Defying conventional wisdom, Mary Schweitzer works to transform dinosaur paleontology into a molecular science. CYNTHIA MATTY-HUBER

‘I don’t care what they say about me’: Paleontologist stares down critics in her hunt for dinosaur proteins

By **Robert F. Service** | Sep. 13, 2017 , 10:30 AM

NORTHEASTERN MONTANA—The first day of Mary Schweitzer's 2017 dinosaur hunt isn't going well. The team has been searching under the high summer sun without success for the fossil-rich strata that braid through the arid rangeland here. Then, toward sundown, the aging Chevy Suburban in which she and four colleagues are riding erupts in a brief, poltergeist-like spectacle—with door locks jumping up and down and multiple dashboard warning lights flashing simultaneously. Finally, the car gives up the ghost and stops completely.

It's a rocky start to a week during which Schweitzer plans to crisscross vast swaths of private property looking for the northernmost outcrops of beds called the Hell Creek Formation. On nearby public land, these same beds have yielded scores of fossils of dinosaurs, but no pa ntc sts a nown to have scoured this section of rangeland, settled in the late 1800s. "We're really lucky to be out here," says Schweitzer, a dinosaur paleontologist at North Carolina State University (NC State) in Raleigh.

She takes the automotive adversity in stride, enjoying the sunset while others in her party phone for help. The challenges of fieldwork are minor compared with the storm of criticism she's endured for the central claim of her work: that her team has recovered fragments of proteins from dinosaurs as old as 80 million years.

The evidence, which she has laid out in a series of papers in *Science* and other journals, challenges traditional notions of what a fossil is: a stone replica of the original bone. If that "stone" includes proteins from the living animal, "I don't know what the definition is anymore," Schweitzer says.

More important, being able to analyze intact dinosaur proteins would transform paleontology into a molecular science, much as ancient DNA research has transformed the study of our human ancestors. "If soft tissue is preserved there is a whole bunch of stuff we can figure out," says Jessica Theodor, a paleontologist at the University of Calgary in Canada. "It's groundbreaking."

Researchers might be able to use molecular methods to work out the dinosaur family tree, and get answers to long-standing questions such as whether dinosaurs were warm- or cold-blooded and when feathers began to be used for flight. Schweitzer's discoveries, if confirmed, may amount to glimpses of dinosaurs in the flesh.

But no one except Schweitzer and her collaborators has been able to replicate their work. Although the study of ancient proteins, or paleoproteomics, is taking off, with provocative new results announced every few weeks, most findings come from samples thousands or hundreds of thousands of years old—orders of magnitude younger than Schweitzer's dinosaurs.

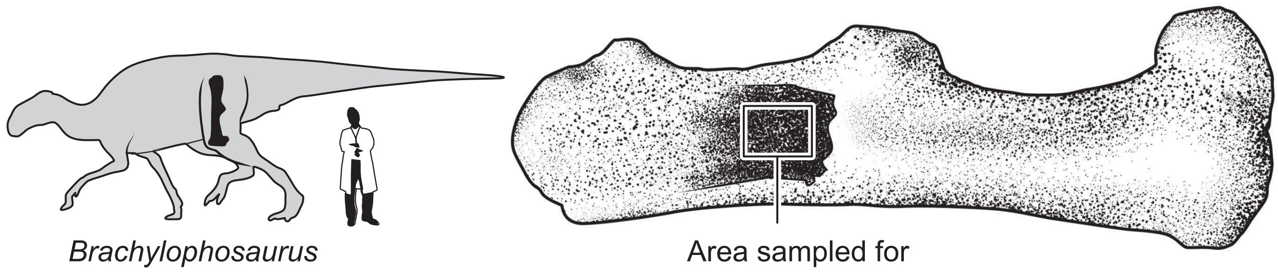
"I want them to be right," says Matthew Collins, a leading paleoproteomics researcher at the University of York in the United Kingdom. "It's great work. I just can't replicate it."

Others are harsher, and suggest that Schweitzer's protein pieces come from bacteria or contaminants. "It's problematic that no other lab has been able to replicate Mary Schweitzer's work," says Jakob Vinther, a paleontologist at the University of Bristol in the United Kingdom, who's tried to do so. "The idiom that exceptional claims require exceptional evidence remains," ad[illegible] Mic[illegible]l Bu[illegible], a paleontologist at the University of Manchester, also in the United Kingdom.

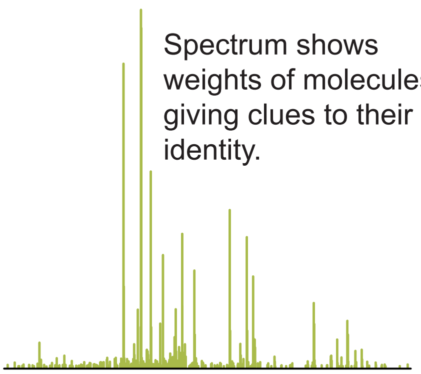
Schweitzer, who came to the field late, and whose unusual background casts her as an outsider in a field still dominated by men, isn't cowed. She has spent decades building her case. Now, on her Hell Creek expedition, she hopes to find new, well-preserved fossils that might harbor ancient proteins—and new evidence to convince the doubters. "I don't care what they say about me," she says. "I know my work is good."

How to hunt proteins in dinosaur fossils

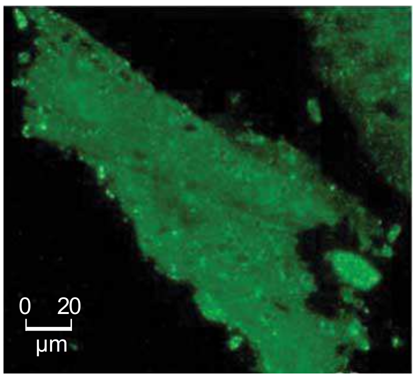
Mary Schweitzer and her colleagues first remove minerals from fossils such as a hadrosaur femur, leaving organic material behind. They then apply an array of optical, chemical, and biological methods to pinpoint ancient proteins, although the results are disputed.



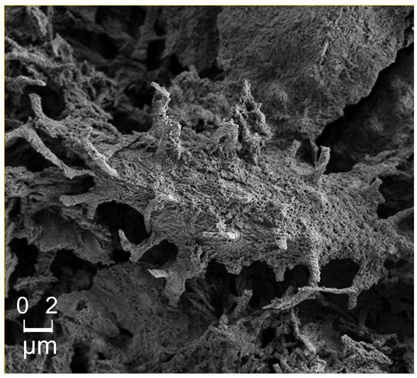
Controversial clues



Mass spectrometry
Identifies protein fragments and provides the sequence of amino acids that make up each fragment.



Antibody fluorescence
Lights up target proteins when fluorescent-labeled antibodies bind to them.



Microscopy
Reveals features in demineralized fossils. Here, what appears to be a bone cell nestles within a fibrous matrix that may be collagen.

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A third-generation Montanan, Schweitzer, 62, grew up outside of Helena as the youngest of three children in a conservative Catholic family. Her father, with whom she was very close, died of a heart attack when she was 16, and Schweitzer turned to fundamentalist Christianity for solace, er ddi herse eeply in her new community. She also rejected evolution and adopted the belief that Earth is only 6000 years old.

After earning an undergraduate degree in audiology, Schweitzer married and had three children. She went back to school at Montana State University in Bozeman for an education degree, planning to become a high school science teacher. But then she sat in on a dinosaur lecture given by Jack Horner, now retired from the university, who was the model for the paleontologist in the original *Jurassic Park* movie. After the talk, Schweitzer went up to Horner to ask whether she could audit his class.

"Hi Jack, I'm Mary," Schweitzer recalls telling him. "I'm a young Earth creationist. I'm going to show you that you are wrong about evolution."

"Hi Mary, I'm Jack. I'm an atheist," he told her. Then he agreed to let her sit in on the course.

Over the next 6 months, Horner opened Schweitzer's eyes to the overwhelming evidence supporting evolution and Earth's antiquity. "He didn't try to convince me," Schweitzer says. "He just laid out the evidence."

She rejected many fundamentalist views, a painful conversion. "It cost me a lot: my friends, my church, my husband." But it didn't destroy her faith. She felt that she saw God's handiwork in setting evolution in motion. "It made God bigger," she says.

In 1990, she volunteered to work in Horner's lab, slicing pieces of *Tyrannosaurus rex* bone into thin sections for analysis. Under a light microscope, Schweitzer saw groups of red circular structures that looked for all the world like red blood cells.

Schweitzer knew this amounted to paleontological heresy: According to the textbooks, when fossils form, all but the hardiest organic matter decays, leaving a mix of leftover minerals plus new ones that have leached in and taken the bone's shape. Meanwhile, the fragile chains of amino acids in proteins quickly fall apart. Feeling "somewhat terrified," Schweitzer didn't want to tell anyone, least of all Horner, what she'd seen under the scope.

She confided in a fellow graduate student, who spread the news. Horner caught wind and called Schweitzer in. "They are in the right place to be red blood cells," she recalls telling him. "But they can't be red blood cells. We all know that."

Horner stared at the slide himself for 5 to 10 minutes. "Prove to me they're not," he said.

Schweitzer says this moment was a turning point in her life. "It was the second most impactful thing anyone has ever said to me," she says. (The first was when her former husband called her work on dinosaurs "irrelevant.") "That's the way science should work. You can't prove something is true. But you can disprove it. I've been trying ever since to disprove it. I still haven't."

To chase the blood cell lead, Horner suggested that Schweitzer pursue her doctorate with him. She earned her Ph.D. in 1995, a few days prior to her 40th birthday. And she began publishing pa s w Horn nd others, laying out evidence that those apparent red blood cells were the visible sign of organic residues lurking in dinosaur fossils.

In their first paper, published in the *Journal of Vertebrate Paleontology* in 1997, Schweitzer, Horner, and colleagues reported that **spectroscopy and chemical analyses of extracts from a *T. rex* femur suggested preserved proteins**, including a form of collagen abundant in modern animal bones.

In 2005, Schweitzer, Horner, and two colleagues tried another technique. They dissolved away the minerals in a *T. rex* fossil sample; what remained, they reported in *Science*, were **structures that looked like millimeter-long blood vessels that flexed and stretched like real tissue when tugged by tiny tweezers**. Horner, now of the Burke Museum in Seattle, Washington, credits Schweitzer for the idea of demineralizing the fossil, a practice rare in paleontology but common for biologists studying modern bone. "The preconceived notion was nothing could possibly remain," he says.

Schweitzer's most explosive claim came 2 years later in two papers in *Science*. **In samples from their 68-million-year-old *T. rex***, Schweitzer and colleagues spotted microstructures commonly seen in modern collagen, such as periodic bands every 65 nanometers, which reflect how the fibers assemble. In another line of evidence, the team found that anticollagen antibodies bound to those purported fibers. Finally, **they analyzed those same regions with Harvard University mass spectrometry specialist John Asara**, who got the weights of six collagen fragments, and so worked out their amino acid sequences. The sequences resembled those of today's birds, supporting the wealth of fossil evidence that birds descend from extinct dinosaurs.

Others challenged the findings, suggesting that the structures seen under the scope might be bacterial biofilms, and that the mass spectrometry results might reflect contamination with modern bird collagen.

But Schweitzer's team pressed on. In 2009, she, Asara, and colleagues reported in *Science* that **they had isolated protein fragments from a second dinosaur, an 80-million-year-old hadrosaur**. Asara's lab identified eight collagen fragments. This time Schweitzer sent samples of fossil extract to an independent lab, which also detected three of the collagen fragments.

Collectively, the sequences showed the purported hadrosaur collagen was more closely related to *T. rex* and birds than to modern reptiles. "This proves the first [*T. rex*] study was not a one-hit wonder," Asara said at the time. Two labs also detected the proteins laminin and elastin with antibody tests, although mass spectrometry failed to turn up sequences for these proteins.

In 2007, Schweitzer's team excavated an 80-million-year-old hadrosaur in Montana. Later, they reported faint signatures of preserved protein fragments, but no independent group has managed to replicate those findings.
MARY SCHWEITZER

On day two of their fossil hunt, Schweitzer and colleagues get a slow start while the Chevy is towed and fixed. Finally, they are back in the field, fanning out over Hell Creek outcrops. Sixty-six million years ago, *T. rex* and *Triceratops* roamed a hot and humid landscape here, traversing a meandering river delta. Now, the ground is parched and exposed, so the paleontologists can spot bone peeking out from eroded hillsides.

Schweitzer scans the ground as she walks, explaining that previously collected fossils are likely contaminated with modern proteins from everything from bacteria to people. If she finds a new skeleton, she'll leave some of it encased in the surrounding material to keep modern contaminants out, and avoid applying the organic glues often used to hold fragile fossils together.

But after 8 hours of fossil hunting, the team spots only a few stray bone fragments. "No *T. rex* today," Schweitzer says.

She needs more fossils to quiet a continuing drumbeat of criticism. In addition to raising the specter of contamination, Buckley and others have argued that antibodies often bind nonspecifically and yield false-positive results. Critics also noted that one of the six amino acid sequences reported in the 2007 paper was misassigned and is likely incorrect. Asara later agreed and retracted that particular sequence.

"T' 's w 'ing," s Maria McNamara, a paleontologist at University College Cork in Ireland. "If you are going to make claims for preservation, you really need to have tight arguments. At this point I don't think we are quite there."

Buckley and colleagues also **dived deeper into the proteomes of ostriches and alligators**, as they reported on 31 May in the *Proceedings of the Royal Society B: Biological Sciences*. They found that a protein sequence in Schweitzer's data reported to be unique to dinosaurs actually matches a sequence from modern ostriches. So the purported dinosaur protein might be a contaminant from modern samples, Buckley says. "You can't rule it out."

Collins adds that Schweitzer's samples don't show the degradation expected in certain amino acids after so many millions of years; his work suggests that proteins could survive a million years or so at most.

Vinther's results also make him skeptical. He searches for organics in dinosaur fossils by using heat to break down molecules into volatile components and running them through a mass spectrometer. He has picked up signs of relatively stable organic molecules such as cholesterol and the pigment melanin, but he has never seen the tell-tale building blocks of proteins.

Schweitzer and her team have detailed ripostes to all of these critiques. NC State postdoc and mass spectrometry expert Elena Schroeter notes that the collagen seen in their dinosaur samples mostly lacks the amino acids Collins tracked. Where those particular amino acids are present, many are indeed degraded.

As for Vinther's criticism, Schweitzer says his method isn't suited to finding trace amounts of proteins, so it's unsurprising that he couldn't replicate her team's results. "They don't follow our techniques, and then they criticize us when they don't get the same results," she says.

She adds that her team is finding more than collagen: It has recovered sequences from eight proteins isolated from what appear to be blood vessels, all matching common vessel proteins such as actin, tubulin, and hemoglobin. It's hard to imagine that all stem from contamination, Schroeter says. "At what point does contamination become so unlikely that it's not a parsimonious explanation?" she asks.

In January, Schweitzer's team reported in the *Journal of Proteome Research* (JPR) that it had redone its 2009 analysis to answer the critics, **analyzing new pieces of bone from the hadrosaur and reworking their lab procedures to avoid contamination**. "We [had] left a full meter of sediment around the fossil, used no glues or preservatives, and only exposed the bone in an aseptic environment. [In the new study,] the mass spectrometer was cleared of contaminants prior to running the sample," Schweitzer says. The team identified eight protein fragments, two of which were identical to those found previously.

At the time, Enrico Cappellini, a paleoproteomics expert at the University of Copenhagen's Natural History Museum of Denmark, called the paper "a milestone." "The methodology and procedures … all were done at state-of-the-art levels." The evidence of protein sequences looks real, he said. "The implications are big."

Af[illegible] the [illegible]R par[illegible] some say they are puzzled by the persistent skepticism. "I don't get it," says Johan Lindgren, a dinosaur paleontologist from Lund University in Sweden, who has recently begun collaborating with Schweitzer. "It seems like there is a double standard," with some researchers ignoring Schweitzer's multiple lines of evidence while making their own bold claims with less backing. "She's extremely careful not to overstate what she's doing."

Theodor agrees. "I do think cultural factors play into it," she says, noting that few women hold senior positions in dinosaur paleontology. "I'm not saying the criticisms are off base, but they're more vitriolic than she deserves." She says Schweitzer should get enormous credit for pushing researchers to rethink their assumptions. "Even if she turns out to be wrong in some detail, she has stimulated a huge amount of work."

Back in the Montana rangeland, Schweitzer's voice sounds heavy as she discusses her critics, as though she's built up scar tissue from these encounters. "It's taken a bit out of me," she says. "Perhaps I'm not cut out for that part." The battles have taken a toll on her funding, too; her National Science Foundation grant runs out in the fall. "I worry constantly about keeping the lab going," she says.

But as she walks over an arid patch of Hell Creek, she perks up again at the prospect of discovery. "It's addictive," she says, scanning the ground for ancient bone. Thanks to a private donor, she's got money for another year and a half. And Capellini has agreed to analyze samples of dinosaur teeth in parallel with her lab, which might offer independent support for her claim that proteins can survive deep time.

So Schweitzer pushes on, walking briskly across the badlands in search of fossils, bits of protein, and, perhaps one day, acceptance. "I'm not much of a fighter," she says. "But I'm very stubborn."

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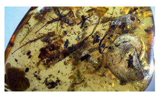

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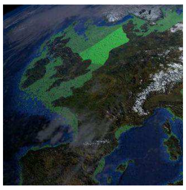

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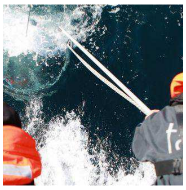

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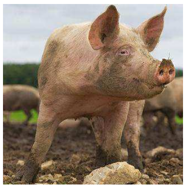

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