Several years ago, paleontologist Shaena Montanari was doing field work in Mongolia’s Gobi Desert when she noticed fragments of dinosaur eggshells in the sand. She knew them by distinctive lines and bumps on their surfaces. Montanari, then a graduate student at the American Museum of Natural History, and now a Royal Society fellow at the University of Edinburgh, realized she could use the fragments to understand the climate that these dinosaurs lived in about 80 million years ago.

That’s because an isotopic signature in the calcium carbonate shells—the ratio of oxygen-18 to oxygen-16—can tell researchers about the same signature in the water the dinosaurs drank. Such water isotopes, geochemists have shown, depend on how wet or dry a certain climate is. Montanari eventually found that the oviraptorid theropod dinosaurs that made those eggshells lived in arid conditions, much like the Gobi has today.

Montanari wasn’t the only one to use these fossilized shells as a window to the past. Chemist Robert Eagle, then of California Institute of Technology, realized that the eggshells might also shed light on an ongoing debate in paleontology: whether dinosaurs were warm-blooded like birds and mammals or cold-blooded like reptiles. Eagle, now at the University of California, Los Angeles, was inspired by a relatively recent advance called clumped isotope analysis, which can accurately predict the temperature at which a carbonate mineral was formed. Pioneered by John M. Eiler and colleagues at Caltech, it has been used to more accurately reconstruct temperature deep into the past by analyzing carbonate in ancient corals and shells in ocean sediments.

Through signatures preserved in eggshells, bones, teeth, and even the molecular remains of primeval plants, researchers are piecing together the physiology and diet of ancient animals and humans, and figuring out how climate and environmental changes might have influenced evolution and extinction events.

Eagle thought clumped isotope analysis on the oviraptorid theropod eggshells could tell him about the body temperature of the creature that formed them. In clumped isotope analysis, scientists use a mass spectrometer to simultaneously measure the stable isotopes of carbon and oxygen in carbon dioxide derived from a carbonate sample. They then determine how often the different isotopes are paired together in the carbonate material. The temperature at which carbonate is formed influences how often certain carbon and oxygen isotopes join up in the crystal, a phenomenon called isotopic “clumping”.

Eagle first validated his hunch by analyzing eggs laid by modern birds. He then visited Montanari at the museum and worked with her to study the Gobi eggshells, as well as ones from titanosaurid sauropods, plant-eaters that resemble Brontosaurus, found in Argentina.
However, scientists need to go further back in time, at least where an animal is positioned in the food chain. The diet of corn or cow, or what ecologists call trophic level, can be ambiguous. For example, nitrogen in collagen can help further distinguish between a wheat-based diet and a corn-based diet. Meanwhile, the isotopic signature of carbon-12, because of this, isotopes preserved in collagen, found in bones and teeth, for dietary clues. That’s because plants known as C4 plants, including most grasses, have a different photosynthetic metabolism than most other vegetation, so-called C3 plants. This gives C4 plants like corn a higher ratio of carbon-13 to carbon-12. Because of this, hair keratin or bone collagen from people eating a corn-based diet—whether on the cob or from a burger made from a corn-fed cow—is infinitesimally heavier than that from people who have a wheat-based diet. Meanwhile, the isotopic signature of nitrogen in collagen can help further distinguish between a diet of corn or cow, or what ecologists call trophic level—where an animal is positioned in the food chain.

To tease apart questions of human and animal evolution, however, scientists need to go further back in time, at least 2 million years, to “answer fundamental questions about how our species came to be”, says biological anthropologist Matt Sponheimer of the University of Colorado, Boulder. They must work with stuff that sticks around longer than collagen or keratin, like the mineral apatite in bones and teeth. Carbon isotopes in apatite indicate the plants at the base of the diet, but without a record of nitrogen—not present in apatite—trophic level can be ambiguous.

For samples so old that nitrogen is no longer preserved, researchers including Dominy and Vincent Balter of Ecole Normale Supérieure de Lyon have been investigating some nontraditional alternatives, including isotopes of magnesium and calcium in the apatite of bones and teeth, as well as iron, copper, and zinc isotopes in that of bones. Balter and colleagues have recently shown that some of these signatures are linked with trophic level in modern mammals.

Although it’s a powerful technique, isotope analysis can’t provide all of the answers. “One of the fundamental problems with this kind of work in biological systems is that biology is messy”, Sponheimer says. For example, Dominy wanted to figure out the diet of the earliest primates, who lived 30 to 40 million years ago, and thought calcium isotopes in bone apatite might be a good trophic level indicator. Researchers have shown that the bone apatite of carnivorous dinosaurs, which may have absorbed some calcium from eating raw bone, has different calcium isotope signatures than that of plant-eating dinosaurs.

To test the approach, Dominy and colleagues went to Costa Rica and Borneo to study some of the closest living relatives of the earliest primates, to see if they could distinguish the calcium isotope signatures of herbivores, omnivores, and carnivores. They analyzed calcium isotopes in the bone apatite of plant-eaters, including lemur-like colugos, and omnivorous tree-shrews—small mammals that resemble squirrels. The researchers compared the signatures with those of meat-eaters, including leopard cats in Borneo, and ocelots, mountain lions, and jaguars in Costa Rica.

Collectively, the cats had a similar calcium isotope signature to those found for carnivorous dinosaurs, suggesting some bone consumption. But the calcium isotopes of the tree-shrews, which eat insects, plants, and small vertebrates, were indistinguishable from those of the herbivores. Dominy thinks this might be because insects, like plants, don’t have much calcium. “That’s a big bummer,” he says, “since we think the earliest primates, the fossils we wanted to focus on next, were probably eating insects and plants—so calcium would probably fail to distinguish between them.”

Paleontologist Shaena Montanari (shown holding fossilized bones) collected dinosaur eggshells in the Gobi Desert and analyzed them to understand ancient climates. Credit: Courtesy of Shaena Montanari.
Climate Clues

Isotopes preserved in resilient molecules made by ancient plants and algae are also helping scientists understand how climate and vegetation may have shaped human evolution. For example, carbon isotopes in long-chain alkanes, found in leaf waxes, reflect the type of vegetation. Their hydrogen isotopes can act like the oxygen isotopes in carbonates and describe how wet or dry the climate was. Clayton Magill of the Swiss Federal Institute of Technology, Zurich (ETH), and Katherine H. Freeman of Pennsylvania State University have recovered these molecules from lake sediments to chart vegetation and rainfall in Olduvai Gorge at a major evolutionary juncture, the emergence of our immediate ancestor, *Homo erectus*, about 1.9 million years ago.

They found that there was a relatively abrupt change from a forested landscape that offered water and shade to a more arid grassland over a few hundred to a thousand years. “Whatever organisms were living there had to be really adaptable to the environment around them. They had to be able to do something that fit both types of ecosystems”, Magill suggests. “Because these organisms had to use some sense of cognition, there was an impetus for learning new behaviors and having new physical adaptations.” He thinks this may have helped drive the evolution of increased brain size and leg musculature, as well as tool use, seen with the rise of *H. erectus*.

Meanwhile, Matthew J. Wooller at the University of Alaska, Fairbanks wants to know whether climate changes caused the last woolly mammoths in North America to go extinct. Whereas most mainland mammoths died out about 11,000 years ago, the population on a remote island in the Bering Sea called St. Paul Island remained until about 5,600 years ago. “It’s a real paleo detective story”, he says. To investigate, he and many collaborators recovered a sediment core from a lake on the island spanning that time period and beyond.

Reconstructing past climate from the core presented them with a challenge, however. Carbonate, found in the shells of bivalves known as ostracods, is rarely preserved in arctic lakes due to acidic conditions at many sites. “We had to be more creative in what we measure isotopes on”, he says.

But lots of flies and mosquitoes live in arctic lakes. Wooller found an alternative paleoclimate proxy in the chitin head capsules of mosquitoes called chironomids, which preserve the isotopic signature of the oxygen and hydrogen from the water they’re living in. The scientists have painstakingly picked the insects’ tiny bodies out of the St. Paul Island core. At just a few millimeters wide, it takes 50 to 100 of them to generate one analysis. Wooller says the results show “lots of wonderful things”, and they are awaiting publication to elaborate.

Meanwhile, he plans to apply another isotopic proxy to understand the migration patterns of mammoths, ancient horses, and ancient bison, which all went extinct in Alaska. The ratio of strontium-87 to strontium-86 varies with geology across landscapes, and this signature is incorporated into annual growth layers in teeth—or, for mammoths, tusks—when animals drink water or eat food from the soil. As a result, it’s a kind of an isotopic GPS. Wooller’s group recently teamed up with researchers at the University of Utah to generate the first baseline map of strontium isotopes across Alaska. “The variation on the landscape is huge”,

Scientists from the University of Alaska, Fairbanks, take a sediment core from Alaska’s Lost Lake to reconstruct past environmental conditions. Shown (left to right) are Matthew Wooller, Joshua Reuther, Katherine Mulliken, and Cassidy Philos. Credit: Juliette Funk.
he says. “If something is moving around on that landscape, then that can translate into isotopic variability in teeth.”

Now, in collaboration with the Alaska Department of Fish & Game, they are planning to work with a herd of wood bison recently introduced into the state. When animals from the herd die, the researchers will analyze the animals’ teeth for strontium isotopes, providing conservation biologists with another way to track the herd and corroborating the method’s use in fossils. “My heart is driven by wanting to know what some of these ancient fauna were doing in the past”, Wooller says. But only research on modern animals, he says, can validate what’s written in the isotopes of creatures that lived long ago.

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