Who Were the Denisovans?

At an unusual meeting at a Siberian cave, researchers find that these mysterious archaic humans lived in the same place as both modern humans and Neandertals—though not necessarily at the same time—and their range probably stretched into east Asia

DENISOVA CAVE, SIBERIA—Bence Viola first saw the ancient molar last summer, just after a piece of it was dug out of layers full of brown dirt, gray rock, animal bones, stone tools, and goat feces. He considered the tooth fragments too big and weirdly shaped to be human. "I thought it must belong to a cave bear," he says.

Several fossils were found that summer in this remote cave in the Altai Mountains. Some, including a toe bone, looked human and were to be sent for DNA analysis to paleogeneticist Svante Pääbo at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Viola, a postdoc at Max Planck, almost didn't include the molar. But he and Pääbo decided to play it safe and test all the new fossils. The layer that held the molar in Denisova Cave was also the resting place of a girl's finger bone, which was so well preserved that Pääbo's lab was able to sequence its nuclear genome and identify it as belonging to a previously unknown type of archaic human. The team called them the Denisovans. For the first time, researchers had a genome in search of a fossil record, so every possible new bone was significant.

Back in Leipzig, graduate student Susanna Sawyer was charged with extracting DNA from the animal bones. In June, she stopped Pääbo in the hall. "I think I found another Denisovan," she said. Preliminary analysis suggested that the molar's DNA was similar to that of the cave girl's. Pääbo shook Sawyer's hand—this was only the third fossil ever found of a Denisovan, the others being the bit of finger bone and another molar, also from Denisova cave.



Cave treasure. Researchers have found the tooth of a Denisovan, plus a sophisticated stone bracelet and tools, in Denisova Cave.

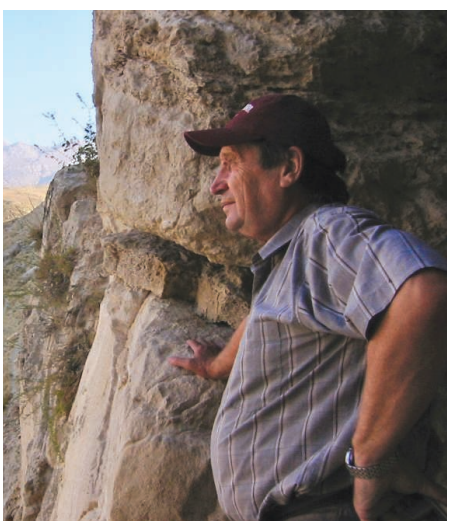
CREDITS (TOP TO BOTTOM): ANN GIBBONS; BENCE VIOLA/MPI EVA; ANN GIBBONS; BENCE VIOLA/MPI EVA

Published by AAAS

Room with a view. Denisova Cave was such prime real estate, it attracted three kinds of humans.

What's more, preliminary analysis of the mitochondrial DNA from the toe bone suggests that it belonged not to a Denisovan but to a Neandertal. That means both types of archaic humans lived in the same cave. And the large, three-room cave also holds sophisticated stone tools and bone artifacts that appear to have been crafted by our own species, *Homo sapiens*. "The one place where we are sure all three human forms have lived at one time or another is here in Denisova Cave," Pääbo said.

Today the cave is off the beaten path, in southern Siberia, 350 kilometers north of the Russian border with both Kazakhstan and Mongolia, and closer to Beijing than Moscow. Now the Denisovan discoveries have shifted the spotlight from ancient humans in Africa, the Middle East, and Europe to those in this remote corner of Asia. As Russian Academy of Sciences (RAS) archaeologist Anatoly Derevianko puts it: "The world is looking eastward."



Meeting of the minds. Archaeologist Anatoly Derevianko (*top*) and paleogeneticist Svante Pääbo worked together to discover the Denisovans.

CREDITS (TOP TO BOTTOM): MAP ADAPTED FROM A. DEREVIANKO AND M. SHUNKOV, *PALEONTOLOGICAL J.* **43**, 8 (2009); BENCE VIOLA/MPI EVA (2)

To that end, Derevianko and his Russian colleagues invited Pääbo and a select group of human origins researchers from different disciplines and countries to a remarkable symposium at an archaeological camp near Denisova Cave in July. Their goal was to try to solve the mystery of the cave girl's identity, to find more of her people, and to explore how the discovery is challenging models of modern human origins. In lively discussions sometimes catalyzed by vodka toasts, they compared what archaeology, genetics, and fossils reveal about the world the Denisovans inhabited 30,000 to 50,000 years ago. Genomic data have already shown that our ancestors mingled with archaic humans, who may have given us valuable immune cell types (see sidebar, p. 1086). But it's not clear when and where this happened.

Invisible human

The gathering gave Derevianko, director of the Institute of Archaeology and Ethnography at the RAS in Novosibirsk, a chance to showcase some of the region's impressive archaeological sites. Driving off dirt roads in troop movers and along rutted roads in indestructible UAZ vans, the Russians took their visitors to a dozen digs. Some were caves at the edge of alpine forests of silver birch and Siberian larch; others were open-air sites in grassy meadows of bee balm, wild mint, and edelweiss.

The trail of ancient humans starts with *H. erectus*, which left primitive "pebble" tools in the Altai almost 800,000 years ago. After a hiatus when the climate was frigid, the descendants of *H. erectus* returned by 300,000 years ago, leaving more tools behind. Some kind of human has lived here ever since.

Starting 80,000 to 70,000 years ago, archaic humans began to use more modern methods to make tools at sites called Kara Bom and Ust-Karakol, where 10% of the tools were blades or burins (a tool used to chisel wood); the Russians see this as the first stirrings of modern human behavior here.

From 50,000 to 30,000 years ago, the archaic people hunted bear, lynx, and wild boar in the Altai Mountains, where they set up seasonal camps in summer, said RAS archaeologist Mikhail Shunkov as he led the tours. They retreated to limestone caves such as Denisova in winter. "With a natural opening for a chimney, the cave was quite a cozy place," Shunkov said, pointing to an opening in the ceiling at Denisova. With a clear view of the Anui River—and any humans or animals passing below—Denisova must have been choice housing, said Pääbo, noting how sunlight streaming through the opening overhead lit the cave like a chapel. "It is kind of cool to imagine that the person whose genome was sequenced had seen these walls," he said.

At about this time, at least two different types or local cultures of artifacts appear, one at Kara Bom and one at Ust-Karakol. The Russians consider both to be sophisticated

Online

sciencemag.org

Podcast interview with author Ann Gibbons.

Published by AAAS

cultures traditionally associated with only *H. sapiens*. Similarly advanced artifacts appear at the same time in Denisova, with stone bladelets used on spears; pendants made of teeth of fox, bison, and deer; and even a bracelet made of a mineral found hundreds of kilometers away. Until recently, the archaeologists had "no doubts that people associated with this industry were anatomically modern," Derevianko says. But now, thanks to the genomic results, it's possible that some were Denisovans, Shunkov says.

To identify the toolmakers, researchers need fossils, but they are few and far between. As a result, "it remains unknown what the Denisovan looked like or how he behaved," says biological anthropologist Maria Mednikova of the RAS in Moscow. So Viola's talk at the meeting, describing the single new tooth, drew intense interest. Like the first molar found, it is very large and lacks specialized features found in Neandertals. Nor does the tooth resemble a modern human molar, as it has many unusual cusps, Viola says. The finger bone fragment that first yielded Denisovan DNA was so small that it yielded little information other than it was a child's because the growth plate was not fused.

In addition to the few Denisovan fossils, Neandertals also left fossils and characteristic Mousterian stone points and scrapers in Denisova and other caves. At the meeting, Russian researchers described new finds of Neandertal tools and fossils in caves just 100 and 150 kilometers away from Denisova Cave, dated to 45,000 years ago. Mednikova adds that the toe bone from Denisova looks most like a Neandertal toe from Iraq, fitting well with the preliminary DNA finding. And yet Derevianko thinks Neandertals didn't stay long here, because their bones and artifacts disappear by 40,000 years ago. He views them as brief visitors, probably coming from the west in Kazakhstan.

Neighbors, or successors?

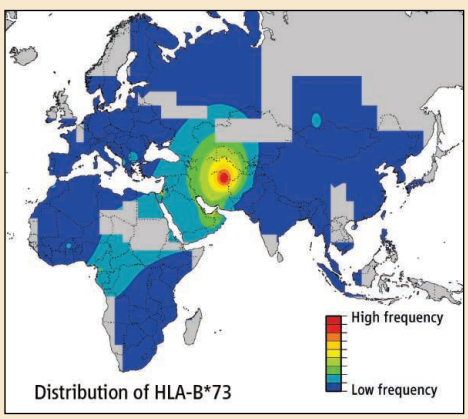
It is now clear that Neandertals, Denisovans, and modern humans once occupied the Altai—but were they all there at the same time? This is hard to answer because there are questions about the dating of crucial layer 11 in Denisova Cave. This meter-thick layer held the Denisovan finger and molars, the Neandertal toe, and the modern human artifacts, although some were found in different galleries of the cave. The bones and teeth are too fragmentary to be dated directly. But radiocarbon dating of seven animal bones with cut marks from layer 11 provides dates of 50,000 years or older in both galleries. Yet the layer's youngest sediments date to as late as 16,000 to 30,000 years ago, as reported in December in *Nature*. Thus layer 11 has artifacts from at least two different periods. And, in the south gallery near the spot where the finger bone was found, an obvious wedge of disturbed sediment suggests some mixing.

For now, Derevianko and colleagues propose sequential occupations: The Denisovans were in the cave about 50,000 years ago, Neandertals came in briefly about

A Denisovan Legacy in the Immune System?

Everybody knows about the dangers of inbreeding (see Hapsburg dynasty, collapse of). In fact the reproductive strategies of many animals are based on avoiding it, as when female chimpanzees move out of their birth groups to mate. Last year, researchers showed that human ancestors took that strategy to its limits by breeding with the now-extinct Neandertals and Denisovans (*Science*, 28 January, p. 392). Now a study published online in *Science* this week (http://scim.ag/Abi-Rached) suggests that such mating was beneficial, boosting the immune systems of early Europeans and Asians and leaving a valuable legacy in the genes of many people alive today. "This is the first suggestion that something that came from archaic hominins into modern humans conferred an advantage," says paleogeneticist Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.

Genomic data from fossils thus far suggest that living people carry only small amounts of archaic DNA. Only 2% to 7% of the DNA of today's Europeans and Asians apparently came from the ancient Denisovans and Neandertals (see main text). The new paper examines Europeans and Asians and finds that archaic people contributed more than half of the alleles that code for proteins made by the human leukocyte antigen system (HLA), which helps the immune system recognize pathogens. "Archaic alleles have significantly shaped modern human immune systems," wrote Peter Parham and Laurent Abi-Rached of Stanford University in Palo Alto, California.



Ancient roots. The allele HLA-B*73, today mostly seen in west Asia, may come from Denisovans.

Immunogeneticist Parham has spent 16 years puzzling over the evolution of one rare HLA allele, called HLA-B*73. This variant is quite different from others but is similar to alleles in the same position in the genomes of chimps and gorillas. So it seems to be ancient, perhaps arising long before our ancestors split from gorillas about 16 million years ago. Yet today, B*73 is concentrated in western Asia, where modern humans have lived for less than 90,000 years, and it is absent from African tribes who usually carry the most ancient gene lineages.

While studying this allele, Parham's team got a big break last year when Pääbo's team published the complete genome of the Denisovan cave girl. She didn't carry B*73—and it hasn't been found in Siberia—but she carried two other linked HLA-C variants, which occur on the same stretch of chromosome 6. If living people have any of these variants, they almost always carry at least two of the three variants—as did the cave girl. So even though she lacked B*73, the researchers propose that all three variants were inherited, often in pairs, from archaic humans in Asia. The Denisovans are the prime suspects, given their presumed distribution in Asia.

The team also examined other HLA alleles in three Neandertals and one Denisovan and found several other ancient variants that today show up in living Asians or Europeans. Parham thinks these variants were beneficial and so, once acquired from archaic people, spread rapidly in small but expanding modern populations. "The fact [that these genes] may have been parachuted into modern humans is an attractive interpretation," says immunologist John Trowsdale of the University of Cambridge in the United Kingdom.

However, others are not quite convinced that the alleles came from archaic humans. Parham's team hasn't completely ruled out other explanations for the gene distributions, such as certain types of selection, says geneticist David Reich of Harvard University. Regardless, he says, "I am happy to see people using archaic genomes for different kinds of analyses." **–A.G.**

On tour. Archaeologist Mikhail Shunkov showcased the many archaeological sites of the Altai Mountains.

45,000 years ago, and modern humans followed. But the researchers agree that the microstratigraphy of the cave needs more analysis. They are redating layer 11 with radiocarbon on more cut-marked animal bones.

Overall, Derevianko and his colleagues see a gradual, local evolution of *H. erectus* into *H. sapiens* in the Altai, with a brief intrusion of Neandertals and Denisovans. This fits a minority view of human origins, called multiregionalism, which posits that the descendants of *H. erectus* evolved into Neandertals and modern humans—and, apparently, Denisovans—in different regions. Then humans coming out of Africa mingled with the other groups and *H. sapiens* emerged worldwide.

As Russian and Chinese archaeologists raised their glasses to toast regional continuity, however, several geneticists shifted uncomfortably or even quietly demurred: That theory is in contrast to the long-prevailing view that *H. sapiens* was born in Africa and swept the globe, wiping out local archaic peoples. And in light of the genomic data, most geneticists now hold a middle-of-the-road view that modern humans arose in and spread out of Africa, then interbred with local archaic peoples to a limited degree (*Science*, 28 January, p. 392). "If you write that I drank a toast to [regional] continuity, I'll kill you," one geneticist told a reporter.

But the geneticists do agree with the Russians that modern humans mingled with both Neandertals and Denisovans. Pääbo's team found in 2010 that living Europeans and Asians have inherited about 2.5% of their DNA from Neandertals (*Science*, 7 May 2010, pp. 680 and 710) and that living Melanesians carry an additional 5% of Denisovan DNA.

If modern humans interbred with Neandertals, researchers speculated that fossils of each group, about the same age and found close to each other in Israeli caves, represented the groups who mixed sometime before 90,000 years ago. Those modern people carrying a small amount of Neandertal DNA then split into at least two groups—one that headed into Europe to replace the Neandertals there, and a second group that headed into Asia to mix with the Denisovans, says population geneticist David Reich of Harvard Medical School in Boston.

At the meeting, the DNA researchers offered some new insights into this story. They found that the three Denisovans, all from one cave, had more variation in their mtDNA than did seven Neandertals from western Europe to Siberia, Sawyer reported. This and another report at the meeting—that Australian Aborigines, like Melanesians, have inherited 5% of their DNA from Denisovans—suggests that the Denisovan home range once stretched far beyond the Altai, into eastern Asia. "This tells us that the Denisovans had large population sizes," despite their puny fossil record, Pääbo says. It also shows that Denisovans and the ancestors of Melanesians must have interbred before 40,000 to 60,000 years ago, when Aborigines first settled Australia.

As for the timing of the Neandertal-human mixing, the newest analyses tend to push that younger. Population geneticist Montgomery Slatkin of the University of California, Berkeley, said that his model runs gave him a wide range of preliminary results, from 65,000 years to 45,000 years ago, but he's still working the numbers. Reich reported that his independent analyses also suggest a younger date. If the mixing happened more recently than 90,000 years ago, it rules out the Israeli fossils as representatives of the groups who mixed.

Others, such as Derevianko and paleoanthropologist John Hawks of the University of Wisconsin, Madison, interpret the genetic data differently. They think that even small amounts of interbreeding confirm the regional continuity model, and that there was more mixing in the past, but its traces were erased by later waves of immigrants who swamped out the archaic genes.

To help decide among these models, several groups are searching for Denisovans beyond Denisova, as far east as China, where Pääbo is now analyzing fossil DNA. As Pääbo climbed down a ladder into a floodlit pit at Denisova and bent his lanky frame low to get a good look at layer 11, a colleague shouted: "Grab a trowel, Svante." Pääbo didn't. But like the others, he is convinced that all types of data—genetic, archaeological, and fossil—will have to be integrated in order to tell the story of the Denisovans and so of our own species. "We're beginning to clarify history in eastern Eurasia," Pääbo said, "and I'm sure that in the next few years, there will be more discoveries."

–ANN GIBBONS

Teamwork. Anthropologist Maria Mednikova (*top*) analyzed fossils, and geneticists Susanna Sawyer and David Reich studied the DNA of the ancient Denisovans.

CREDITS (TOP TO BOTTOM): ANN GIBBONS; MARIA DOBROVOLSKAYA; ANN GIBBONS

Published by AAAS