



Archaeologists David Anthony and Dorcas Brown measure a skeleton from the Russian steppe whose 5000-year-old DNA revealed close ties with Europeans.

REVOLUTION IN HUMAN EVOLUTION

As it smashes disciplinary boundaries, ancient DNA is rewriting much of human prehistory

By Ann Gibbons

At 6:30 p.m. on a blustery March evening in Boston, most of the researchers on the second floor of Harvard Medical School's New Research Building were at a beer hour, but David Reich's lab was still bustling. Molecular biologist Nadin Rohland was working late, trying to finish sequencing 390,000 bases of DNA from each of 92 Bronze Age human bones. The samples had been streaming in from archaeologists, and she was hustling to keep up. "We're really busy," Rohland said as she pre-

pared equipment for the next day's round of sequencing.

Next door, postdocs from three countries sat at their large computer screens, trying to figure out how to analyze sequences faster. "Exactly a year ago, we had DNA from one farmer from Germany, one farmer from Luxembourg, and two or three hunter-gatherers," said Iosif Lazaridis from Greece. "Suddenly we had 50. Now it's in the 100s—I don't know if we've crossed the thousands." Pontus Skoglund, from Sweden, joked: "There's too much data!"

Just 5 years ago, extracting and decipher-

ing a single fossil's genome—and making sure the result was not muddled by contamination with modern DNA—was a titanic effort. Now, thanks to technological breakthroughs that have vastly accelerated sequencing and made the results more trustworthy, DNA researchers the world over are awash in data (see p. 359). The result is a series of revelations about humanity's past. Ancient DNA has led to the discovery of new types of ancient humans and revealed interbreeding between our ancestors and our archaic cousins, which left a genetic legacy that shapes our health and appear-

ance today. And because investigators can now sequence entire ancient populations, as Reich's lab is doing, ancient DNA is adding layers of complexity to the story of how ancient populations migrated and mixed across the globe. "The whole field is exploding in terms of its impact," says Christina Warinner of the University of Oklahoma, Norman. "The data that's coming out is completely rewriting what we know about human prehistory."

The findings are forcing a shotgun marriage between ancient DNA specialists and other researchers trying to unravel the past, including anthropologists, archaeologists, and population geneticists. For them, the technique poses unsettling challenges as well as opportunities. Ancient DNA has contradicted prevailing views—that the invention of farming reflected the spread of ideas rather than people, for example. But it is also enabling these scientists to answer questions they could not previously address, and many are now seeking collaborations with ancient DNA researchers. "Before 2010 I didn't know anything about DNA," says archaeologist David Anthony of Hartwick College in Oneonta, New York, who provided bone samples of ancient herders from Russia to Reich's lab. "I've had to ramp up my knowledge—ancient DNA is becoming a tool for archaeology almost like radiocarbon dating."

Such collaborations aren't always easy. Despite their interest, archaeologists, for example, are still outsiders to the world of DNA, says archaeogeneticist Johannes Krause of the Max Planck Institute for the Science of Human History in Jena, Germany. "Archaeologists can't analyze that kind of data," he says. "And they aren't completely in charge anymore." But they can't ignore the burgeoning new field. "The new data can rewrite history."

WHEN PALEOANTHROPOLOGIST Chris Stringer was a 22-year-old grad student in the early 1970s, he took his calipers to museums around Europe, applying a new, systematic measurement procedure to all the skulls of Neandertals and modern humans he could get his hands on. Traveling in a battered Morris Minor, with long hair, barely enough cash to stay in youth hostels, and a modern human skull as passenger, he some-

times aroused suspicion. Some researchers at first refused access to fossils, and the Czech police searched his belongings, saying that his visit was of "no value to the people of Czechoslovakia." In Rome, thieves stole the modern human skull.

But Stringer persevered, carefully measuring the rare bones. He noted that Neandertals had long, low skulls with pronounced brow ridges and projecting midfaces, whereas the younger modern humans had globular skulls and flat faces. He

of contamination—Stringer, for one, had left a trail of his own DNA on fossils across Europe. But Pääbo and his colleagues ultimately managed to create a composite genome from three female Neandertals and compare it with modern human DNA. The team found that Neandertals did indeed have a genome distinct from our own—but that living Europeans and Asians had inherited 1% to 3% of their DNA from Neandertals (*Science*, 7 May 2010, p. 680).

That genetic legacy meant that Stringer's view of the fossils was incomplete: Neandertals had interbred with modern humans at least once. More recent ancient DNA analyses suggest that such interbreeding happened at least three times, probably 37,000 to 85,000 years ago in the Middle East and Europe (*Science*, 22 May, p. 847). One modern human who lived in Romania roughly 40,000 years ago even had a great-great-great-grandparent who was a Neandertal, Pääbo's team reported in June.

The same year Pääbo's team published the Neandertal genome, it sequenced a sliver of a fossil pinky bone from Denisova Cave in Siberia and found genetic evidence of a new kind of human, related but not identical to Neandertals. The team called them Denisovans and found that they, too, had mixed with modern humans so that Melanesians carry up to 5% Denisovan DNA (*Science*, 26 August 2011, p. 1084).

Although ancient DNA proved Stringer wrong about interbreeding among our ancestors, he embraces the new results. As anthropologist Marcia Ponce de León of the University of Zurich

in Switzerland puts it, "ancient DNA answers questions that morphology alone cannot answer conclusively—and raises questions that have never been asked before."

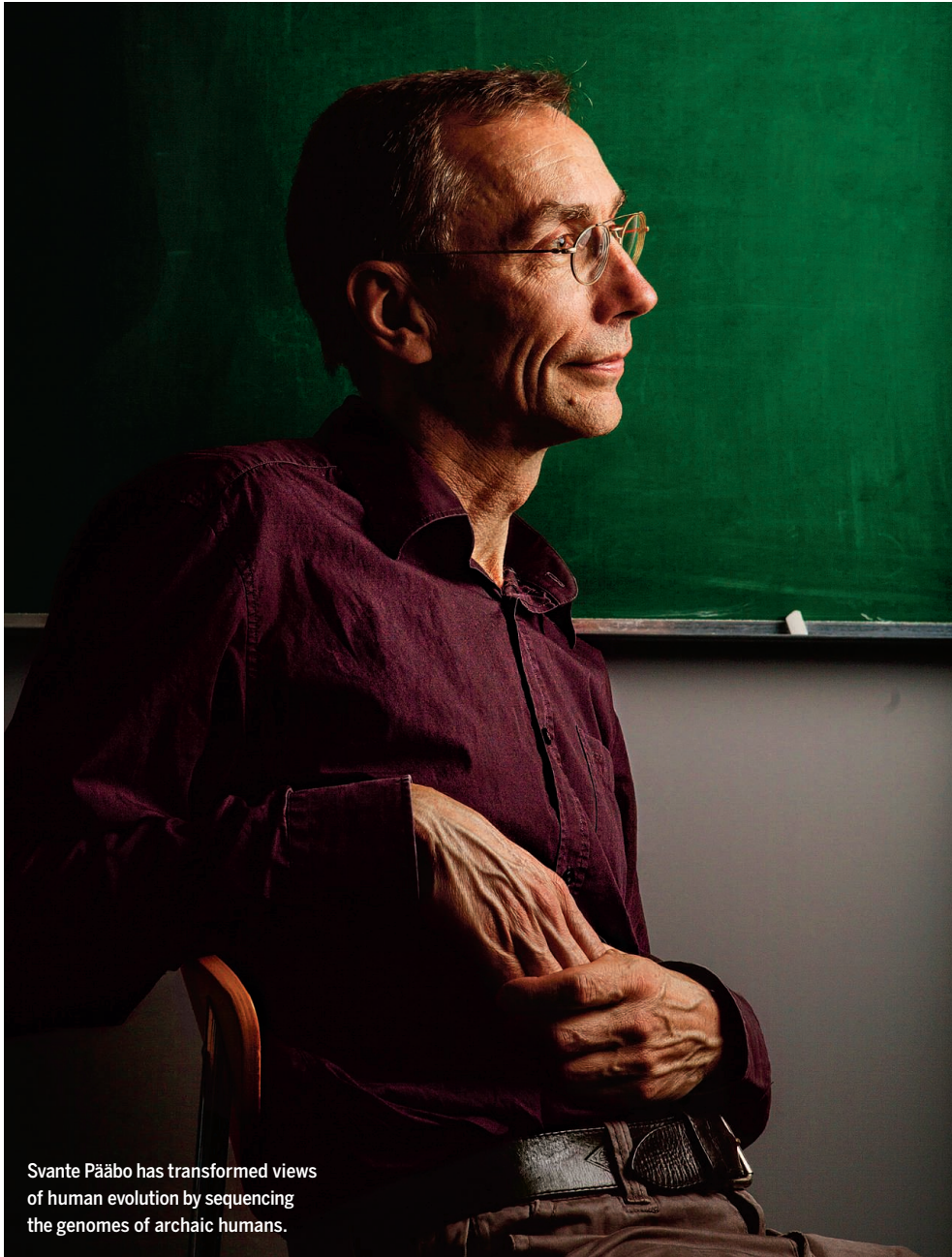
Ponce de León, who specializes in state-of-the-art morphological analyses, notes that that lesson emerged yet again earlier this year, when she and others reanalyzed the skull and sequenced the genome of Kennewick Man, an 8500-year-old skeleton found on the shore of the Columbia River in Washington state. Some scientists had argued that Kennewick's long, low skull resembled those of Polynesians or the Ainu people of Japan rather than the broader, rounder skulls of today's Native Americans. They suggested that Kennewick Man might



Excavations of a Bronze Age settlement in Russia's Samara Valley unearthed dozens of skeletons, including an adult male (above). Their DNA is helping reveal the origins of Europeans and Asians.

concluded that Neandertals in Europe were not the ancestors of modern humans there, as was then widely believed, but a separate species that had been completely replaced by modern humans. That put him at odds with researchers who focused on different anatomical traits and thought that Neandertals had interbred with our ancestors, and even were members of our own species, *Homo sapiens*.

The infighting went on for years. Meanwhile, paleogeneticist Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, took a different, risky route to answering the question: He studied DNA from Neandertal fossils. First, he had to overcome the problems



Svante Pääbo has transformed views of human evolution by sequencing the genomes of archaic humans.

have been part of an early wave of migration to the Americas and only distantly related to today's Native Americans; their anatomical research influenced court decisions preventing the reburial of the bones according to Native American customs.

Ponce de León and others confirmed the anatomical differences seen in Kennewick Man's skull. But in the same study, evolutionary biologist Eske Willerslev of the University of Copenhagen and others found that Kennewick Man's genome shows that he was closely related to Native Americans, including at least one of the five tribes that originally fought to rebury him. "Ancient DNA analyses provide thousands of independent, often neutrally evolving, features per individual," Ponce de León says. "In morphology, we typically have comparatively few features, which are highly interdependent and only partially reflect the genome."

All the same, Stringer cautions, "it's not time to throw away the artifacts and fossils." They yield insight into ancient people's activities that genes just can't provide. "No amount of ancient DNA would tell us whether the Neandertals buried their dead, or whether the ancestors of Australasians used boats to reach New Guinea and Australia," he says.

WHEN POPULATION GENETICIST Joshua Akey was a graduate student in the 1990s, his fieldwork involved sitting in front of a computer and downloading data on the genes of living people. He was scanning for genes that had been targets of natural selection, and he succeeded: He and his colleagues analyzed variation in the genomes of the 270 people collected by the International HapMap Project and spotted 174 genes, including two linked to cystic fibrosis and

diabetes, that apparently had been shaped by natural selection. "I remember thinking how cool it was to be able to use patterns of variation in contemporary individuals to learn about what influenced our ancestors' ability to survive and reproduce," says Akey, now at the University of Washington, Seattle.

He recalls, though, that "all the data could fit in an Excel spreadsheet." That's because living people preserve only a fraction of the genetic diversity of ancient ones. "Population geneticists were trapped in time—they could only look at what was here today," Skoglund says. Now he, Akey, and others can look deep into the past by analyzing the genetic makeup of people who lived long ago. "It seems like science fiction to be able to generate large amounts of sequence data from individuals who lived 30,000, 40,000, 50,000 years ago," Akey says.

As soon as these investigators post sequences from ancient people into public databases, the data feed whole schools of evolutionary researchers downstream, who fish for signs of evolution and adaptation in our genomes. Population geneticists who have never measured a fossil and computational biologists who have never worked in a clean room now sit at their computers unraveling the complex genetics of what made us modern.

Today, Akey continues to seek genes that were favored or weeded out by natural selection. But now he's on the alert for something that hadn't been on his radar before:

genes that our ancestors lifted from archaic humans. Adaptation is usually a slow process, as beneficial mutations often require hundreds or thousands of generations to spread through a population. But the Neandertal and Denisovan genomes have shown that in some cases our modern human ancestors were able to take an evolutionary shortcut: As they spread around the globe, they met other kinds of humans who were already adapted to the local environment. By breeding with them, our ancestors were able to snag beneficial genes.

"We're figuring out how interactions with Neandertals and Denisovans helped our ancestors survive," Akey says. Such "adaptive introgression" has been well documented in plants and bacteria. Its importance in human evolution was highlighted last year, when researchers discovered that Tibetan highlanders had inherited a "superathlete"

gene variant called *EPAS1* from Denisovans. This ancient variant, which helps Tibetans use oxygen more efficiently, was found in Denisovans but not in Neandertals or other people around the world, according to work by population geneticist Rasmus Nielsen of the University of California (UC), Berkeley, and his Chinese collaborators.

Other archaic genes helped our ancestors resist disease. “When modern humans started dispersing around the globe, they encountered unique pathogens that archaic humans were better adapted to,” Akey says. Luckily for modern humans, they picked up some immune genes from Neandertals, such as a version of *STAT2*, a gene involved in the interferon response that fights viral infections; moderns also acquired different types of human leukocyte antigen genes, which help the immune system detect foreign invaders. Researchers are now trying to figure out just how these archaic gene variants change immune function, but the effect must have

been beneficial: The DNA record shows that these genes spread rapidly through Europeans and Asians.

Neandertals, whose ancestors had at least 200,000 years to adapt to Europe’s gray skies and frigid winters, also bequeathed some skin genes to the modern humans they encountered, including a gene called *BNC2*, which is associated with light skin in Europeans and allows skin to synthesize more vitamin D. (Many of today’s Africans, whose ancestors didn’t mingle with Neandertals, do not carry these gene variants.) Akey’s team also found that Neandertals contributed other genes that protect skin against abrasion



A sliver of pinky bone from Denisova Cave generated a high-quality genome.

or water loss (*Science*, 28 February 2014, p. 1017). These chunks of archaic DNA have provided “a rich reservoir” of genes that have allowed Europeans and Asians (including the ancestors of Native Americans) to adapt rapidly to various environmental conditions, according to a June report in *Nature Reviews Genetics* by Nielsen and Fernando Racimo of UC Berkeley.

Not all such archaic genes are beneficial: Mayas in Mexico, some Native Americans, and about 25% of Asians retain an allele from Neandertals that boosts their risk for diabetes. The gene variant plays a role in the breakdown of fats and may have been beneficial when diets were lean and our ancestors needed to store fat efficiently. Also, several independent studies have noted that long stretches of the modern genome are archaic “deserts” lacking any Neandertal or Denisovan signal. Researchers suspect that natural selection weeded out deleterious archaic genes in these regions, and the DNA here may be what distinguishes us from those archaic people. “This has the potential to contribute to our understanding of what makes modern humans modern,” Akey says.

To date, Pääbo has assembled a catalog of about 31,000 base-pair changes, or single nucleotide polymorphisms (SNPs), in which modern humans carry a different version from Neandertals and Denisovans. Several teams are doing lab work in stem cells and mice to try to figure out what some of these genes do (*Science*, 3 July 2015, p. 21).

The revolution in ancient studies has brought Akey full circle—back to scanning data from living humans: He is now col-

The most wanted genomes

Genomes from ancient humans in these artists’ reconstructions could reveal much about our evolution but remain just out of reach—for now



Homo floresiensis, aka “the hobbit”

Found in Liang Bua cave on the Indonesian island of Flores in 1998, this hominin was just over 1 meter tall but has sparked an outsize controversy. Many think the bones represent an amazingly tiny species of ancient human, but a few researchers say the hobbit, who lived as recently as 18,000 years ago, is a diseased member of our own species. DNA sequencing could put that argument to rest, and reveal any kinship with other archaic humans. But independent efforts have so far failed to retrieve any genetic material from the hobbit bones.



Homo heidelbergensis

In the 1990s, archaeologists found the remains of about 32 archaic people with large brows and short, compact bodies in a cave in northern Spain. Many researchers thought that the 440,000-year-old fossils were ancestral to the Neandertals. But in 2013, researchers at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, managed to sequence the mitochondrial genome from a *H. heidelbergensis* legbone, and found it looked more like that of another ancient group, the Denisovans. Max Planck researchers are now trying to sequence its nuclear genome to check this puzzling result.

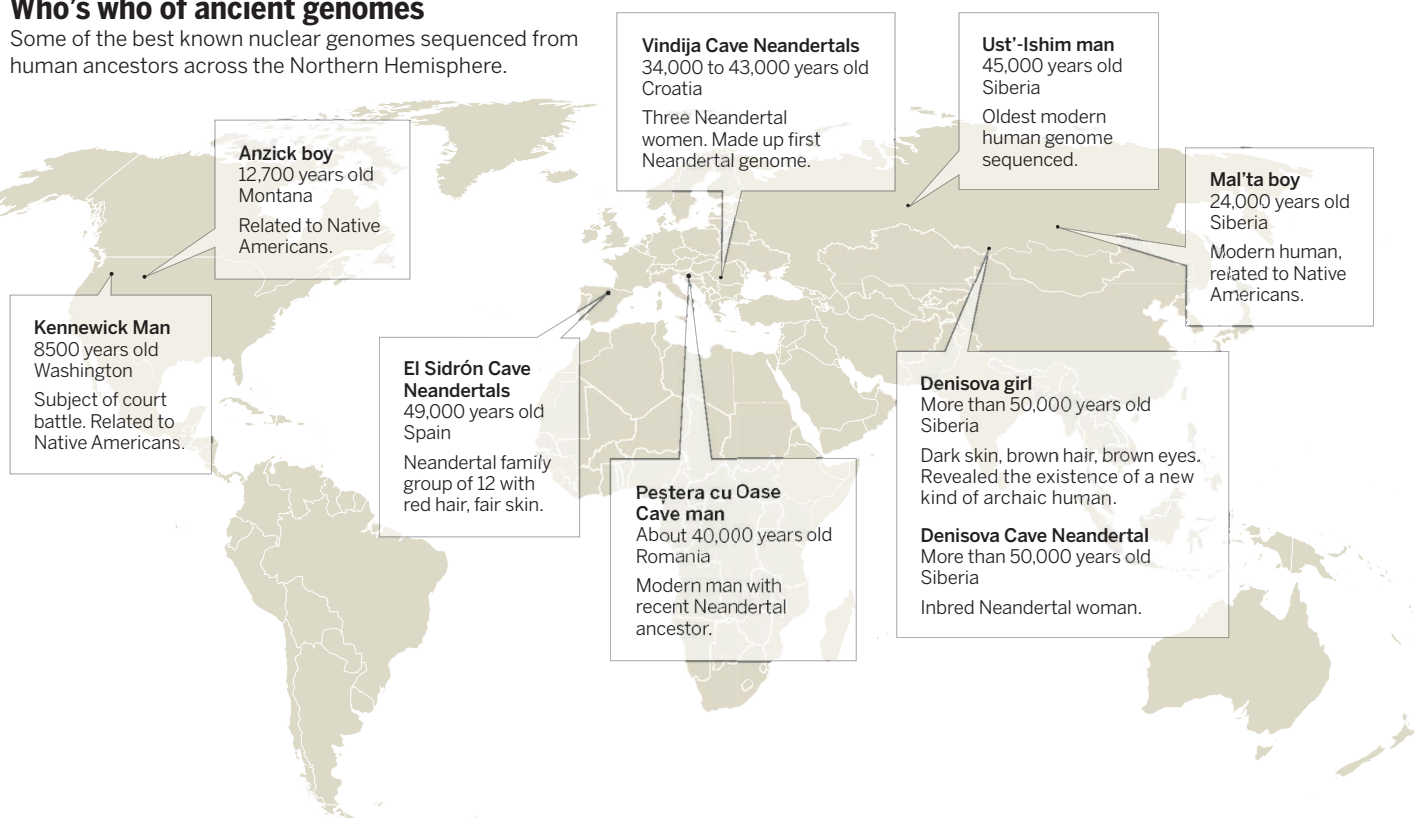


Homo erectus

This iconic human ancestor, the first with a notably enlarged brain, lived 1.8 million to 1 million years ago, so sequencing its DNA seems a bit like science fiction. But researchers are eager to do so, because it is thought to be ancestral to later hominids, including Neandertals, Denisovans, and modern humans. Comparing *H. erectus* with its descendants could show what these three types of humans once had in common, how they have diverged, and the stepwise changes that led to *H. sapiens*. To glimpse *H. erectus*’s genome, researchers are now analyzing long stretches of very old DNA in its descendants.

Who's who of ancient genomes

Some of the best known nuclear genomes sequenced from human ancestors across the Northern Hemisphere.



laborating with researchers worldwide to see whether archaic versions of genes boost the risk of schizophrenia, diabetes, and autoimmune disorders. For better or worse, “we’re all amalgamations of the past, with little bits and pieces of DNA that originated all over the world and, in some cases, from different species,” Akey says.

FOR ARCHAEOLOGIST ANTHONY, in contrast, ancient DNA is a tool for unveiling ancient populations—in particular, a mysterious group of tall young herders he has excavated from beneath earth mounds in the Samara Valley of Russia. He and Dorcas Brown, his wife and research partner, used all the tricks of their trade to probe how these elite members of the Yamnaya herding culture lived and died about 5000 years ago. With their Russian collaborators, they measured bones, analyzed isotopes, and examined their grave goods. Finally, the pair sealed bone fragments of the Yamnaya into plastic bags and stored them on a shelf.

But Anthony was haunted by all he still didn’t know about the nomads who moved across the northern steppe on horseback. “I wanted to know their eye color, skin color, hair color,” he says. “And were the people buried in these elite graves related to each other?”

So when he got a phone call in 2013 from a colleague of Reich’s who was seeking

bones for DNA analysis, he agreed to let the researchers grind up small samples of the Yamnaya’s limb bones for sequencing. To quickly probe their population history and appearance, Reich’s team sequenced not the full genomes but a set of 390,000 key SNPs from each of 69 ancient Europeans and Asians, including nine Yamnaya. The results, published in February, showed that the Yamnaya were the source of a massive migration of herders who swept into the heartland of Europe on horseback about 5000 years ago—and that most Europeans can trace at least some of their ancestry to this group. Anthony got some of his questions answered: The Yamnaya had brown eyes, brown hair, and light skin. And the people in the elite graves were members of the same clan, showing that family ties influenced status. “One of the wonderful things about ancient DNA is that it gives these old samples new life,” he says.

Sometimes, though, the results can be hard for archaeologists to handle. “When we circulated the final version of our paper, the European archaeologists who had given us samples were distressed,” Reich says. They were startled when the genetic data showed the Yamnaya from the Russian steppes were the ancestors of the Corded Ware people in Germany, because it seemed to echo an erroneous idea about Aryan culture propagated by the Nazis. But once archaeologists realized

that the genetic evidence was “unambiguous,” they added cautionary notes and signed onto the paper, Reich says.

DNA repeatedly shows that people who live in a place today rarely are related to those who lived there thousands of years earlier. “People in every inhabited continent 10,000 years ago looked different to people in these same regions today,” Stringer says. For example, when Willerslev’s team sequenced the genome of a 24,000-year-old Siberian boy from Mal’ta in 2013, it found no genetic connection to anyone living in Central Asia today. But the Mal’ta boy was related to Kennewick Man and Native Americans, suggesting that he represented an ancient source population for migrations of Paleoindians to the Americas.

Anthony hopes ancient DNA will help him continue to learn about the Yamnaya. Could these elite herders drink alcohol? Did their dogs travel with them? To answer such questions, ancient DNA researchers need data from many more archaeological sites. So at the last annual meeting of the Society for American Archaeology, Anthony could be found recruiting samples for Reich’s lab. There are “samples lying all over Europe and North America, in labs sitting in the dark,” he says.

Most archaeologists, he says, are happy to be asked. “People are excited that their samples could be a source of ancient DNA.” ■