleagues from the universities of Barcelona and Leipzig, have not only analysed the DNA sequence of an early human gene variant but also produced the relevant protein and studied its properties. This led them to discover that the activity of a certain gene variant in the melanocortin receptor in two Neanderthals was significantly reduced. Gene variants with a similarly reduced activity are also observed in modern humans – with visible consequences: individuals with such gene variants have red hair. The paleogeneticists therefore assume that some Neanderthals may have been redheads.

OUR POPULATION HISTORY IN THE LIGHT OF ANCIENT DNA

The recent paleogenetics findings lead to an entirely new view of the evolutionary processes that once gave rise to the *Homo sapiens* and helped the species to play an important role on our planet as the last representative of its genus. The findings show that the many hundreds of thousands of years of human evolution followed a different path to the long-accepted one. Since the researchers managed to extract information from genetic material in the found bones, the scientific consensus that had been arduously built up bone by bone has been crumbling. *Homo sapiens* no longer stands as the pinnacle of evolution but rather as a scion of the various *mésalliances* of the past.

Its predecessors live on in the DNA of modern-day humans. In 2010 Svante Pääbo and his team sequenced DNA from the

tiny fragment of a finger bone that they had discovered in the Denisova cave in southern Siberia. "Using gene analyses we were able to show that it was a hitherto unknown human form," explains Pääbo. They were also able to demonstrate that this Denisovan, as the researchers called him, had mated with the ancestors of the modern-day inhabitants of Australia, New Guinea and East Asia. Genome comparisons prove that there must have been an exchange of genes (**gene flow**) between Neanderthals, Denisovans and *Homo sapiens* (Fig. C). "In light of this information, we now need to look at the modern human as part of a hominid metapopulation," says Pääbo. "Only the last 20,000 years are really unique in that we as humans have been alone in the world." And the paleogeneticist predicts: "In the future, we will certainly find out a lot more about population history from a minimum number of finds."

Keywords

DNA sequencing, genome, selection, gene variants, gene flow

Recommended reading

Svante Pääbo, *Neanderthal Man: In Search of Lost Genomes*, Basic Books; 1 edition (March 24, 2015)
<https://www.scientificamerican.com/article/special-evolution-issue-humanity-s-journey/>

Recommended videos

The Neanderthal in us – <https://www.youtube.com/watch?v=-1Nrm-M-m14&list=PL77BD5A2A35A72DCB&index=139>
The mysterious hominids from the Denisova Cave – https://www.youtube.com/watch?v=eweVB0XPC_8&list=PL77BD5A2A35A72DCB&index=102

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