With a wealth of good specimens now at their disposal, paleontologists are probing elegantly preserved fossil bone tissue for clues to how long-extinct animals grew and lived

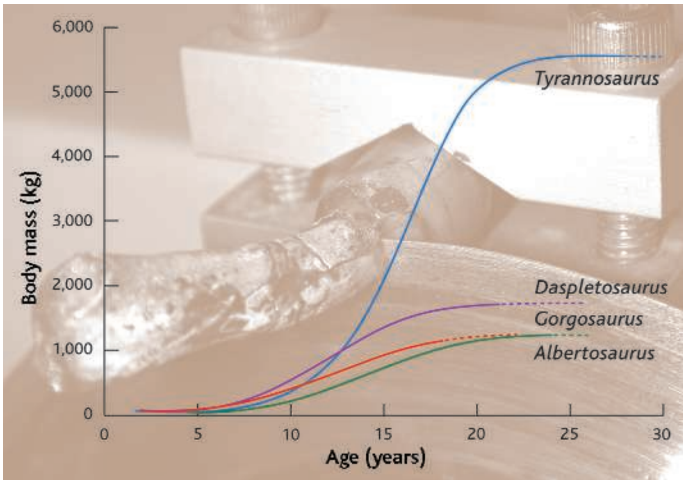
Dinosaurs Under the Knife

With high-domed skulls built like battering rams, dinosaurs called pachycephalosaurs look for all the world as if they must have butted heads. Paleontologists imagined the males sparring for mates as bighorn sheep do, and the idea was bolstered by radiating bony structures that apparently strengthened the head against impacts. But did they actually knock noggins?

To find out, Mark Goodwin of the University of California, Berkeley, and John Horner of Montana State University in Bozeman did something that would give most museum curators the heebie-jeebies: They sawed open the skulls to examine the fossilized bone tissue. The answer was trapped within the domes, Goodwin says, and histology—the study of tissues—was the only way to get it.

Preserved in the bone, as in many fossils, was a beautiful record of the original tissue, down to the level of individual cells. That's beyond the resolution of computed tomography scanners. By studying pachycephalosaurs of various ages, Horner and Goodwin determined that the radial structures were ephemeral features associated with fast-growing bones of juveniles. There was no sign of stress to the skull bones, they reported in the spring issue of *Paleobiology*. "I didn't see any evidence that they head-butted," Goodwin says. However, he and Horner did find bundles of so-called Sharpey's fibers, which anchor ligaments and also thick pads of keratin to bone. Horner and Goodwin speculate that this may have secured a crest to the top of the head, perhaps for display.

More and more paleontologists are putting their fossils under the knife—the rock saw, actually—to gain new insights into their biology. "The microstructure includes a tremendous amount of information," says Armand de Ricqlès of the Université Paris VII. After removing a slice of bone, they glue it to a glass slide and then grind it until it is transparent. Studying this "thin section" of bone tissue with microscopes can explain the origin of strange structures, such as the thick heads of pachycephalosaurs and the plates of stegosaurs, and help test hypotheses about their function. "I get quite excited about the potential of using bone microstructure to flesh ancient animals out and make them more real," says Anusuya Chinsamy-Turan of the University of Cape Town, South Africa.



Supersize me. Bone-based growth curves show how *T. rex* dwarfed its kin.

Paleohistology is already shedding light on the question of how sauropods and tyrannosaurs attained their gigantic sizes and other evolutionary patterns. It can tell adult animals from juveniles, and it provides the only way to determine how old extinct animals were when they died and how quickly they grew—key questions for studying their population biology and ecology. "We're on the cusp of being able to learn a lot about the biology of these animals—things we thought we'd never be able to tease out of the bones," says Lawrence Witmer of Ohio University College of Osteopathic Medicine in Athens. This week at the Society of Vertebrate Paleontology (SVP) annual meeting in Denver, Colorado, paleontologists unveiled a bumper crop of histological studies, from a possible determination of the sex of a *Tyrannosaurus rex* to identification of an island of dwarf sauropods. "This field is about to explode," says paleobiologist Gregory Erickson of Florida State University, Tallahassee.

Diverse tissues

Paleohistology has a long history. For 150 years, paleontologists have used the technique to classify ancient fishes. But not until the early 20th century did they begin to compare the microstructure of various fossil land animals. Studies by Rodolfo Amprino of the University of Turin, Italy, led to an observation in 1947 that is now called "Amprino's rule": The rate of an animal's growth strongly influences the type of tissue deposited in its bones. A rapidly growing bone has many blood vessels. Its characteristic "fibrolamellar" texture is marked by quickly deposited fibers and holes that are then filled by bony structures called primary osteons. In contrast, a bone growing more slowly has a texture called lamellar-zonal with fewer blood vessels and a finely layered appearance.

Dinosaurs typically have bone tissue that more closely resembles the quickly growing bones of large birds and mammals than the slow, lamellar-zonal tissue of reptiles. In 1969 and in later papers, de Ricqlès suggested that the dinosaur bone tissue might indicate fast, continuous growth and an active metabolism like that of mammals and birds. This idea played an important role in the "renaissance" that changed the perception of dinosaurs from sluggish reptiles to active, possibly warm-blooded animals. Tissue type turned out not to be a simple indicator of metabolism, but it does indicate the general pace of growth.

Bone tissue offers another way to understand the growth of ancient animals. Bones

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sometimes lay down dark lines, called lines of arrested growth (LAGs), which represent periods when growth slowed or stopped for a while. LAGs are common in amphibians and reptiles, but modern birds typically lack them because they complete their growth in less than a year. In 1981, Robin Reid of Queen's University of Belfast reported that dinosaurs showed the lines, too. By counting them like tree rings, paleontologists can infer how many years a bone has grown, and by extension how long the dinosaur lived. This technique of skeletochronology is now widely used by biologists studying modern reptiles and amphibians thanks to Jacques Castanet and others in de Ricqlès's laboratory in Paris, which has trained many paleohistologists.

Interpreting fossils can be tricky. For one thing, an animal's body continually dissolves primary bone—to extract calcium or to repair microfractures—and then deposits secondary bone, erasing the bone's early history. To account for missing LAGs, researchers must make assumptions about their spacing and about bone deposition rates—no simple task, because in living animals, deposition rates vary widely from species to species and even between bones in the same individual. Temperature and diet affect bone growth, too.

One solution is to look at many specimens of various ages, so that juvenile bone fills in the missing picture for adults. "As long as I have enough individuals and a diversity of bones, I can reconstruct what was going on," says Kristi Curry Rogers of the Science Museum of Minnesota in St. Paul. By counting LAGs, researchers can assemble a series with individuals of various ages. Then, using techniques for estimating an animal's mass from the size of its bones, they plot how various types of dinosaurs typically grew over time. Most growth series are partial, but the hadrosaur *Maiasaura* is known from embryo to adult.

When growth curves were published in the 1990s, they revealed startling facts about dinosaurs. In a 1999 *Journal of Vertebrate Paleontology* paper, for example, Curry Rogers showed that the giant sauropod *Apatosaurus* reached full size—25 meters long—in just 8 to 11 years, not the decades that had long been assumed. "People would have laughed!" says Kevin Padian of the University of California, Berkeley. The quick growth rate complicates a long-standing puzzle: How did sauropods, with their relatively small mouths and simple teeth, manage to get so big, particularly during the Jurassic, when only cycads and other plants of meager nutrition were growing?

Researchers have also established a general pattern for dinosaurs, compared to other groups. In a pair of 2001 *Nature* papers, two groups—Padian's team and Erickson and colleagues—used different techniques to plot growth curves for several dinosaur species. Both concluded that dinosaurs grew faster than reptiles. The larger dinosaurs packed on weight at a pace comparable with that of mammals, but none grew as blindingly fast as modern birds do. Growth curves also show that, like birds and mammals, dinosaurs grew fast when young, then slowed down and stopped growing as adults. By contrast, nondinosaurian reptiles such as crocodiles grow more slowly.

Core research. Martin Sander extracts a paleohistology sample from a mammoth bone.

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Growth curves have been used to investigate how dinosaurs evolved various patterns of growth. In August, Erickson and several co-authors reported in *Nature* how *T. rex* evolved to its formidable size, relative to other tyrannosaurids (*Science*, 13 August, p. 930). Rather than extend its growth phase, *T. rex* accelerated its adolescent growth spurt—packing on up to 2 kilograms a day. Sauropods show similar changes, according to a paper by Martin Sander of the University of Bonn, Germany, and colleagues, in press at *Organisms, Diversity & Evolution*. "That's not information you can get from gross anatomy," notes Allison Tumarkin-Deratzian of Vassar College in Poughkeepsie, New York. "The only way you can pin down accelerated rates of growth versus extended period of growth in the fossil record is by looking at histology."

Giants and dwarfs

As a rule, dinosaurs and other vertebrates evolved to get bigger through the ages. But the bones show that some bucked the trend. At this week's SVP meeting, Sander and Octavio Mateus of the Museum of Lourinha in Portugal and colleagues announced that small sauropods discovered in Germany are not juveniles but "the first unequivocal case of dwarfing for any dinosaur." The 10 individuals range in size from 1.8 to 6.2 meters long—much smaller than the 23-meter-long brachiosaurids to which they are closely related—but the tightly spaced growth lines in their bone tissue clearly show that they were full grown. The growth curve, based on seven leg bones, suggests that the dwarfs may have been sexually mature at as young as 2 to 3 years of age.

The dwarfs lived about 150 million years ago, on an island about half the size of New Zealand. So they could provide new data about the relationship between land area and the maximum size of animals. Curry Rogers and colleagues are working on the histology of other possible "island dwarf" sauropods, titanosaurs from Argentina and Romania.

Birds are another group that reduced their body size relative to their dinosaurian ancestors, and histology is helping researchers figure out how that happened. By comparing their tissue with those of the most birdlike dinosaurs, Padian and others have argued that they shrank by shortening the amount of time they spend growing most rapidly. (Most birds reach full size within a few weeks.) "It's a very smart idea," says Luis Chiappe of the Natural History Museum of Los Angeles County. So even though they grow at a faster rate than dinosaurs, they end up smaller—which would have been a key

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step toward evolving the ability to fly.

Following up on research begun by de Ricqlès in the 1970s, Chinsamy-Turan is also looking at the beginnings of another fast-growing group: the mammals. Research on their therapsid ancestors shows that some of these so-called mammallike reptiles were still growing like reptiles, while others show some distinct evidence of more mammalian growth patterns. Now she's looking at Mesozoic mammals from the Gobi desert. "I have begged and really pleaded" to get access to these rare specimens, she says, to compare them to modern mammals.

Dino tissue. Vessel-rich *Troodon* leg bone (*above*) grew faster than a riblike *Deinonychus* bone (*inset*).

CREDITS: (TOP TO BOTTOM) GREGORY ERICKSON; JOHN HORNER

Paleo-exotica

Sometimes zeroing in on ancient bones turns up exotic results. At the SVP meeting, Horner, and Mary Schweitzer and Jennifer Wittmeyer of North Carolina State University in Raleigh, described tissue, never reported from a dinosaur before, from the femur of a *T. rex*. The tissue has a random structure and is much richer in blood vessels than surrounding tissue. The researchers propose that it functioned like tissue that female birds use to store calcium for making eggshell. If so, it would be the first time paleontologists have determined gender—and reproductive status—from a dinosaur bone. Some skeptics, however, think the tissue structure might be the result of injury or disease.

Other novel tissues have been reported from flying reptiles. Pterosaur bones have plywoodlike tissue made of layers stacked so that bone fibers run at right angles in alternate layers. Such crisscrossing structures are common in fish scales, but Horner, Padian, and de Ricqlès were the first to describe them in a four-limbed vertebrate. In 2000 in the *Zoological Journal of the Linnean Society*, they speculated that the tissue is an adaptation for the biomechanical demands of flight.

Another unusual tissue has been found in squat, armored dinosaurs called ankylosaurs. Examining the bony plates called scutes, Sander and Torsten Scheyer, also of the University of Bonn, found bundles of structural fibers arranged parallel, perpendicularly, and obliquely to the scute surface—a light, strong design that would have resisted impacts from all directions, they speculate in next month's issue of the *Journal of Vertebrate Paleontology*. "It's a highly developed, composite material, like a bulletproof vest, that would prevent penetration of sharp objects," Sander says.

Histology can also be used to test hypotheses about the function of bizarre structures that no longer exist in the world. *Stegosaurus* plates have long attracted attention, and a prevalent idea is that they were used to regulate body temperature. Horner, Padian, de Ricqlès, and Russell Main, now a graduate student at Harvard University, decided to test that idea. After making thin sections of stegosaur plates and the smaller scutes of related dinosaurs, the team discovered that Stegosaur plates had evolved simply by expanding the keel of scutes. "We saw nothing special about the stegosaur plates" that would be an adaptation for thermoregulation, Main says. Moreover, structures originally described as blood vessels probably weren't. The plates were probably used instead for species recognition, the group proposes in a paper in press at *Paleobiology*.

Indirectly, bone histology can even shed light on long-vanished animals' behavior. Curious about whether baby hadrosaurs would have stayed in the nest or struck out on their own after hatching, Horner's team looked at the bone tissue of *Maiasaura* embryos, as well as embryos of alligators and ratite birds. "We didn't have much evidence until we looked at the histology of the bones," Horner says. Unlike the ossified bones of alligators and ostriches, the tissue at the end of the hadrosaur limb bones consisted of calcified cartilage, suggesting that hatchlings couldn't walk immediately. They reported these findings in the *Journal of Vertebrate Paleontology* in 2000.

Walking, running, jumping, flying: Bones actively respond to the stresses of these and other physical activities. On the one hand, this can complicate the interpretation of bone tissue when researchers are trying to establish growth rates. But because physical activity affects bone, it may also be possible to extract that history from bone tissue, for example by studying the orientation of the strutlike trabecular tissue inside bones, which is often oriented perpendicularly to the major axis of strain. "It's tricky and requires a certain amount of interpretation," cautions John Hutchinson of the Royal Veterinary College in London. "There's still a lot of work that needs to be done in modern animals to see how strain impacts bone remodeling."

A good amount of that work is going on. For example, Main is studying goats to determine how biomechanics affects their bone histology. He hopes to find signals that could enable fossils to reveal posture, among other details. Other researchers are seeking similar clues in alligators, crocodiles, and birds. "Modern animals are some of the great unsung heroes of dinosaur paleontology," says Curry Rogers.

Better known heroes are playing a key role too, especially when they are abundant. Horner, for example, continues to mine a rich deposit of hadrosaurs, with individuals of all ages and sizes. "We're cutting hundreds and hundreds of slides," says Horner, who has a technician working on histology full-time. Once his group and others nail down what's normal for bone tissues, they may be able to probe the many influences that affect bone, extracting information about sexual dimorphism, climate, gait, and much else. "We've just begun to scratch the surface," says Chinsamy-Turan.

–ERIK STOKSTAD

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