



## PALEOGENETICS: The Dawn of Stone Age Genomics

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DNA from a 38,000-year-old Neandertal is revitalizing the once-moribund field of ancient DNA, and it promises a fresh perspective on how we differ from our closest relatives

# The Dawn of Stone Age Genomics

**WHEN GERMAN QUARRY WORKERS CHIPPED** the first Neandertal bones out of a limestone cave in 1856, DNA analysis wasn't even a glimmer in any scientist's mind. Now, two reports, one on page 1113 and the other in the 16 November issue of *Nature*, describe the first successes in sequencing nuclear DNA from a Neandertal bone—a feat once considered impossible. The results from the two groups, working collaboratively but using different approaches, support the view that Neandertals are a separate branch of the hominid family tree that diverged from our own ancestors perhaps 450,000 years ago or more.

Because the extinct Neandertals are our closest relatives, comparing their DNA to ours may one day reveal the mutations that set *Homo sapiens* apart from all other species, as well as the timing of key evolutionary changes. But it's early days yet, and this week's papers chiefly suggest the potential of Neandertal genomics. They also fan the flames of the debate about how different Neandertals were from modern humans, and whether the two groups interbred during the thousands of years they coexisted in Eurasia (see sidebar, p. 1071). "This is great stuff," says molecular evolutionist Alan Cooper of the University of Adelaide, Australia. "It opens the way for much more work on identifying uniquely human genetic changes."

Coming on the heels of dramatic sequencing successes with ancient mammoth and cave bear DNA, the papers also herald a renaissance for a field that has been stymied by issues of poor sample quality and contamination. The Neandertal studies use metagenomics, which makes unnecessary the onerous task of purifying ancient DNA. They also employ faster, cheaper sequencing methods, and their achievement demonstrates the feasibility of deciphering ancient genetic material. "It has people talking about new ideas, new extraction techniques, new ways to prepare samples, new ways to think about old DNA," says Beth Shapiro, an ancient DNA specialist at the University of Oxford in the U.K.

Both teams are planning major additional projects. In July, the team led by Svante Pääbo, a paleogeneticist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, announced that it plans to produce a very rough draft of the entire Neandertal genome in 2 years. With that draft, he and others will be better able to tell which of the 35 million bases that differ between chimp and humans are mutations that occurred in just the past 500,000 years and therefore likely define our species. "Perhaps we can find that last little bit that made us special," says Pääbo.

Meanwhile, the other team, led by Edward Rubin, head of the Department of Energy Joint Genome Institute in Walnut Creek, California, has support from the U.S. National Institutes of Health to gather DNA from several Neandertal fossils to study specific regions deemed key to understanding human evolution. At least one other team, led by Cooper, has its own Neandertal project and is working to gather DNA from other ancient humans as well. "A whole new world has opened up with regard to what can be done with ancient DNA," says Thomas Gilbert, a paleogeneticist at the University of Copenhagen, Denmark.

But despite the seductive promise of new techniques, researchers warn that ancient DNA has been a fickle mistress. Over the past 20 years, successes have been followed by frustration after frustration. It's hard to find suitable DNA, and it's also quite tricky to avoid contamination with modern genetic material and to cull errors. These issues may come back to haunt Pääbo and Rubin, says genomicist Stephan Schuster of Pennsylvania State University in State College. "The divergence [between living people and Neandertals] is so small compared to the DNA damage and the sequencing error" that





**Rare find.** Neandertal bone (*inset*) from this Croatian cave had well-preserved DNA, which has now been sequenced.

it's hard to be confident of any results, he says. "If we've learned anything, it is that we generally haven't perceived the full extent of the problems and complexities of ancient DNA research," admits Cooper. "We're still very much in the learning curve."

### Ups and downs

Ancient DNA made its first appearance in 1984, when Allan Wilson of the University of California (UC), Berkeley, was able to tease out 100 bases from a quagga, an extinct species that looked like a cross between a horse and a zebra. A year later, Pääbo succeeded in extracting genetic material from a 2400-year-old Egyptian mummy.

The world was wowed by these successes, "but there was not much future in the field or the approach," Pääbo recalls. DNA degrades after death, as water, oxygen, and microbes attack it, and the sequencing methods of the time demanded more DNA than was readily available from ancient specimens.

The polymerase chain reaction (PCR), which uses an enzyme to make millions of copies of a particular DNA fragment, seemed to be just what the field needed, offering a way to amplify and read a tiny bit of sequence. The technique powered analyses of quagga, Tasmanian wolves, moas, and other extinct species during the 1990s.

But reliable results from more ancient specimens proved hard to come by. The reaction also amplified age-induced errors and extraneous DNA. A few spectacular failures cast doubt on the whole field: Supposedly 25-million-year-old DNA from amber-encased bees and even older DNA from dinosaurs turned out to be from living humans instead. Ancient human remains were especially problematic because of the specter of contamination: Anyone who handled bone could leave traces of their DNA upon it, and it was impossible to distinguish old from modern sequence.

Then in 1997, following new methodological guidelines, a team led by Pääbo, then at the University of



**DNA-free.** Clean-room garb in Spain's El Sidron cave helps reduce contamination by human DNA.

Munich in Germany, and his student Matthias Krings restored the appeal of ancient DNA by decoding 379 bases of Neandertal mitochondrial DNA (mtDNA) (*Science*, 11 July 1997, p. 176). The bases were quite different from the equivalent modern human DNA, suggesting that Neandertals were a distinct species that split off from a common ancestor a half-million years ago and did not interbreed with modern humans. That and subsequent mtDNA and fossil studies supported the leading view that *H. sapiens* arose in Africa and spread around the globe, replacing other kinds of humans.

But in part because modern humans and Neandertals overlapped in Europe and west Asia for at least a few thousand years, and perhaps up to 10,000 years, some researchers had continued to argue that the two species interbred. They pointed out that 379 base pairs were too few to be conclusive. Also, because mitochondria are passed on only by the mother, nuclear DNA is needed to rule out the possibility of mixing.

### Making the dream real

But getting nuclear DNA from ancient bones was a tall order. Back in 1997, "it was just a dream," Pääbo recalls. Because the amount of nuclear DNA in a cell is just 0.05% that of mitochondrial DNA, it's even harder to get enough nuclear DNA to sequence, particularly because

often the DNA has disintegrated. Also, Neandertal bones are rare, and curators are reluctant to pro-

vide samples. But Pääbo's team devised a hierarchy of tests that required just a tiny amount of material to begin with.

First they tested a tiny, 10-milligram sample for intact proteins, as their presence suggests that DNA was preserved as well. Then they examined 150 milligrams to determine the ratio of Neandertal to modern human DNA, using existing Neandertal mtDNA as a guide. Two of the 70 samples they examined passed both tests with flying colors. So Pääbo's team sliced out a larger piece of one, a 38,000-year-old bone from Croatia, and extracted the DNA.

Meanwhile, Rubin had begun to think that the metagenomics approaches that he was pioneering to study microbial diversity would work with fossil DNA too. He suggested to Pääbo that Neandertal genomics might now be possible. After Rubin's postdoc James Noonan successfully sequenced 26,861 bases of cave bear DNA (*Science*, 22 July 2005, p. 597), Pääbo gave a sample of the Neandertal DNA to Noonan to work on.

The two teams embarked on parallel but independent analyses using different methods. Noonan first created a library of Neandertal DNA incorporated into live bacteria. As each bacterium replicated, it made copies of a particular fragment. The team employed a new, massively parallel technique called pyrosequencing, which uses pulses of light to read the sequence of thousands of bases at once. Sophisticated computer programs then compared the sequence fragments to available DNA databases and identified the potential Neandertal ones based on their similarity to modern human sequence. The team used several tests to rule out contamination with modern human DNA, such as checking that fragments had the correct flanking sequence and the expected amount of DNA damage for their size. In all, Rubin's team was able to extract 65,000 bases of Neandertal DNA.

**Not quite gone.** Genome data may one day shed light on how Neandertals lived.



Pääbo employed pyrosequencing too, but he used a different method to prepare the DNA. Schuster and Hendrik Poinar of McMaster University in Hamilton, Canada, had successfully used this technique to read an astonishing 13 million bases from a 27,000-year-old mammoth (*Science*, 20 January, p. 392). This procedure avoids using bacteria, which for unknown reasons sometimes fail to incorporate certain stretches of DNA and so may not provide a complete sequence. Instead, Pääbo's team coated tiny beads with Neandertal DNA fragments, one fragment per bead. Then each bead's DNA was amplified, independently, by PCR, and read using pyrosequencing.

Ed Green of Pääbo's lab and his colleagues sequenced 225,000 fragments of DNA, totaling millions of bases. But by comparing the sequences with those in existing databases, they found that "the vast majority [of the DNA]—94%—has nothing to do with the human genome," says Pääbo, and came from sources such as soil microbes. Still, they identified a staggering 1 million bases of Neandertal DNA.

Green kept tabs on contamination in part by comparing stretches of mtDNA that showed up in the sequencing to known modern human and Neandertal mtDNA. They found little modern human mitochondrial sequence and say they are confident their Neandertal DNA is genuine.

Both teams compared the new sequences to the modern human genome and to the chimp genome and tallied the sequence differences between each pair of species. Places where the two human genomes match but the chimp's differs likely mark mutations that resulted in uniquely human changes, perhaps including our upright skeletons, bigger brains, lack of hair, and

so forth. Differences between the two humans are signposts to changes that were key to their individual evolution. Eventually those changes could lead researchers to the genetic basis of *H. sapiens* speciation.

As expected, the Neandertal and human genomes proved more than 99.5% identical. Rubin's team's analysis of 65,000 bases revealed that the two humans shared 502 mutations that were different from chimp bases. And 27 bases varied between modern humans and Neandertals, indicating sites where evolution occurred after the two species diverged. Assuming that chimps and humans split 6.5 million years ago, the most recent common ancestor of the two human species lived 468,000 to 1 million years ago, most likely dating back 700,000 years, Noonan and his colleagues report.

In Green and Pääbo's much larger analysis, 10,167 bases were shared by just the modern human and Neandertal, and 434 were unique to modern humans. Taking a slightly different approach from Rubin, the Leipzig team found a more recent divergence time, about 465,000 to 569,000 years ago. This matches the mtDNA analyses, too, but doesn't quite settle the question. Not everyone agrees with the 6.5-million-year-old divergence date for humans and chimps, and a different date would change the timing of the split between modern humans and Neandertals.

As to the question of admixture, Rubin's group found no sign of it. There were no sites where the Neandertal possessed a rare single nucleotide polymorphism (SNP) found only in Europeans, which one would expect had interbreeding occurred. However, given the size of the study, there's still a chance that such shared SNPs exist but haven't yet been found, Rubin explains. So his study refutes the notion that Neandertals were major contributors to

the modern human genome but can't rule out a modest amount of gene flow.

In contrast, the Leipzig group did find some evidence of hanky-panky between the two humans—although it's far from conclusive. They used the HapMap and another large catalog of modern human variation developed by a private company to guide them to potential SNP sites in the Neandertal. They found that at 30% of those sites, the Neandertal had the same base as living people, but the chimp had a different base. That's too much similarity, given how long ago the two lineages split. "Taken at face value, our data can be explained by gene flow from modern humans into Neandertals," says Pääbo. He thinks there may have been one-sided mating: Modern human males invaded the Neandertal gene pool by sometimes fathering children with Neandertal females, but not necessarily vice versa.

To those who have long argued for Neandertal admixture—and been in the minority—this is vindication. "These comprise some of the strongest genetic evidence of interbreeding with Neandertals that we have yet seen," says Milford Wolpoff, a biological anthropologist at the University of Michigan, Ann Arbor. But Stanford paleoanthropologist Richard Klein disagrees. "I don't think either paper bears much on the issue of admixture," he says. Schuster is even more circumspect: "Both papers are overinterpreting the data."

Rubin hopes that other researchers will do their own analyses on these publicly available data to help clarify the results. But Montgomery Slatkin, a theoretical population geneticist at UC Berkeley, thinks that even with more studies and more sequence, "it will be very difficult to distinguish between a low level of admixture and no admixture at all."

### Concern about contamination

Anxiety about the sequence being wrong fuels this pessimism. Researchers need to be sure that what they called "Neandertal" isn't really "technician" DNA. And contamination is hard to avoid. "Bone acts like a sponge; a drop of sweat on the surface will penetrate very deep," Schuster explains.

With nonhuman ancient DNA, researchers can easily pick out and discard modern sequences, but that's not possible with Neandertal DNA, which is nearly identical to our own, notes paleogeneticist Carles Lalueza-Fox of the University of Barcelona, Spain. He is not convinced that the tests for

**From bones to genomes.** Plates of bacteria can reproduce DNA from bones like this skullcap (inset) from the Neander Valley.





## A Neandertal Legacy?

The perennial question about Neandertal-human relations is, "Did they mate?" (*Science*, 11 February 2005, p. 841). The lack of a strong Neandertal signature in the modern human genome means that such interspecies dalliances were probably rare, but the Neandertal nuclear DNA sequenced to date raises the possibility that interbreeding did happen (see main text). If so, there may be traces of Neandertal genes in living people, especially if the Neandertal variants were favored by natural selection. Now a handful of other studies are finding genes that may fit the bill.

"There is now a relatively long list of candidates" for such adaptive genetic variants, contends anthropologist John Hawks of the University of Wisconsin, Madison. But not all researchers agree. Population geneticist Laurent Excoffier of the University of Bern in Switzerland counters that it's "highly unlikely" there were enough matings between Neandertals and modern humans to have left significant traces in the modern genome.

The most recent candidate was reported last week in the *Proceedings of the National Academy of Sciences* by a team led by geneticist Bruce Lahn of the University of Chicago in Illinois. Lahn's team had earlier claimed that a variant of the brain-related gene *microcephalin* first appeared in modern humans about 37,000 years ago and quickly spread around the world because it was favored by selection (*Science*, 9 September 2005, p. 1662). In the new

work, Lahn estimated that the variant actually arose in hominids more than 1 million years ago, long

**Kissing cousins?**  
Neandertals (*foreground*) may have left some genes behind.

contamination are foolproof. "It might never be possible to determine if the amplified sequence is real or one of the many potential sources of contamination," agrees Shapiro.

All the same, researchers are making some headway. Lalueza-Fox sequenced mtDNA from everyone who had ever touched a Neandertal specimen and compared it to the DNA obtained from the Neandertal. He found that most of the contamination came from the field, not the lab. His solution: Treat the excavation site like a crime scene. Archaeologists in his team now wear face masks, coveralls, and sterile gloves; they use sterile blades and quickly freeze bones destined for DNA sampling. The dress code has reduced human contamination from about 95% to 5%, says Lalueza-Fox.

Even if contamination can be contained, ancient DNA studies must contend with errors. Sequencing itself makes mistakes. And that's where Rubin's bacterial libraries come in handy. With an ever-reproducing source of DNA, his team can sequence the same fragment multiple times and therefore tell right from wrong bases. With

before it appeared in our own lineage. He suggests interbreeding, probably with Neandertals, as a likely explanation. "It seems to be the most compelling case to date for a genetic contribution of Neandertals to modern humans," says Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.

Similar candidates include a gene shown to have conferred a reproductive advantage in living Icelanders, a variant of a gene called *MAPT* implicated in neurological disease. As with *microcephalin*, the *MAPT* variant appeared in modern humans about 30,000 years ago but apparently arose in hominids much earlier and so may have come from Neandertals, according to recent work by John Hardy of the National Institute on Aging in Bethesda, Maryland.

There are several genetic variants whose roots go back as far as 2 million years ago but appeared more recently in modern humans, says geneticist

Michael Hammer of the University of Arizona in Tucson. He says this pattern is best explained by occasional matings among different hominid groups within Africa as well as between African migrants and Eurasian hominids, including possibly *Homo erectus*. Even Chris Stringer of the Natural History Museum in London, who has argued that modern humans migrating from Africa replaced Neandertals with little or no interbreeding, now says that some interspecies matings are "feasible."

Just why genes from Neandertals or other ancient hominids would have benefited modern humans remains a mystery. But if the geneticists are correct, it could mean that before Neandertals went extinct about 30,000 years ago, they left modern humanity with lasting gifts.

—MICHAEL BALTER



Pääbo's method, the sample gets used up.

More problematic are those errors that have arisen from age-related decay. "Many, and perhaps most, observed differences between a Neandertal genome sequence and the human reference will be caused by [ancient] chemical damage to the Neandertal sample," says Webb Miller, a computer scientist at Pennsylvania State University. One way to detect such errors is to sequence and compare several different specimens, because each fossil should have a unique pattern of DNA damage, says Miller.

Here, too, Rubin's methods can help. He envisions several libraries, each from a different Neandertal. Researchers would pull out the same fragment from each library to compare with each other and with living people. A pilot project has already demonstrated probes that ferret out specific target sequences, so the team needn't analyze the billions of bases shared by Neandertals and living humans, or

among different Neandertals. "We will be able to identify and confirm sequence changes in more than one Neandertal without having to sequence several Neandertals to completion," Rubin says. "Seeing the same change in multiple Neandertals will give us confidence that we got [the sequence] right."

Such talk of multiple sequencing has some fossil guardians anxious. "If everybody that wanted a chunk of Neandertal got a chunk of Neandertal, that would put the whole Neandertal fossil record at risk," warns paleo-anthropologist Tim White of UC Berkeley.

At this point, however, even the paleontologists seem eager to see what genomic studies can do. This month, Lalueza-Fox will bring one of his "clean-room excavated" bones to Pääbo to see whether its DNA qualifies for sequencing, and he's thrilled with the potential of sequencing. "For the [150th] Neandertal anniversary, we are moving from paleogenetics to paleogenomics," Lalueza-Fox explains. "It is incredible considering this was impossible just a few years ago."

—ELIZABETH PENNISI