# DNA From an Extinct Human

## The first-ever analysis of ancient DNA from a Neandertal bone is a technical tour de force and offers a new kind of evidence for the view that Neandertals were an evolutionary dead end

MUNICH, GERMANY—A chance discovery by quarry workers who blasted their way into a limestone cave one summer day in 1856 is making scientific history once again. The unusual skeleton they found in the cave's rubble soon became the first recognized example of a now-vanished type of human. Named Neandertal, after the Neander Tal (Neander Valley) in Germany where it was found, the skeleton was dramatic proof that humans evolved, just like all other living things. Ever since, researchers have fiercely debated what these ancient beings were like, how they lived, and whether they are ancestors of modern humans or an evolutionary dead end.

Now, 141 years later, this same skeleton is yielding the world's first glimpse into the genome of an ancient type of human. In today's issue of *Cell*, Matthias Krings and Svante Pääbo at the University of Munich and Anne Stone and Mark Stoneking of Pennsylvania State University report that they have recovered and analyzed a tiny snippet of DNA from the skeleton. Its sequence, says Pääbo, is "very different from [the corresponding region of] modern humans." While one sequence from a single individual is not definitive proof, the data lend a new kind of support to the now-favored view of Neandertals: that they were a side branch of the human family tree, not our direct ancestors.

The work is creating a stir among human origins researchers, not only for its implications for their field but because it demonstrates the feasibility of recovering DNA from at least some human fossils. The emerging field of ancient DNA has been badly tainted in recent years by spectacular claims, such as DNA from a dinosaur, that were later discredited. But researchers familiar with the new work are convinced that this Neandertal sequence is the real thing. "I really looked for holes in the methodology, but I just couldn't find any. It seems to be an authentic sequence and certainly as far as I can tell the most rigorous ancient DNA study I've ever seen," says evolutionary biologist and ancient DNA researcher Blair Hedges of Penn State.

Although duplicating the Pääbo group's achievement will be difficult for many reasons, Hedges and others say that this gives the whole field a needed shot in the arm. "It's one of my dreams that this would be possible," rejoices paleoanthropologist Christopher Stringer of The Natural History Museum in London, who belongs to one of several other teams that have tried—unsuccessfully—to recover Neandertal DNA. "It's a marvelous achievement. ... For human evolution, this is as exciting as the Mars landing. ... It's the biggest breakthrough in Neandertal studies."

Stringer is particularly excited because the data tend to support his view in the debate about modern human origins, although they won't put the argument to rest. He is the leading advocate of the currently favored idea called "Out of Africa"—that modern humans arose recently in Africa and then replaced existing human populations around the world, including the European Neandertals, without interbeeding with them. That theory predicts that Neandertals were a separate species, as the differences between the Neandertal DNA and that of modern humans now suggest. "You can't prove [Neandertals] were a separate species from just this sequence, but it's very unlikely they contributed to the modern gene pool," says molecular anthropologist Maryellen Ruvolo of Harvard University.


J. OBERHEIDE/ARGUM

**DNA wizards.** A team lead by Svante Pääbo *(right)* managed to analyze DNA from a Neandertal arm bone.

### Technical triumphs

The groundwork for this astonishing success goes back several years. Archaeologists Hans-Ekhard Joachim of the Rheinisches Landesmuseum (where the Neandertal type specimen is kept), and paleontologist Ralf Schmitz of the Rhine State Department of Archaeology organized a committee to study what new research could be done on their precious skeleton, thought to be somewhere between 30,000 and 100,000 years old. They approached Pääbo, a pioneer in the ancient DNA field, about trying to recover DNA. After extensive studies of the preservation of various bones, Schmitz and Heike Krainitzki of the Technical College for Preparators in Bachum sent a sample of an upper arm bone to Pääbo's Munich lab last July.

Pääbo decided to search for a particular sequence called the control region in the DNA of mitochondria, cells' energy-producing organelles, which have their own tiny genome. Over the past decade, this inconspicuous region, which doesn't code for any protein, has become a crucial tool for inferring evolutionary relationships among species and populations (*Science*, 16 May, p. 1032). That's because mitochondria are inherited only from the mother and do not undergo genetic recombination, so their sequence stays the same from one generation to the next—except when mutations change it.

As a result, the number of nucleotide differences between two mitochondrial sequences generally reflects the degree of evolutionary divergence between them. The control region is particularly useful because it mutates faster than most other parts of the mitochondrial genome and so has more power to reveal differences between populations. Coincidentally, mitochondrial DNA (mtDNA) is usually the only type to survive in long-dead specimens, thanks to its abundance—500 to 1000 copies per cell rather than only two copies, as in DNA of the nucleus.

But the difficulties in recovering any DNA from ancient bone are vast. The biggest problem is that DNA begins to degrade from the moment of death as water, oxygen, and microbes attack it. In most cases, all the DNA is probably gone after 50,000 to 100,000 years. So the best Pääbo's team could hope for in their Neandertal samples was trace amounts of highly damaged, short fragments. Indeed, they later calculated that their standard sample of bone extract contained only about 50 copies of the mtDNA piece they were after, in fragments of about 100 base pairs. Moreover, the damaged state of ancient DNA makes the method used to amplify it—the polymerase chain reaction (PCR)—far more error prone.

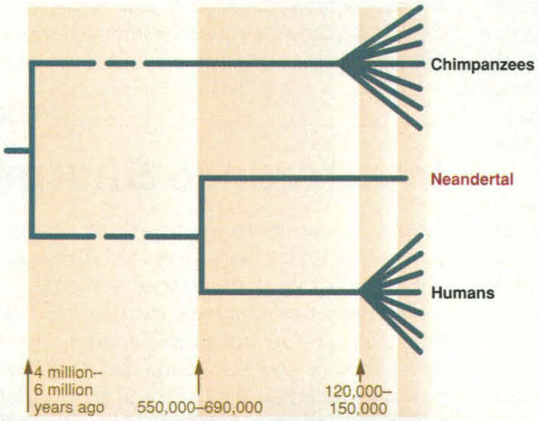
Worst, it's tough to distinguish DNA intrinsic to an ancient sample from the modern DNA that unavoidably contaminates it—the source of many false claims in the past. Ancient human samples are especially tricky, because their sequences might not differ much from that of contaminating modern human

DNA, so it's hard to get a believable result.

Past failures had left Pääbo skeptical that recovering Neandertal DNA was even possible. Krings, the graduate student who took on the project, was skeptical, too. But initial tests on the bone's amino acids, which require only minuscule amounts of sample and measure the extent of DNA-killing water damage, were surprisingly promising.

Krings then went after the DNA, strenuously trying to reduce contamination. He repeated what he had done for the amino acid tests—bleaching the outside of the bone to help rid it of contaminating DNA from past handlers, sterilizing instruments, and working in a room where no modern DNA work is done. He ground part of the sample to powder and extracted DNA from it; then he used PCR to amplify part of the control region and cloned the products, which allows more careful sequencing.

When he saw the sequences, "something started to crawl up my spine," he says. Mixed among a few clones that looked like modern human DNA—and were most likely contamination—were some unlike any in living humans. "I can't describe how exciting it was," says Krings. Then came 3 months of painstaking work to piece together the total sequence of 379 base pairs.



SOURCE: S. PÄÄBO

KEITH GARRETT/NATIONAL GEOGRAPHIC SOCIETY

**Out on a limb.** Neandertals, like this one from France *(above)*, were not the ancestors of modern humans, according to new mitochondrial DNA data *(top)*. The single Neandertal DNA sequence is distinct from all those known for humans and chimps.

The clincher came when Stone repeated the entire procedure in Stoneking's lab at Penn State—and came up with the identical sequence. "That's when we opened the champagne," says Krings. That sense of triumph was fully justified, say other researchers. "It's really on the edge of what's possible technically," says Richard H. Thomas, a molecular systematist at The Natural History Museum in London. Adds paleoanthropologist Dan Lieberman of Rutgers University in New Brunswick, New Jersey, "The fact that they managed to find DNA from a region of prime importance is proof that there is a God who likes paleoanthropology."

Finally, the team compared the Neandertal sequence with 986 distinct sequences from living humans. They found, on average, three times more differences between the Neandertal and modern human sequences than between pairs of modern humans. Specifically, pairs of modern human sequences differed at an average of only eight positions, while human-Neandertal pairs differed at an average of 25.6 positions. And the range only barely overlapped: The most divergent modern humans differed in only 24 nucleotides, while the closest modern-Neandertal pair had 20 differences. Also, the type of nucleotide substitutions and their locations were different. These data put the Neandertal sequence outside the statistical range of modern human variation and, says Pääbo, make it "highly unlikely that Neandertals contributed to the human mtDNA pool."

To find out when the mitochondrial DNA lineages of Neandertals and our own ancestors must have separated, the Pääbo team also used the sequence data as a molecular clock. They used the estimated date when the human and chimp lineages diverged (4 million to 5 million years ago) and the sequence differences between humans and chimps (taking into account the types of changes that occur in this sequence) to come up with a rate at which the control region accumulated mutations over time. With other assumptions and correction factors, they calculated that the sequence ancestral to both modern and Neandertal mitochondria began to diverge some 550,000 to 690,000 years ago, compared to only 120,000 to 150,000 years ago for the ancestral sequence of all modern humans. To put it in relative terms, the last common ancestor of Neandertals and modern humans is four times older than the last ancestor of all modern humans.

Such molecular clocks are full of questionable assumptions, but that relative difference is significant, says evolutionary geneticist Ryk Ward of Oxford University. Specifically, it suggests a deeply rooted split in the human family tree and implies that the two lineages diverged before the first known Neandertal at about 300,000 years ago, and long before the first modern humans at less than 200,000 years ago, says Stringer.

### Out of Africa supported

All this has major implications for how modern humans arose, although this one study is unlikely to end the debate. Although recent fossil and genetic evidence has tended to support the Out of Africa theory, an opposing theory holds that modern humans evolved continuously in many parts of the world. In this view, called regional continuity, modern humans interbred with archaic people in Europe and Asia, including with Neandertals during the roughly 70,000 years they apparently coexisted in the Middle East and parts of Europe and Asia. Recently, each of these models has inched toward the other: Some Out of Africa contenders have begun to admit that populations on continents other than Africa could have contributed genes to modern populations, and multiregional proponents now advocate something short of widespread mixing.

But the new data suggest no mixing at all, at least in mitochondrial genes. "Neandertals in Europe could not have contributed to the modern human mitochondrial genome," says Stanford University geneticist Luca Cavalli-Sforza. That "destroys one of the fortresses of the regional continuity model," he says, which postulates that Neandertals in Europe are among the ancestors of living Europeans.

Indeed, one of the paper's most important findings is that Neandertal DNA shows no particular similarity to that of Europeans. "If regional continuity were correct, "we'd assume that Europeans would be closest [in their sequence] to Neandertals. But the results show Neandertals are equidistant to all races," says Stringer. As a result, most researchers who spoke with *Science* consider the new data as support for the idea that modern humans replaced, rather than intermingled with, Neandertals. "The multiregional guys will have a hard time wriggling out of this one," says Ward.

But there is some wriggle room left. For starters, the fact that Neandertal sequences differ from those in modern humans doesn't by itself settle the tricky question of whether some members of the two groups interbred in the distant past. The genetic variation seen between the modern and Neandertal sequences is within the range of other single species of primates, notes Ruvolo, who says "there isn't a yardstick for genetic difference upon which you can define a species."

It's true that the Neandertal mtDNA is different from that of living humans. But that may simply support what geneticists have long known: Living humans are strangely homogeneous genetically, presumably because they originated recently from a small group or their ancestors underwent a population bottleneck that wiped out many variations. Thus, genetically diverse ancient populations could have intermingled long ago, then over time modern humans lost many of those genetic variants.

That's why multiregional partisans say it's not possible to rule out their theory with data on a single sequence from one individual. "This is an extremely important piece of work. They're first. But we just don't have the data to answer the question of whether it supports one hypothesis or another," says paleoanthropologist Milford Wolpoff of the University of Michigan, Ann Arbor. He argues that Neandertals may have contributed to the modern gene pool, but their sequences disappeared through random genetic loss, selection, or both. Or the particular Neandertal sequence analyzed might be at one extreme of a diverse spectrum in Neandertals that includes other, more modernlike sequences. But most population geneticists consider these possibilities remote, says anthropological geneticist John Relethford at the State University of New York College at Oneonta.

And of course, because mtDNA comes only from the mother, it's possible that Neandertal fathers—but not mothers—contributed nuclear genes to the modern gene pool. Most researchers think such a one-sided genetic interchange is quite unlikely or "odd," as Ruvolo puts it. But it's impossible to test directly, as the chances of recovering nuclear DNA are basically nil, says Pääbo—a fact sure to disappoint potential entrepreneurs dreaming of "Neandertal Park," as resurrecting any extinct creature would require intact nuclear DNA, among other impossibilities.

Other population geneticists say they would like to see more data to be sure. "The icing on the cake would be Neandertal number two," says Penn State's Blair. "Get one of those North African Neandertals or something really far away, and see if it clusters with this one." Genetic data from archaic moderns would also be helpful. Says Relethford, "I'd like to see DNA from the first undisputed early modern Europeans, the Cro Magnon from about 30,000 years ago. That's a real good test. Their mtDNA should look more like us."

No one has that, quite, but Bryan Sykes of Oxford University and Stringer think they have isolated mtDNA from a 10,000-year-old late Cro Magnon from Cheddar, England—and it shows only one base pair difference from that of modern humans. This as yet unpublished work shows that "we can put Cro Magnons at 10,000 nicely in the present variation," says Stringer.

But although Pääbo's group has shown that it is possible to get believable results with ancient, human DNA, he and others call for caution in going after more. Fossils are precious, and it's crucial to test the preservation of tiny samples or of animal bones found with human fossils before grinding them up, says Ward. What's more, adds London's Thomas, "all [researchers] have to do is read the paper closely to see it's a vast amount of work to do this right."

Even if the new result doesn't quite settle the debate about whether Neandertals mixed with modern humans, it does underscore how different they were from our own lineage. And that implies that "up to 200,000 or 300,000 years ago, humans evolved just like gorillas and everything else," says Ward, perhaps with several contemporaneous, diverse human species. That picture is emerging from other new lines of evidence, too. For example, new dates on *Homo erectus* fossils in Asia show that this species also coexisted with modern humans until as recently as 30,000 years ago. These results are forcing paleoanthropologists to renounce the once-preferred linear model of human evolution in which a single primitive species gradually gave rise to the most advanced form—us. Rather, it seems, there was once a bushy human family tree—and all the branches but one went extinct.

"It's what happens to [other kinds of] populations," says paleontologist Rob Foley of Cambridge University in the United Kingdom. "But in the case of Neandertals, we get excited about it." So it seems that the Neandertal sequence reinforces what this skeleton told the world when it was first discovered: that humans evolve just like everything else.

**–Patricia Kahn and Ann Gibbons**

ASTRONOMY

# Interference Sharpens the View

Last week, astronomers released the first images made with the help of a new orbiting radio telescope. Although the images—which show a powerful jet of subatomic particles spewing from a quasar—are not groundbreaking themselves, they are a powerful demonstration of the new system, indicating that it can provide detailed views of some of the most energetic and mysterious objects in the universe.

The radio antenna, called HALCA, is the first space-based one designed for interferometry, a technique that allows scientists to combine data from far-flung telescopes and create the equivalent of an enormous collecting dish. The larger the dish, the more detailed the images. HALCA works in concert with ground-based telescopes, allowing astronomers to simulate a dish with a diameter greater than 30,000 kilometers and a resolving power—the ability to detect fine details—more than 100 times that of the Hubble Space Telescope.

The image to the left (reduced in size for comparison) was taken by the Very Long Baseline Array (VLBA), a collection of ground-based radio telescopes. It shows quasar 1156+295, which is 6.5 billion light-years from Earth. The image to the right is a view of the same quasar in which data from HALCA are combined with those from the VLBA. It shows in unprecedented detail the particle jet emanating from the quasar, which may harbor a black hole, says astronomer Jonathan Romney of the National Radio Astronomy Observatory in Socorro, New Mexico, which is part of the international team that uses HALCA. No one is sure how the jets are formed, he says, but a clearer picture of their source may help scientists solve that mystery.

Astronomers hope that HALCA, built by scientists at the Japanese Institute for Space and Astronautical Science and launched in February (*Science*, 31 January, p. 620), will allow them to make the most precise observations yet of objects that emit radio waves, including other quasars and black holes.

**–Gretchen Vogel**