

ANCIENT DNA

The Trials and Tribulations of Cracking the Prehistoric Code

OXFORD, U.K.—Proponents of the burgeoning field of ancient DNA analysis, who gathered here last month for Ancient DNA III, heard much about attempts to make their discipline more scientifically rigorous. Some, however, were not too timid to use ancient DNA studies to help tackle two big issues: the origins of Europeans and native Americans.

The Trouble With DNA . . .

Soon after the polymerase chain reaction (PCR) made it possible to amplify tiny samples of DNA, geneticists began reaching back in time. Since the late 1980s, spectacular reports of DNA tens of millions of years old have hit the headlines. But the hype—and the embarrassment when some claims did not hold up—is causing ancient DNA researchers to fear that their field won't be taken seriously. That concern sparked a lot of discussion at the Oxford meeting of ways to add more rigor to ancient DNA studies—and when to treat claims with caution. "The field has established itself and shown that you can go back in time and gather data on molecular evolution," says Munich University's Svante Pääbo. "But in other areas, the field is in trouble."

One of the key questions researchers are still grappling with is just how long DNA can survive. Biochemist Thomas Lindahl of Britain's Imperial Cancer Research Fund at South Mimms has studied the main mechanisms that degrade DNA—hydrolysis and the loss of purines, one of the chemical building blocks of the molecule. He has found that, by the standards of most biological molecules, DNA can be remarkably long-lived. "Results of ancient DNA up to 50,000 to 100,000 years old are credible," he says, adding that beyond this time it is much harder to envision how the molecule could be protected from decay, as even encased in materials such as amber, oxidative damage would be expected. Lindahl believes researchers claiming to have older DNA should explain how it was preserved.

These concerns have not stopped researchers attempting to extract samples of "super-old" DNA. So far, three types of super-old DNA have been reported: compression fossils of leaves from Idaho, dated at around 17 million years; insects encased in amber, such as those found in the Dominican Republic, dated at 25 million to 30 million years, and a weevil from Lebanese amber dated at up to 135 million years; and dinosaur DNA at more than 65 million years.

The results of such studies are hotly contested, and some experts in the field are arguing that rigorous procedures are needed to

verify new claims. "If the field is to become fully respectable it is vital to ensure the reproducibility of results," Pääbo says. He suggests researchers set up formal partnerships between laboratories to independently analyze each ancient DNA sample using standard methods. "If results cannot be verified, the authors of any publication should state that," he says.

A fresh analysis of super-old DNA samples reported at the meeting indicates the need for such verification. Jeremy Austin, a zoologist at the Natural History Museum in London, has analyzed DNA extracted from two 25-million-year-old Dominican bees trapped in amber, together with two bees in copal, a younger subfossil form of amber from East Africa, dated at about 2000 years—well within the known life-span of DNA.

Austin used genetic probes to examine a well-characterized gene present in several different species, and then he applied four main controls to check that the DNA he had amplified using PCR was really ancient bee DNA. He tested the materials surrounding the samples and the PCR reagents themselves for the presence of DNA. He analyzed the size range of fragments amplified by PCR, because contamination can distort the expected range. Finally, he analyzed the amplified DNA sequences to see if they belonged to another known species. The result: "All four samples failed on at least one of these criteria," says Austin. "Of the DNA sequences obtained, two were from fungi, one was from humans, and the last one was unknown, but showed no resemblance to insect species."

In the face of such difficulties, researchers are looking for reliable ways to identify which sources of ancient DNA may be worth sampling. One such test, developed by Hendrik Poinar of Munich University, involves analyzing protein material associated

with a source of ancient DNA. The test looks at aspartic acid in the protein, in particular at the ratio of the natural *l*-form of aspartic acid to the *d*-form. Conversion from *l*-form to *d*-form through hydrolysis is known to correlate with the loss of purines from DNA. "A high ratio between the *d* and *l* forms of aspartic acid indicates that DNA in the same sample is likely to be degraded and not worth analysis," says Pääbo. "It's a much less laborious test than looking directly at DNA," he adds.

In spite of the growing awareness of the difficulties facing the field, researchers remain intrigued by its potential. In the increasingly popular area of ancient human DNA, for example, anthropologists are hoping that genetic analysis of human remains may help in understanding the origin and dispersal of people around the world over the past 30,000 years. Indeed, evidence from ancient DNA is already proving an attractive adjunct to large-scale population studies of modern DNA. But in these still early days, the new data are throwing up as many questions as answers.

One Wave of American Migration?

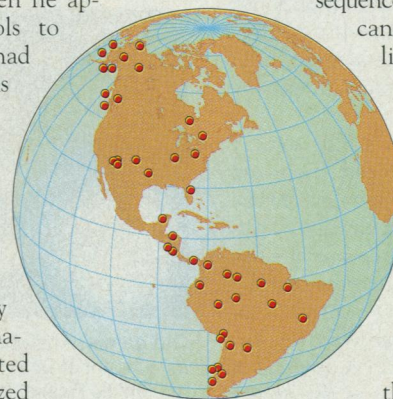
One researcher using ancient DNA analyses to assist in a study of modern DNA is Andrew Merriwether of the University of Pittsburgh. Merriwether is leading a team carrying out the largest survey yet of mitochondrial DNA sequences from modern native Americans.

His aim is to shed some light on the source and date of the original Asian migrations into the Americas. So far, his results, supported by some ancient DNA studies, have proved very contentious, because they do not agree with models derived from other methods, such as language and dental morphology.

The most widely held theory is that the first Americans crossed the Bering Strait from Asia within the last 30,000 years in three distinct waves of migration: First, the Amerind people arrived about 30,000 years ago,

followed by the NaDene, 20,000 years later, and finally the Eskimo-Aleuts, who arrived in northern Canada and Alaska within the last 5000 to 7000 years.

Some studies of DNA from the mitochondria of native Americans have supported this theory. They found that all native Americans belong to just four distinct lineages, labeled A to D. In comparison, Asian populations have many more lineages, which



New wave. Genetic study of Native Americans from sites around the Americas is challenging theory that migration occurred in three waves.

suggests that native Americans were derived from migrants who carried only a small sample of Asian mitochondrial types. More importantly, researchers found that the Amerinds have all four lineages, the NaDene have only A, and the Eskimos have A and D. This suggests there were different migrations at different times.

But more recent studies suggest there may have been more founding lineages in the New World. Merriwether's study, presented at the Oxford meeting, is based on an analysis of mitochondrial DNA from 1300 native Americans from more than 40 populations across the whole of the continent. Merriwether's team also extracted ancient DNA from bone fragments and mummified tissue remains from 300 individuals at three main burial sites: the Ohio River Cultures in the United States, dated to A.D. 1300; Mayan sites in Central America, dated at A.D. 1250; and Azapa Valley in South America, dated between 5000 B.C. and A.D. 500.

Using an additional genetic probe that can detect differences in the mitochondrial genome at a different site, Merriwether was able to identify not only the traditional four lineages, but to divide them into nine distinct genetic subtypes. And most of the lineages, and indeed many of the subtypes, showed up all over the linguistic map, not segregated at all into the three classic migrations. "The key finding was that many of the populations have all four lineages and a number even have all the subtypes. And all types can be found in North, Central, and South America," he says. "It is not realistic to believe that the same lineages ended up in all these populations across two continents by separate migrations."

The ancient DNA studies also suggest that the lineages were widespread among old populations. "Preliminary evidence from the ancient samples suggests that lineage A was present in humans at all three burial sites, and lineages C and D were also present at the Maya sites," Merriwether says. Anthropologist Ann Stone of Pennsylvania State University has also carried out DNA analysis of human remains at a burial site of the Upper Mississippi Culture, dated at A.D. 1300. She has successfully extracted DNA from rib bones of 50 individuals, and preliminary analysis of mitochondrial DNA suggests that all four lineages were present at this site.

Merriwether argues that the distribution of the different mitochondrial types within the modern native American populations in both North and South America is better explained by a single wave of migration. Rosalind Harding, a population geneticist at the Institute of Molecular Medicine in Oxford, agrees: "The more you look at the data, the more it looks as if the migrations didn't happen in jumps."

Not everyone is convinced, however.

Antonio Torroni of the University of Rome, one of the researchers who identified the four mitochondrial DNA lineages that supported the three-wave theory, believes that Merriwether's genetic probe may not provide a reliable indication of the origin of mitochondrial lineages. Some of the sites studied to assign lineages may have undergone more recent mutations, Torroni argues. "Fuller analysis of the mitochondrial genome is needed to determine whether the new subtypes really represent lineages distinct from the four main types," he says.

Merriwether agrees that there are still important questions to resolve concerning the timings, sizes, and routes of migration into the New World, adding that "ancient DNA is emerging as the best candidate to choose between the different scenarios."

When Did Modern Europeans Arrive?

Ancient DNA is also being used to support another controversial study in the battleground of population genetics: a new analysis of how and when modern Europeans migrated into the continent from the Middle East. Modern humans are thought to have replaced Neandertals in Europe during the middle or upper Paleolithic period 30,000 years ago. Then around 10,000 years ago, farmers are thought to have spread across the continent, also from the Middle East, largely replacing the earlier foraging populations.



PAUL HANNY/GAMMA LIAISON

Old genes. Analysis of the Tyrolean ice man's mitochondrial DNA suggests his ancestors left the Middle East at least 30,000 years ago.

Studies of European languages and analyses of blood group and other proteins have indicated that most modern Europeans derive from this influx of Neolithic farmers. But new evidence from mitochondrial DNA, gathered by a group led by Bryan Sykes of the Institute of Molecular Medicine in Oxford, suggests that the ancestors of modern Europeans left the Middle East at least 30,000 years ago—long before the Neolithic farmers.

Until recently, studies of mitochondrial DNA in Europeans have failed to turn up distinctive genetic lineages that could be used to track migration patterns, because the variations between most Europeans showed

no relation with particular populations. "Europeans appear really rather homogeneous," says Laurent Excoffier, an anthropologist at the University of Geneva.

But Sykes's team has developed a new method that reveals previously hidden lineages. They have found differences among European populations in a region of the mitochondria that shows one of the highest mutation rates. This region poses problems because it also exhibits a high rate of reversions—where a mutation changes a DNA base but a second mutation reverts it back to the original—making it possible to draw many different patterns of relations.

Sykes's team got around this problem with an analysis that tries to predict how these mutations developed over time, so that different sequences can be grouped together as coming from a common origin. "Our approach produces just one network of relationships from one set of sequences, which makes it much easier to interpret," he says. "It's an important new approach to the question of European origins," says Chris Stringer, a paleontologist at the Natural History Museum in London.

The team has analyzed the relations between mitochondrial sequences from more than 800 modern Europeans. Using the network approach, they have identified at least three main groups of related sequences. By analyzing the mutations in these groups and their rates of mutation, they were able to estimate the time since the groups diverged from a reference Middle Eastern sequence. Surprisingly, all three groups appear to have arisen at least 30,000 years ago, which suggests that they predate the spread of Neolithic farmers, says Sykes, although a cluster of group II sequences does appear to be much younger, at around 10,000 years old.

The team is now using ancient DNA samples as a critical test of their calculations. They carried out an analysis of mitochondria from the Tyrolean ice man Ötzi, who was frozen in an Austrian glacier 5000 years ago and discovered in 1991. Ötzi's mitochondrial sequence, they found, belonged to the group I type—the most common group in modern Europeans. The analysis was confirmed by another laboratory.

Studies have also begun on human skeletal remains dated to the upper Paleolithic period from Gough's Cave in southwest England. The results of the first analysis, which will be repeated by an independent laboratory, suggest that this ancient mitochondrial sequence is also a member of the group I types, supporting the view that this group existed at least 30,000 years ago and has long been in Europe. "The goal now is to study human remains at well-established Neolithic sites to see what mitochondrial types are present," says Sykes.

—Nigel Williams