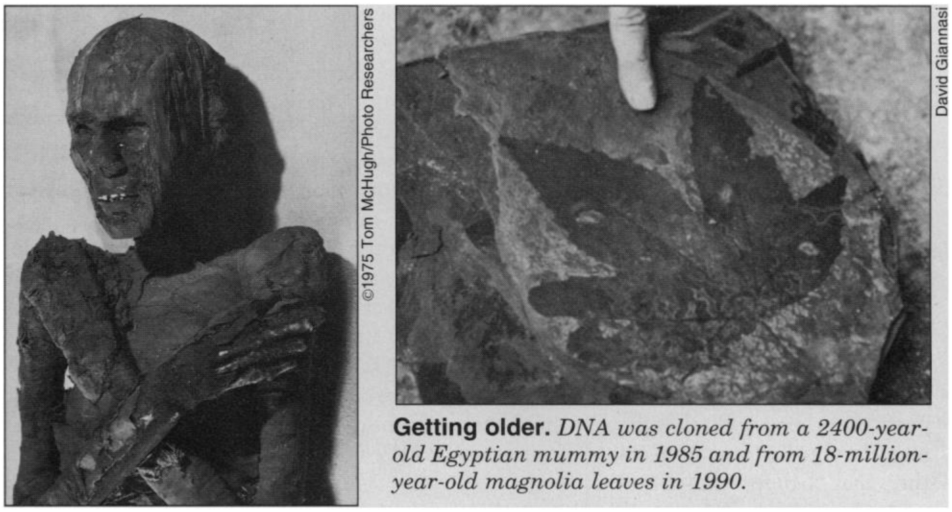
Ancient DNA: Still Busy After Death

When researchers working with DNA from the tissues of long-dead organisms got together recently, they found they had created a new field



©1975 Tom McHugh/Photo Researchers

David Giannasi

Getting older. *DNA was cloned from a 2400-year-old Egyptian mummy in 1985 and from 18-million-year-old magnolia leaves in 1990.*

Nottingham, England—WHEN RICHARD Thomas, an expatriate American who heads the DNA laboratory at the Natural History Museum in London, planned a workshop on the recovery of DNA from archeological material and museum specimens, he had in mind a quiet, technical meeting. "We were hoping to get 35 people," he says. But that was before the science section of *The New York Times* published a fanciful "recipe" for recreating a dinosaur from ancient DNA—and mentioned Thomas's upcoming workshop at the University of Nottingham. The result: "We were inundated by people," says Thomas. "We were stunned and amazed by the reaction from the press. We had to spend a fair amount of our time telling them, 'No, we are not going to reconstruct the dinosaur.' "

While some members of the press may have left disappointed, conference delegates did not. They found that molecular biology may be on the brink of revolutionizing archeology and paleontology, just as it had earlier revolutionized population genetics and evolutionary biology. Some 40 presentations at the conference* showed that students of ancient DNA are overcoming the problems of technique and contamination and turning their pursuit into a full-fledged field that offers unique answers to serious questions about kinship, the migrations of ancient peoples, and the taxonomic relations and rates of evolution of long-extinct species.

Conference delegates could also take pleasure in the number of their peers. Along with the press, some 70 scientists who are studying ancient DNA or planning to move into the field came to the workshop; several dozen more were turned away for lack of space. "The field is growing exponentially at the moment," says Thomas. "We're already planning the next conference at which we expect to be able to examine serious data sets instead of bits and pieces of information."

It has taken less than a decade to get from bits and pieces to the expectation of data sets. Although attempts to extract DNA from museum specimens of extinct creatures began in the early 1980s, the first real coup was not until 1984. That year, the late Allan Wilson's group at the University of California, Berkeley, successfully cloned DNA from preserved tissue of the quagga, a curious-looking beast that resembles a cross between a horse and a zebra and became extinct 108 years ago. Humans came next. In 1985 a 3.4-kilobase section of DNA was cloned from a 2400-year-old Egyptian mummy by Svante Pääbo at the University of Uppsala in Sweden. Then, in 1989, a new group formed at Oxford University by molecular biologist Bryan Sykes and archeologist Robert Hedges drove back the genetic clock even further when they extracted DNA from a much tougher material—human bone—5500 years old.

But even that date would soon seem unimpressive. Last year Edward Golenberg, a population geneticist now at Wayne State University, smashed the age record when he extracted DNA from 18-million-year-old fossil magnolia leaves from the bed of an ancient lake in Idaho. That DNA could survive for such a staggering length of time was totally unexpected—almost unbelievable. But careful comparison of the DNA fragment with modern magnolia leaf DNA showed that it really was ancient magnolia DNA and not some laboratory contaminant.

The same cannot be said of every claim that DNA has been extracted from ancient materials. Earlier meetings on ancient DNA have been "confrontational," says Thomas, as scientists disputed the validity of their techniques. Not at Nottingham. There, he says, with considerable satisfaction, people at long last talked openly about the problems they had with their samples.

The technique that has made the study of ancient DNA really take off is, of course, the polymerase chain reaction (PCR), the ultrasensitive DNA-amplification technique that hit the headlines in the late 1980s (see *Science,* 22 December 1989, p. 1543). The technique is so powerful that even a single molecule of DNA can be picked up and amplified a millionfold.

At the conference, dramatic illustration of how that power can confuse as well as clarify came from Oxford's Sykes. He presented data from experiments that looked at DNA in detail, rather than assuming that a positive PCR product indicated the presence of the expected ancient DNA. Extracts of DNA from a pig bone found in the wreck of King Henry VIII's battleship the *Mary Rose* proved OK—that DNA was indeed porcine DNA—but a neolithic cow bone produced human DNA, as did an Anglo-Saxon horse. Contamination of that sort could have happened at any point, from excavation to extraction and subsequent PCR exponentiation. Even more worrying, a 6th-century human bone produced a pig PCR product. "Since the archeologists were not pigs," says Sykes dryly, "the contamination must have come from somewhere else." The likeliest

**Ancient DNA: The Recovery and Analysis of DNA Sequences from Archeological Material and Museum Specimens,* sponsored by the British Museum (Natural History), the Natural Environment Research Council, Cetus Corp., and Perkin-Elmer, held at the University of Nottingham, England, from 8 to 10 July.

place: the laboratory, where pig DNA had been amplified previously.

About one in three of the positive PCR amplifications that Sykes tested were in fact false positives, which has led his group to take the problem extremely seriously—in fact, the team now does all its bone extracts in a distant laboratory where no PCR takes place. Labs that don't do that, he says, will always be suspect. "Careless labs will look productive," Sykes warns, but only "for a little while."

The flip side of that observation is that certainty is possible, provided sufficient care is taken. In at least one case described at the conference, evidence from old bones was enough to convince a British court. In December 1989, shortly after Sykes and Hedges published their seminal work on extracting DNA from old bones, the skeleton of a young girl turned up in a backyard grave in Cardiff, Wales. Facial reconstruction and dental records hinted at an identity, but could not confirm it. The police enlisted Erika Hagelberg, the biochemist turned molecular biologist who had done the DNA extractions for Sykes and Hedges.

Hagelberg extracted DNA from the victim's bones. She passed the DNA to the man who invented genetic fingerprinting, Alec Jeffreys, at Leicester University. Jeffreys compared the bone DNA with samples from the putative victim's parents. The match was good enough to identify the girl as Karen Price, who had disappeared in 1981. No contaminant would have matched, and while 9 years isn't exactly ancient, Hagelberg stresses that the case proved for the first time that old bones can produce not just bits of DNA, but a genetic record strong enough for the identification of an individual.

Normally, Hagelberg uses the power of PCR and genetic markers to identify not individuals but racial groups—and that's with bones 2000 years old. Her work focuses on the Pacific and on how people hopped from island group to island group. She has a small genetic marker that seems to identify Polynesian people. Her aim is to look at burials in Melanesia from 2000 years ago and "ask whether these people are ancestors of modern Polynesians, or are they ancestors of modern Melanesians, and how does this fit with anthropological and linguistic evidence."

Burial grounds are something of a happy hunting ground for molecular paleontologists, as the workshop heard repeatedly. At the Windover grave site in Florida brain material has been recovered from 91 individuals buried between 7000 and 8000 years ago. William Hauswirth, a molecular biologist at the University of Florida who has long been involved in the site, says ancient DNA is especially important because it allows him to go back to the time before Western contact. More than 95% of the American Indian population died off in the century after 1492; you have to use ancient DNA if you want to understand the genetics of the original Americans. "Are they of common stock? When was the New World peopled? We can begin to answer those questions without making as many assumptions as people today have to make," says Hauswirth. "Our story is far from complete," he admits, but it is "as far along as anybody's. We've been able to show that people buried 1000 years apart are very likely related to each other." Next will come studies of the flow of genes into and out of the population, and perhaps some insight into the marriage habits of the Windover people.

W. Hauswirth/ U. of Florida

Florida real estate. *Although dead for 7000 years, the brains of Native Americans buried at these archeological sites around the Windover peat bogs in central Florida still yield DNA that can be cloned.*

Another version of such anthropological pursuits is typified in the work of Martin Richards, a molecular biologist at Oxford who wants to know where the Anglo-Saxons came from. They appeared in Britain around the 5th century, and the received wisdom used to be that they were Germanic tribes who invaded England. Nowadays, many archeologists see little evidence of an invasion from Germany, and little influence of German culture. "There is no received wisdom these days," says Richards. "It's wide open, and that's what makes it exciting." DNA derived from Anglo-Saxon burial grounds might reveal that the Anglo-Saxons are, indeed, German invaders, or it might show that they are related to the Romano-Britons who were there before them.

Indeed, one thing that emerged from the workshop was the power of PCR to illuminate seemingly intractable problems, such as the patterns of inheritance in cultures that left no written record. Susanne Hummel, an anthropologist at the University of Göttingen in Germany, told the workshop that in some societies church records reveal that where boys inherit, more girls die young. "I don't want to say they are killed," Hummel explains. "That sounds too active. But perhaps they just take less care, and the child dies." What of communities with no records? Their graveyards, if they showed an excess of girls among the young children but not among still-born infants, might hint at a male pattern of inheritance.

The challenge is that while it is easy enough to determine the sex of a relatively complete adult human skeleton—because of clear differences in the structure of the pelvis—children have not yet developed those differences. DNA, however, and more particularly male-specific sequences from the Y chromosome, could do the trick, except, of course,

If Not a Dinosaur, a Mammoth?

Nottingham—However much scientists may protest that it cannot be done, the public and the popular press clearly expect ancient DNA to create Jurassic Park for real. But if you accept that re-creating dinosaurs is simply impossible, then how about something a little easier—a close encounter with a resurrected wooly mammoth, for example. After all, frozen mammoths are not uncommon in Siberia—and they went extinct a mere 10,000 to 15,000 years ago.

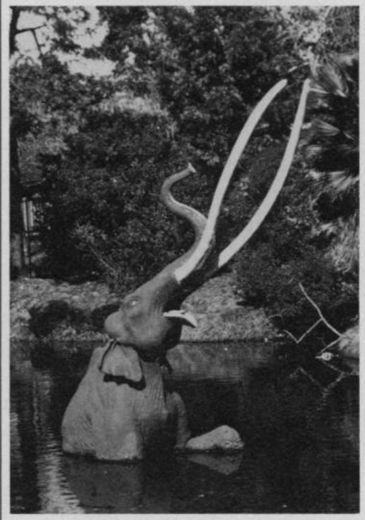
"In my lifetime it seems quite unlikely," says a cautious Russell Higuchi, a molecular geneticist at Cetus Corp., who spoke on mammoth DNA sequences at the Nottingham conference. He does have quite a lot of mammoth DNA—enough to show that the mammoth is related equally to African and Indian elephants—but so far it's all from mitochondria. His DNA comes from a baby mammoth called Dima, thawed from the Siberian tundra in 1977, and was "the first sample [of ancient DNA] that PCR was applied to," says Higuchi. He worked on samples from Dima back in the early 1980s when he was a posdoc in Allan Wilson's lab at Berkeley but at the time he was not able to clone the DNA and investigate it properly. A few years later, Wilson was complaining to two former students who had joined Cetus about the difficulties of reading the mammoth DNA. As Higuchi heard it from Wilson, "They looked at each other and said, 'Well, we have this great new technique called PCR.'" That was enough to send Higuchi off to learn PCR and return to his mammoth remains.

At first, the amplified DNA carried a disappointing message: "A large fraction was human," he says. More recently, Higuchi has employed new and improved PCR techniques on the same sample and now has some 350 nucleotides of mammoth mitochondrial sequence. It differs at four or five places from the Indian elephant's code, and by a similar amount from the African elephant, confirming the three-way split.

Ancient DNA from mastodon bone—obtained by Noreen Tuross, a molecular biologist at the Smithsonian Institution—might shed further light on the evolution of the elephants, but Higuchi doesn't plan to take the work much further; for him, it's been a bit of fun. As for reconstructing an entire mammoth, he thinks that's impossible. He likens it to finding a large encyclopedia ripped into many shreds and written in a language you barely understand. Your mission: to reassemble it in the dark, without using your hands.

Is there nothing he can offer a public hungry for creatures from lost worlds? "The amount of mammoth DNA is enough that in theory a dedicated graduate student could reassemble the entire mitochondrial genome. So we could have elephants walking around carrying mammoth mitochondrial DNA sequences." Great, but there's a downside: "It would make absolutely no difference," he says. "They'd still be elephants." ■ J.C.

Mark Stouffer



No rescue. *The job of saving the mammoth is unlikely to get beyond its mitochondria.*

that while a failure to find a Y-specific sequence could mean that the skeleton is female, it could also mean that the relevant bit of DNA was not amplified. Nevertheless, Hummel is confident that she can overcome the difficulties, and if she can, that could add another piece—inheritance patterns—to the puzzle of early European cultures.

While archeology and anthropology are likely to be the first big beneficiaries of the study of ancient DNA, simply because materials are more recent and easier to work with, evolutionary biologists are also caught up in the boom. Alan Cooper, a molecular taxonomist at Victoria University in New Zealand, told the conference how ancient DNA reveals that New Zealand's two flightless birds—the kiwi and the extinct moa—are not as closely related as had been assumed. The kiwi is closer to Australia's emus and cassowary than to the moa, which suggests that the ancestors of moas and kiwis arrived in New Zealand independently. Elsewhere, researchers are looking at DNA from species that went extinct to see what it can tell them about present-day endangered animals. And several are peering into DNA from old plants.

Terry Brown, a molecular biologist at the University of Manchester Institute of Science and Technology, has been working with archeological colleagues (one of whom is his wife) and has isolated substantial quantities of DNA from wheat seeds that date back at least 2000 years. Again, though, finding the DNA is just the beginning: "There are so many things that we could do with the ancient wheat DNA that we hardly know where to start." Pinning down the wild progenitors of wheat and examining the spread of different cultivars—perhaps traded by early Neolithic plant breeders—are just two of the tasks he hopes to tackle soon.

There are plenty of possibilities too for the world's oldest DNA. Golenberg can isolate pieces of nuclear DNA several thousand base-pairs long from about a quarter of his samples of 18 million-year-old magnolia leaves. He plans to investigate the pattern and rate of molecular evolution "not by inferring it but by seeing how genes and gene sequences change over a real-time difference." He also thinks that DNA may help to resolve some puzzles of plant paleontology, such as cases where taxonomists have a fruit and a leaf and think they are from the same species, but cannot prove it. "With the sequence," says Golenberg, "you can get an answer that is not open to debate."

In the meantime, Golenberg continues to explore. He had just come back from a site in Nebraska, where he made extractions of organic material from some leaves that date back 100 million years. One hundred million years extends back into the Cretaceous period, which means that even though extracting dinosaur DNA is just a newspaper writer's fantasy, it may soon prove possible to obtain DNA from dinosaur fodder. Golenberg does not know if there is any DNA in his leaves yet. "I'm not that optimistic," he says.

In any case, age isn't everything. "The object is not necessarily to see who can get the oldest DNA," Golenburg insists, "but actually to start working up research projects that can make sense." That was the message that participants at the Nottingham conference took away with them: Despite the remaining technical problems, ancient DNA is no longer just a curiosity but an area where systematic studies can produce insights unavailable by any other technique. For archeologists, anthropologists, and paleontologists the message is clear—the time has come to ensure that textbooks on the polymerase chain reaction and gene cloning are on the bedside table. ■ JEREMY CHERFAS