Dino DNA: The Hunt and the Hype

Several groups are racing to get the first DNA out of dinosaur bones, but other researchers say their efforts are taking attention away from the real scientific value of ancient DNA

Eighteen months ago, Mary Schweitzer, a biology graduate student at Montana State University's Museum of the Rockies, was examining a thin section of *Tyrannosaurus rex* bone under her light microscope, when she noticed a series of peculiar structures. Round and tiny and nucleated, they were threaded through the bone like red blood cells in blood vessels. But blood cells in a dinosaur bone should have disappeared eons ago. "I got goose bumps," recalls Schweitzer. "It was exactly like looking at a slice of modern bone. But, of course, I couldn't believe it. I said to the lab technician: 'The bones, after all, are 65 million years old. How could blood cells survive that long?' "

Yet even as she was doubting the evidence before her eyes, Schweitzer was moving ahead to another daring thought. If red blood cells had survived fossilization, it should be possible to get at the dinosaur's DNA. "The minute I saw those structures, getting the DNA became my goal," she says. "It was obvious that was the thing to try for."

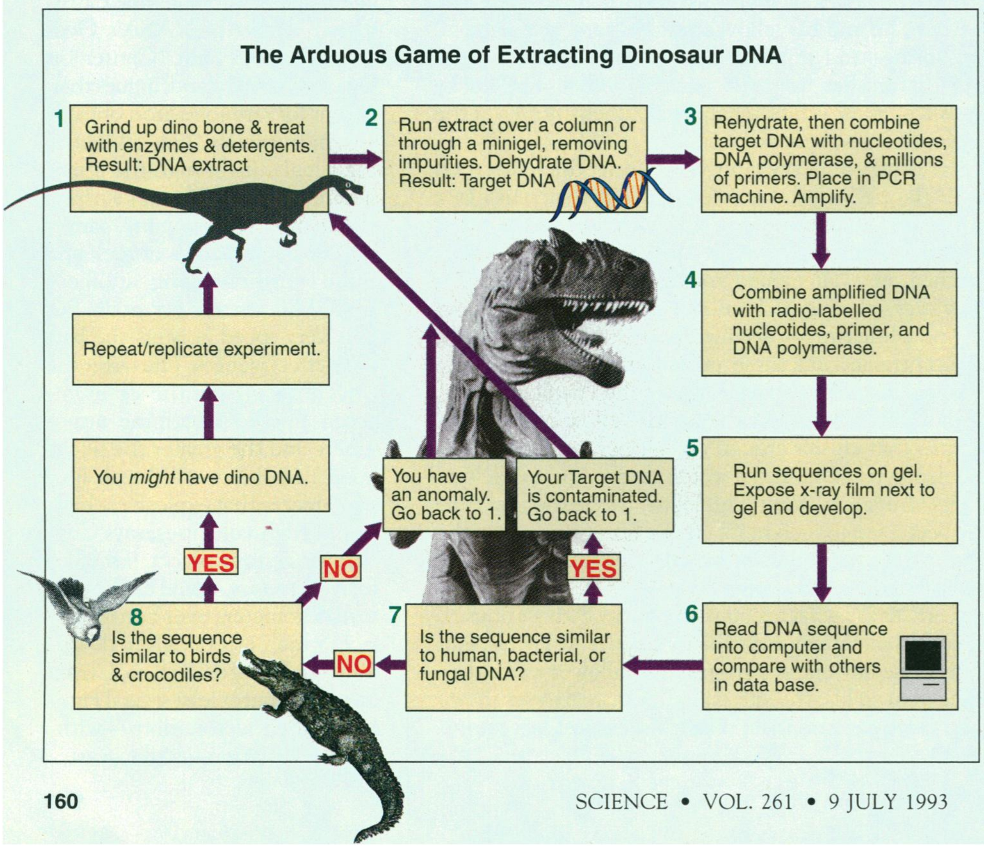
Does that mean the real Jurassic Park is right around the corner? Maybe, and then again, maybe not. From the interior cavities of the *T. rex*'s bone (which her colleague, paleontologist Jack Horner, provided), Schweitzer has already extracted a molecule that might be dinosaur DNA. And their discovery has set off a furious race with other labs to be the first to publish on dinosaur genes. But before you start thinking that next summer's big amusement will be live dinosaurs at your local zoo rather than a Steven Spielberg movie, be warned that all these efforts are plagued with technical problems. No one knows enough about developmental genetics even to consider cloning a dinosaur should any genetic material from the age of reptiles be recovered. More important, most researchers think it's unlikely that any such material has survived in the first place. "Given what we know about the decay of the DNA molecule, the onus remains on those who are searching for dinosaur DNA to prove that they've found it," says molecular biologist Noreen Tuross of the Smithsonian Institution.

Beyond the technical problems lies a deeper concern about the attempt to retrieve dinosaur DNA, an effort some researchers disparage as "Disco Science." That is the possibility that this summer's intersection of science and Hollywood (which has already landed Horner on the cover of *U.S. News and World Report*) will overshadow some less dramatic—but potentially more fruitful—attempts by other scientists to get DNA from extinct animals and people that are hundreds or thousands of years old, rather than millions. These less glamorous specimens could provide answers to questions about population movements and biodiversity that dino DNA can't even begin to address.

None of this criticism means researchers won't be able to extract DNA from dinosaurs. In fact, they may well succeed. In a typical dinosaur fossil all the bone's original organic structures have been replaced by minerals and silica. But in a few cases, such as Horner's and Schweitzer's *T. rex* skeleton, the interior cavities of the bone were not completely mineralized and appear to contain organic matter. Horner suspects the bones are thick enough to protect their internal cellular structure—and perhaps their genetic material—from water and oxygen, two elements lethal to the survival of DNA.

What is more, the route into the bones isn't the only chance for success. Other scientists hope to sidestep the skeleton and go for the extinct animals' blood in a move taken straight from the pages of Michael Crichton's novel. At least two research groups are attempting to remove blood from the gut of amber-preserved biting insects, whose last meal might have been a dino. The net result of these efforts is that "someone's going to get dinosaur DNA, and soon," says the leader of one competing group, Raul Cano, a molecular geneticist at California Polytechnic State University.

Cano's team, not to miss a trick, is pursuing both bone and blood possibilities. Cano and his graduate student Hendrik Poinar, who recently smashed the age record for ancient DNA by recovering a gene fragment from a 120-million-year-old amber-preserved weevil, say they have—like Schweitzer and Horner—already managed to extract minuscule amounts of DNA from a fossilized dinosaur bone. But, again like Schweitzer and Horner, they can't say whether it is dinosaur DNA or DNA from something else. Because the polymerase chain reaction (PCR) technique that enables scientists to study the bits of DNA they harvest is exquisitely sensitive, it can pick up any stray DNA—a human technician's, for instance, or DNA from a fungus on the original bone—and amplify

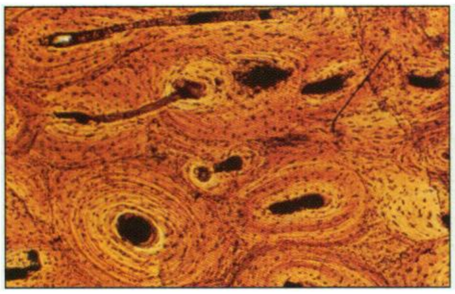


ALLOSAURUS MODEL, S. CZERKAS, NATURAL HISTORY MUSEUM L.A./ILLUSTRATION, D. DE FRANCESCO that instead (see sidebar). Consequently, neither group is rushing to claim victory. Says Horner: "It's easy to extract DNA from a dinosaur bone. It's trying to prove that it's from the dinosaur—and not from some contaminant—that's hard."

In spite of these roadblocks, big game DNA hunters keep pushing forward, and not just for the glory of being first, they say. Horner and Cano both say they want to use the genes to reconstruct dinosaur evolutionary history. That, says Horner, "is the big question: Where do dinosaurs fit?" They could be most closely related to either birds or crocodiles—or both or neither. Michael Parrish, a paleontologist and biochemist at Northern Illinois University, argues that with the molecule in hand they will be able to "answer a lot of questions about phylogeny." In addition, he points out that if they could get DNA from more than one species, researchers could then go on to study questions about the classification of specific dinosaurs.

But many other researchers think this is a highly unlikely scenario, arguing that DNA, a notoriously unstable molecule, would be hard put to survive in any boney material, no matter how well preserved, for 65 million years or more. It will be tough enough to get DNA from one dinosaur specimen, they say, let alone from the numbers needed to do any sort of species comparison. "There is no evidence—none—that even a very common, stable macromolecule like collagen has survived for that long," says the Smithsonian's Tuross. "It takes a leap of faith to believe that something like collagen has been destroyed and yet DNA persists." Backing Tuross up, biochemical studies of the rate of decomposition of DNA in solution show that under normal conditions, with typical exposure to air and water, the molecule is seriously degraded if not entirely broken down after 40,000 to 50,000 years.

Yet there are hints that organic molecules can survive the millennia. Schweitzer has seen fibers on her *T. rex* bone that look like collagen, although she has not tested them. And biochemist Gerard Muyzer, from the University of Leiden in the Netherlands, reported last fall that he and his colleagues had extracted a bone protein, osteocalcin, from the bones of several dinosaurs including ceratopsians and hadrosaurs. Muyser's group used two types of antibodies that reacted specifically to osteocalcin from modern bones, and found that the antibodies also labeled the ancient protein. The researchers noted that burial in an impermeable matrix and the absence of high heat made it possible for the material to survive; a similar burial might perserve delicate DNA.



LEE PARKER

Blood from a bone? This bone section from a duck-billed dinosaur may hold blood cells—and therefore dinosaur DNA.

But preservation is only one worry. A more persistent concern among researchers in the young field of molecular paleontology is that the current dinosaur-sized hype may squash their own less glamorous but scientifically more rewarding investigations. "It's the new Disco Science question: Who's going to be the first to get dinosaur DNA?," says Bob Wayne, an evolutionary biologist at the University of California, Los Angeles, and an editor of the *Ancient DNA Newsletter*. "But the trouble is that these very topical questions tend to obscure other research on more recent materials—such as mammal pelts in museum collections—which are much more likely to contain real DNA from the original source."

Kelley Thomas, a molecular evolutionist at the University of California, Berkeley, agrees that "the real value of this work is not so much in the age or type of DNA you can get, but in what it has to offer the study of conservation biology and genetic continuity."

For example, graduate students in Thomas' lab are attempting to recreate the genetic history of the Alaskan and Northwest coast Steller sea lions and various salmon species—organisms whose populations have crashed in the past and are under pressure again. "The museum specimens are snapshots of the genetic diversity that was once found in those populations," explains Thomas. Using DNA taken from the museum specimens, researchers can see if there is a relationship between genetic diversity and a healthy population, Thomas says. "We've assumed that because the species has passed through a bottleneck we've hurt it. Now we

Difficulties With Dinosaur DNA

Getting DNA from the 65-million-year-old bones of a *Tyrannosaurus rex* is a little like rescuing someone from a medieval prison, says Mary Schweitzer, a Montana State University graduate student who is attempting just such a feat. "Basically, the DNA is like someone trapped in a cage at the bottom of a big catacomb—and all these other biogenic materials [proteins, fatty acids, and polysaccharides] stand between you and it. You have to break through all of them to free the DNA." And you more or less have to do it with both hands tied behind your back: the fossil material, chemicals, equipment, and room must be as free of human and other nondinosaurian organic particles as possible. Otherwise, the ultrasensitive polymerase chain reaction (PCR) used to amplify the DNA is liable to pick up one of these contaminants—and release the wrong prisoner.

To make sure she's liberated the right molecule, Schweitzer compares the extracted DNA sequences with those of hundreds of living organisms. If the sequence turns out to be similar to that of a known fungal gene, for example, she knows the sample has been contaminated.

That's how DNA hunters know they've gone wrong. But how do they know when they're on the right track, given that there are no living dinosaurs to provide a handy sample of DNA for comparison? The answer is that they rely on paleontological theory, which (according to most researchers) holds that dinosaurs and crocodiles came from the same stock, and that the dinosaurs' only living descendants are birds. Therefore researchers look for DNA that is similar, but not identical, to DNA from these groups of organisms.

If they can find such a sequence—a dauntingly difficult task—researchers must then face the even harder task of reproducing the results. Because fossil material likely to contain DNA millions of years old is extremely scarce and because the amount of DNA in such samples can vary, researchers often have trouble replicating studies—even their own. As molecular geneticist Svante Pääbo of the University of Munich, a pioneer in ancient DNA studies, notes: "The whole problem of the field is that it is not scientific in the sense that many of these things are not reproducible. People find things they and others cannot repeat, and so that leaves them on shaky ground."

Raul Cano, whose lab at California Polytechnic State University is in the thick of the race to clone dinosaur genes, agrees that the solution is going to require work from more than one group. "It's going to take hard work by several labs to finally verify dinosaur DNA. And in the end, the only way you can be absolutely certain that you've got it is to find a frozen dinosaur."

–V.M.

can determine if those assumptions are true." The answer could be important for many endangered species.

Wayne, for his part, is extracting DNA from museum pelts of a now extinct wolf-like animal that inhabited the Falkland Islands until the latter part of the last century. "Charles Darwin wrote the first description of this species," says Wayne, "but about 30 years after he reported it, hunters wiped it out." Since then, various scientists have argued that the animal was actually a wolf, a large fox, or some type of domestic dog that had gone feral. "By extracting the DNA from hairs on the pelts," Wayne says, "this can be resolved definitively."

The questions being addressed aren't limited to animal species; molecular geneticists have had good luck retrieving viable DNA from human remains and using these to address questions of migration patterns. In the May 1993 *Proceedings of the (UK) Royal Society*, Erika Hagelberg and John Clegg of Cambridge University published initial results of a 3-year study of the prehistoric colonization of the South Pacific islands of Oceania. Previous efforts to trace the settlement patterns of the early island-hoppers relied on linguistic and archeological evidence—and indicated that the original settlers were voyagers from Southeast Asia. But Hagelberg sampled mitochondrial DNA sequences taken from human skeletons at a variety of sites in Oceania (some dating as far back as 2700 years before present). By comparing these skeletal genes to mitochondrial DNA from modern inhabitants, Hagelberg has shown that some of the earliest settlers probably came from Melanesia, and not just Southeast Asia.

Other investigators are looking for 13,000-year-old DNA from ground sloths to untangle their family tree. Still others are taking genetic material from 800-year-old skeletons of early Americans—the Hohokum people—to learn if they are related to current Native Americans. Though none of these projects are as romantic as the hunt for dinosaur DNA, together they constitute what Wayne terms "the real nuts and bolts research" of ancient DNA studies. "What we really want to do is reconstruct the historical variability of species, to get a feel for the variation in gene frequency over time. It's opened up a whole new avenue of research for museum collections, a new way of studying evolutionary history," he says.

And while these cautious researchers may never inspire a Michael Crichton novel or Steven Spielberg film, they are already in a position to do what the dinosaur DNA hunters are still struggling to accomplish: get hard data and replicate it.

–Virginia Morell

COMPUTER SCIENCE

Electronic Time-Stamping: The Notary Public Goes Digital

You've just discovered the secret of cold fusion. You're not ready to go public, but you'd like to establish intellectual (not to mention patent) priority for your inspired idea. Down the hall, your colleague in the biology department is worried about fending off another congressional investigation into whether or not one of his postdocs has altered lab notes, which would be easy to do, since the notes exist solely as computer files. Across town, a surgeon is "revising" a patient's records in the hospital's computer system after a botched operation. Meanwhile, in the high-rise offices of Shady Deals Investment Corp., a ruthless corporate climber is backdating a memo to prove he warned his boss against the company's latest disastrous venture.

The paperless world in which all these things could be happening at once in a medium-sized town isn't far off—and it's getting closer every day. Along with it comes the question: How do you establish trust in documents that exist only in the easily altered memory of a computer? So far the answer has been to make paper printouts of crucial documents, which can't be altered easily without leaving traces. But two researchers at Bell Communications Research (Bellcore) in Morristown, New Jersey, have proposed what they say is a more elegant solution based on mathematical ideas in computer science.

Digital time-stamping, as co-inventors Stuart Haber and Scott Stornetta call their approach, makes it possible to prove that a particular document existed at a particular time in a specific form, without requiring the document to exist in hard copy. How is this trick pulled off? The answer is that instead of authenticating a piece of paper or a magnetic tape, the new scheme creates a time-stamp from the data themselves. "A document with its digital time-stamp not only shows when the thing was created, but also assures anybody looking at it that the document hasn't been changed since then," says Haber. And that may be just what's needed in the increasingly electronic world of finance, insurance, and scholarship. Says Mack Hicks, a vice president for data processing in the technology division of BankAmerica, "This is the first technology I've seen for notarizing electronic documents."

Haber and Stornetta's interest in time-stamping was prompted partly by the much-publicized, lengthy scientific fraud case over a 1986 paper published in *Cell*. During that case, Secret Service analysts testified that data in the lab notebooks of Tufts University immunologist Thereza Imanishi-Kari had been altered. Haber and Stornetta realized that if it had happened today, "she'd probably be keeping all the data in computer files, and there wouldn't be anything on paper for the Secret Service to come back and look at," says Haber. The two wondered whether it's possible to devise a digital stamp that, like an old-fashioned seal on an envelope, would reveal if an electronic document had been tampered with since the seal was applied.

Like a wax seal, the stamp had to do more than give a time; it also had to certify the document's content. The answer, Haber and Stornetta decided, lay in a mathematical procedure called a one-way hash function. One-way hash functions, of which there are many types, are procedures for taking long strings of characters (which is what all documents in a computer amount to) and boiling them down to shorter, random looking character strings. A hash value contains no clue to its input; publishing or storing a hash value at the time a document is created gives away no secrets. At the same time, however, each document's hash value is for all practical purposes unique, like a human fingerprint; alter a document by even one character, and its hash value changes completely.

If all of this seems a bit abstract, try an elementary example. A simple hash function that converts the word *science* into a string of 6 digits might work this way: First, convert the letters into 2-digit numbers, where a=01, b=02, and so on. Then add a digit to describe the position of each letter, turning *science* into the string 119, 203, 309, 405, 514, 603, 705. Next, square the numbers and add them together: $119^2 + 203^2 + 309^2 + 405^2 + 514^2 + 603^2 + 705^2 = 1439706$. Finally, keep only the last 6 digits, yielding a hash value equal to 439706. Clearly, changing even a single letter would change the hash value.

One way to time-stamp a document, Haber and Stornetta realized, would be to send its hash-value fingerprint to a central time-stamp service (say Bellcore), which would attach the time of arrival and then put both in permanent storage. Any question about a document's date or integrity could be settled by checking with the time-stamp service. But that solution was unsatisfactory, Haber and Stornetta felt, mainly because it required the time-stamp service to be absolutely trustworthy. With the connivance of the time-stamp service, after all, a customer could easily alter a document, re-