

Blood from a Stone

By Rebecca Morphis

Can dinosaur tissue survive for 65 million years?
An NC State paleontologist thinks it can.
She might be right.

Mary Schweitzer noticed something unusual about the chunk of fossilized bone as soon as she lifted it out of the box of assorted fossil fragments.

Schweitzer, an assistant professor of paleontology at NC State, could see a distinct mineral layer in the several-inch-long piece of *Tyrannosaurus rex* femur. It reminded her of medullary bone, a type of avian bone she'd read about. When female birds ovulate, they produce a thick layer of calcium in their bones to prevent osteoporosis during the shelling process. Many paleontologists believe birds evolved from dinosaurs.

If it *was* medullary bone, it would be even stronger evidence of that link and, possibly, a way to distinguish gender using fossilized bone. "I just looked at [lab technician Jennifer Wittmeyer], and I got kind of pale," recalls Schweitzer, who has a joint appointment at the N.C. Museum of Natural Sciences. "I said, 'Oh my gosh, we have a girl.' She looked at me like I was crazy."

Schweitzer got the femur fragment and the other fossils from her Ph.D. adviser, Jack Horner, the Montana State University professor who inspired a character in *Jurassic Park*. The 65 million-year-old remains were uncovered at one of his dig sites in Montana's remote Hell's Creek formation, an area pockmarked with eroding mountains, crawling with rattlesnakes and as hot as its name suggests. The paleontologists depended on a helicopter to get their finds back to Bozeman, Mont.; the 42-inch-long femur cracked when they tried to get it through its door.

Schweitzer called Horner, who mailed her a larger section of the femur so she could see if the layer was just in an isolated section or if it extended throughout the bone, as it should if it actually was medullary bone.

To get a closer look, she dissolved the

outer layer of bone in a weak acid solution, stopping before too much of the fossil vanished. The partial removal, which took seven days, revealed what appeared to be a network of blood vessels, still tinted red, branching its way through the bone.

They had found in the fossil evidence of soft tissue, which shouldn't have survived more than 100,000 years, let alone 65 million.

They dissolved the rest of the mineralized material, revealing pliable, transparent tissue threaded with veins and elastic enough to snap back into place when stretched. Wittmeyer was examining the dissolved material using a powerful microscope when she noticed another surprise. She crossed the hall to see Schweitzer.

"She said, 'I think our stuff is contaminated because I'm seeing this stuff in the scope that looks like little bugs,'" Schweitzer says. "If the stuff is truly organic, then certainly microbes can feed on it. I looked at the scope, and I backed up, and I looked at the scope again. And I said, 'Jen, this is not possible. That is not a microbe, that is an osteocyte [a type of cell found in bone].'"

If further testing confirmed the observation, it would mean the tissue wasn't just preserved, but exceptionally so. It could possibly still contain viable genetic information.

"It was just one of those moments, like what else can happen with this dinosaur? It is going to get up and start walking around the room."

In the frenzied weeks that followed—"I took so many pictures of what we were seeing that I crashed the hard drive of our computer," Wittmeyer says—they confirmed the existence of similar tissue in four other fossils. In March, the journal *Science* published the report of their discovery. Further analysis of the specimen and the bones of several ovulating ostriches yielded another study. This one showed that the unusual layer of

bone they noticed initially bore remarkable similarities to medullary bone, particularly that found in large, flightless birds. Both discoveries made headlines in U.S. newspapers such as *The New York Times* and foreign media outlets such as the BBC and China's Xinhua General News Service.

But it is the soft-tissue discovery that could revolutionize everything we thought we knew about the way living flesh decays over time.

"Molecular people said for years that you wouldn't find molecules that old," says paleontologist Kevin Padian, a professor in the integrative biology department at the University of California-Berkeley. "Yeah, in the conditions in their labs, [molecules] would deteriorate. But nature is our laboratory, and sometimes the conditions there wind up being better than a lab. The importance of this is the lesson it has for all scientists. First thing, these things can be found. There are structures there we didn't think could be present. And we can actually perform experiments to determine what they are. It teaches us that if we don't keep our minds open to these possibilities, we'll make mistakes."

The next step in Schweitzer's research is analyzing the soft tissue to see what it contains. Her findings could answer questions that have dogged paleontologists since the first dinosaur fossil was scientifically described in 1824. Were they warm-blooded or cold-blooded? How fast could they move? Are they male or female? What did they really look like? If proteins or DNA survived the ravages of time, they could hold important genetic clues to guide scientists.

Still, the greatest significance, Padian says, may be in what already has been reported. The mere existence of these molecules could encourage other scientists to look more closely for the impossible by taking precious fossils off museum shelves, crushing them and examining them in the laboratory.

In this sense, Schweitzer's work is part of a larger trend. Paleontologists are using advanced technology to study finds that were once just described and cataloged. The pace of traditional hunt-and-gather paleontology is quickening. As detection techniques and erosion aid scientists in the search for dinosaur fossils, an unprecedented

number are being excavated everywhere from the deserts of western China to the western United States.

Indeed, six or seven new dinosaur species are being described every year. And some of the specimens are extremely well-preserved, giving scientists new clues to help them piece together the dinosaurs' world. Some have been found with fossilized impressions of their skin, others with eggs still inside them.

But with no living, breathing dinosaurs to study, everything we know about these ancient creatures must be inferred from the fossils' surroundings, from how the bones were found, how many were found and how these findings jibe with what scientists know about the behavior and physiology of present-day animals.

Technological advances permit a more precise look into the bones themselves and how they function. Looking at cross-sections of fossilized material under a microscope, scientists can discern patterns in the microscopic structure that indicate age, growth and metabolism. CT scans permit another route inside a fossil. Former NC State paleontologist Dale Russell used CT scans in 2000 to reveal the outlines of two ventricles and an aorta in a fossilized thescelosaurus heart, suggesting that it had four chambers, and strengthening the case of paleontologists who believe dinosaurs were warm-blooded.

And computer modeling allows scientists to simulate dinosaurs' muscle structure to calculate how they might have stood and how quickly they might have moved. Such research has altered our assumptions—dramatically in some cases. Recent modeling has revealed that it is unlikely big herbivores, such as the allosaurus, held their head high as giraffes do. Analysis of how their neck bones fit together suggests they held their necks horizontally.

Yet most of this new research still reflects a central tenet of paleontology: The fossils being studied contain no living material to parse for answers about dinosaurs. The conditions for fossilization were supposed to be clear. When a living creature dies, its tissues decay and dissolve. This happens quickly if it is exposed to air and more slowly if it has been covered by something—a layer of mud, for example—shortly after its death. Eventually, though, evidence of skin and flesh

disappears, leaving just the skeleton. As moisture, air and microbes that accelerate decay seep into the bone itself, minerals replace its living tissue. Eventually, no tissue remains, and the bone becomes a rock.

Schweitzer's research, from the beginning of her studies, challenged this basic assumption. Starting from scratch nearly 15 years ago, she helped pioneer the field of molecular paleontology, developing methods to find and analyze molecules inside fossils. Critics used to say she was crazy to devote herself to such a quest.

"Basically, I was just too dumb to know it wasn't possible," Schweitzer, 50, says in one of the flashes of wry, self-deprecating wit that punctuate her conversations. Dressed in jeans and a blue tank top, with her gray hair pulled into a ponytail, she's hunkered down at her desk—crackers for lunch—for another 12-hour day writing grant proposals and preparing to return to Montana to work on her soft-tissue samples.

Her fascination with dinosaurs began when she was 5 years old. Her brother, then a college freshman, sent her frequent letters and two books: *Roy Chapman Andrews and the Dinosaurs of the Cliffs* and *The Enormous Egg*. "I devoured them. When my brother came home for Christmas . . . and all his buddies came over, I told them all I was going to be a paleontologist when I grew up."

But she took a circuitous route. She left her hometown of Helena, Mont., to study speech therapy at Utah State University in Logan, Utah. After graduating in 1977, she moved back to Montana, got married and had three children. In her 30s, and living in Bozeman with her family, she returned to school for a teaching certificate. She planned to become a high school science teacher. She finished the Montana State program in December 1988 with several months to kill before the new school year began and she could start a permanent job. For fun, she took Jack Horner's introduction to paleontology course.

The course rekindled her passion for dinosaurs and ignited her curiosity. "It was the hardest class I think I've ever taken, and Jack made me so mad, which is partly why I pursued it," she says. "He made me feel incompetent, and I got mad, and I learned."

Schweitzer began to volunteer at the Museum of the Rockies in Bozeman. She read everything she could about dinosaurs, messed around with junk fossils to teach herself about microscopic bone characteristics and pestered Horner with questions. "Finally, he told me, 'Mary, I can't answer your questions. Go back to graduate school and do it yourself.'" Already disenchanted with teaching after working as a long-term substitute, she did just that.

Schweitzer, then 35, registered for classes at Montana State. She developed a thesis proposal and, using junk bone for practice, continued her self-education on research methods. Looking for advice on how to prevent bone sections from slipping off glass slides, she took samples from another T. rex to Gayle Callis, a veterinary histologist who specializes in the examination of modern bone. Callis was giving a talk at a conference soon after, and she put one of the samples under a microscope when someone asked her about the oldest bone she had ever worked with. A pathologist attending her talk pointed out that the sample contained something that looked like red blood cells.

Callis returned the sample to Schweitzer, who peered at it through her microscope with a mixture of heart-stopping wonder and complete disbelief. Horner was summoned; he asked Schweitzer if she thought she really had found preserved dinosaur blood cells.

"No," she told him.

"Fine," he said. "Then prove that they're not."

It was years before she was confident enough to publish her results. With no similar research on which to base her studies, Schweitzer started from scratch, learning forensic lab methods used to characterize biological materials and figuring out ways to apply them to her sample. She enlisted the help of numerous specialists, from chemists to oncologists, to figure out ways to adapt methods used on modern tissue to something ancient and probably much more fragile.

Among other methods, Schweitzer and her colleagues pioneered the technique of using antibodies—created by injecting rats with dinosaur tissue and extracting the antiserum they produce in

response—to characterize ancient molecules. When the antiserum is mixed with tissue taken from existing animals, it bonds with the molecules it most resembles. The more specific the bond, the closer the relationship and the more likely the ancient tissue is related to the modern tissue.

Critics were skeptical of her methods and findings. “I’ve had reviewers [for academic papers submitted to journals] say basically, ‘I don’t care what your data say, I know this is not possible, so it’s got to be wrong,’ ” she says. “It’s hard to get up every morning and read that people think you’re crazy.”

But Schweitzer’s desire to show her kids that you shouldn’t quit when the going gets tough prompted her to redouble her effort. “She just really tested this thing six ways to Sunday,” says Padian, who met Schweitzer when she was in graduate school. “Every time someone would say ‘What about this?’ she would figure out a way to go out and test it. I just can’t say enough about the competence and resolve that took. Most people doing research on this level are working on an ongoing project with lots of people in white lab coats using big grants. She started from scratch.”

In the end, Schweitzer didn’t find complete cells. But her multiple tests indicated the presence of heme, the oxygen-carrying part of the hemoglobin molecule. Her findings, published in a scientific journal, made newspaper headlines, and her methods set the standard for research into ancient molecules.

Despite her revolutionary work, Schweitzer struggled to find enough grant funding to continue her research in Montana after graduating in 1995 and had a difficult time finding an academic job that would be a good match. “What I do is so odd that I don’t fit in anywhere,” she explains. “I’m not a biologist, I’m not a chemist, I’m not a molecular biologist, I’m not a paleontologist, and I’m certainly not a geologist, but I’m a combination of those things.”

Her money was about to run out when NC State advertised to fill two vacant paleontology positions. Dale Russell had retired and Reese Barrick had taken a job at another university. Schweitzer applied. NC State would be the only university to offer her a job.

“What she was proposing was a little out

there, no doubt about that, but we would rather have someone at the cutting edge, who can move the field forward rather than keep it at the status quo,” says John Fountain, head of NC State’s marine, earth and atmospheric sciences department, home of paleontology. Also, Fountain says, he thinks that not having paleontologists on the search committee helped them to look at her research with fresh eyes. “We could see that the science behind what she was saying was pretty darn good.”

Schweitzer is now investigating her latest find in her usual careful way: at least three repetitions of a single test before she believes the results and six or seven different technologies or approaches to verify that the method works. “A lot of this stuff is establishing methodology and establishing criteria and baselines, and it takes a huge amount of time,” she says.

Nonetheless, since their paper’s publication, Schweitzer and Wittmeyer have found evidence of soft tissue in other specimens in the same painstaking way, proving their initial results weren’t a fluke and that the survival of ancient molecules may be fairly common.

In the few weeks between the end of classes at NC State and the researchers’ return to Montana for the summer to use the Montana State lab, Wittmeyer worked at a feverish pace to stabilize the samples for safe transfer, by freeze-drying or getting them down to a powdered version.

They hoped to have a much better idea of what the soft tissue actually is by the end of summer. Using a high-powered transmission electron microscope, they’ll generate a map of the tissue’s elements. They’ll also take a closer look at its structure—determining if it still has its cellular wall, for example—and begin chemical tests to make sure it’s the genuine article and not tissue left by a contaminant. If everything goes well, Schweitzer hopes to recover some complete proteins from the tissue. It may also be possible, she allows, to recover some DNA. But that’s not really what she’s after.

“I know DNA is supposed to be the holy grail of this type of research,” she says. “It is not a high priority on my list. . . . It just doesn’t turn my crank.”

With good reason. You need a lot of DNA

to do any real testing or sequencing. When there's only a small quantity of genetic material to work with, scientists generate more by creating clones of the existing strands of DNA. But the cloning process amplifies the strongest, most complete DNA in the sample, which usually comes from a contaminant in a dinosaur fossil. "If you have 10 broken, degraded, horribly altered molecules of dinosaur DNA and you have one [modern molecule] that happens to sneak in there . . . enzymes are going to preferentially amplify that or any healthy piece of DNA because that is what it looks for," Schweitzer says.

Proteins are a different story. The action molecules of living species, they carry out the functional tasks of an organism, which means that proteins identified from a fossil might reveal a great deal about the mysterious creatures they came from—skin color, for example, or metabolism. They can also be analyzed in smaller quantities than DNA, by a far wider variety of methods, and are less subject to the danger of contamination. And the proteins are structured uniquely for specific functions, so it's possible to tell what a protein did even if its function has been severely impaired or eliminated altogether.

Schweitzer is as cautious as she is hopeful about predicting what they might find in the extracted tissue. It's possible, she says, that even if it is the original tissue, it could have been altered so much over the millennia that it barely resembles its former self. And there's also a strong possibility that the surviving molecules differ in some crucial ways from the ones destroyed—in ways even the most careful methods won't reveal.

"In a lab, if you freeze living tissue, 70 percent of that tissue will be destroyed," Wittmeyer explains. "But you'll still have 30 percent to work with. But what's different about that 30 percent? It has something special that allows it to survive the cold. So it's not quite like everything else."

Even if they don't learn anything useful from the samples, Schweitzer's findings suggest the need to take a hard look at the conventional wisdom surrounding the fossilization process. "At the very least we have a new mode of fossilization," she says. "At the very most we have evidence that tissue degradation does not progress the way we

thought it did when we put that tissue inside a bone."

Understanding how the molecules survived so long may be more useful than the molecules themselves. And though it's a stretch at this early stage, it could have implications for fields such as human health. What if researchers could create conditions that slow the decay of specific molecules, perhaps also slowing the progression of a disease or delaying some effects of aging? Knowing how to slow the mineralization process, researchers might develop better treatments for conditions in which the human body itself mineralizes—clogged arteries, for example.

Any information to be gleaned, though, will require a shift in the way people treat fossils, as much as objects of art as objects of study. "I hope this will mean that people will stop putting varnish on them," Schweitzer says, decrying a preservation method that makes bones attractive for a display but could damage or destroy surviving tissue.

She understands the reluctance to dissolve fossil collections in acid solutions to see what's inside. They're valuable. Complete skeletons can bring in millions of dollars. Still, Schweitzer says, "to really understand dinosaurs, you have to destroy the bone. The most interesting and intriguing things about dinosaurs as living animals have to come from the structure."

But a shortage of specimens isn't the only problem plaguing Schweitzer's research. She has been frustrated in her attempt to win grants to support her research, most of which are available through nonprofit and government organizations such as the National Science Foundation (NSF).

The groundbreaking nature of her research can work against her when seeking grant money, says Enriqueta Barrera, program manager in the NSF's earth sciences division. The division bases its funding decisions on peer reviews of the proposals as well as the recommendations of a panel of scientific experts. "Sometimes it's going to be very hard to get five people or the entire panel to agree on something that seems to be far out, cutting edge. That would fall in the category of risky research," she says, adding that it's always trickier to find funds to support such projects. "Because we're working with taxpayer money, we want to make sure the investment is a good one."

In the past, panels looking at Schweitzer's proposals recommended that she use animals more recent than dinosaurs in her research, Barrera recalls. But Barrera thought her proposal was intriguing. She found the funds to give Schweitzer a small grant for exploratory research—which doesn't have to go through the review process—to help get her started.

Barrera believes Schweitzer's recent discoveries will help convince reviewers to invest in her research. But, for now, the clock is ticking. Schweitzer has written or co-written 19 grant proposals the past two years. If one doesn't yield funding, she can't pay Wittmeyer's salary, and she has concerns about supporting the research of her eight graduate students. "I almost wonder if it is even right or ethical to encourage students to follow this line of investigation, because they are not going to have an easy road," she says. "What I tell my students is, 'If you do not have so much passion for what you would do, that you would do it for free, then don't do it. It is passion and intense curiosity that will give you the strength to just keep going even though people say you are nuts.' "

As she crosses her fingers for the funding to continue, Schweitzer's own passion is undimmed.

"There is just so much to learn," she says. "I remember when I first went to Jack, and he told me that I needed to go figure out the answers to my own questions, I said, 'Jack I'm just not creative. I can't come up with my own research problems. I mean, everything has been done already.' And now, my problem is just the opposite. I have so much trouble just thinking about one thing long enough to get it done, because for every piece of information I learn, I get 5 million new questions."

Now, she says, it's about finding a way to get them answered.

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