Gender-Specific Reproductive Tissue in Ratites and Tyrannosaurus rex

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Unambiguous indicators of gender in dinosaurs are usually lost during fossilization, along with other aspects of soft tissue anatomy. We report the presence of endosteally derived bone tissues lining the interior marrow cavities of portions of Tyrannosaurus rex (Museum of the Rockies specimen number 1125) hindlimb elements, and we hypothesize that these tissues are homologous to specialized avian tissues known as medullary bone. Because medullary bone is unique to female birds, its discovery in extinct dinosaurs solidifies the link between dinosaurs and birds, suggests similar reproductive strategies, and provides an objective means of gender differentiation in dinosaurs.

A relatively small (femur length, 107 cm) Tyrannosaurus rex [Museum of the Rockies (MOR) specimen number 1125] was discovered at the base of the Hell Creek Formation (dated at ~70 million years ago) as an association of disarticulated elements with excellent preservation (1). At death, MOR 1125 was estimated to be 18 ± 2 years (2), on the basis of lines of arrested growth (LAG).

Interior femur fragments from MOR 1125 were reserved without preservatives for chemical and molecular analyses. Gross examination revealed a thin layer of bone tissue lining the inner (medullary) surfaces of the bone fragments that was structurally distinct from other described bone types (Fig. 1D) but possessed characteristics in common with avian medullary bone (MB).

MB is an ephemeral tissue, deposited on the endosteal surface of avian long bones (3–10). Its formation in female birds is triggered by increasing levels of gonadal hormones produced upon ovulation (4, 10, 11), but it can also be artificially induced in male birds by the administration of estrogen (3, 4, 12). Because MB is densely mineralized and extremely well vascularized, it provides an easily mobilized source of calcium necessary for the production of calcareous eggshells (13). We compare MB from emu and ostrich (14) at different stages of the laying cycle with newly identified dinosaur tissues, because these basal birds share more primitive features with nonavian dinosaurs than do extant neognaths (15–18).

Our investigations show that ratite MB differs from that seen in better-studied neognaths. We observed substantial variation between emu and ostrich MB tissues and between both ratites and reported neognath tissues (Fig. 1 and fig. S1). MB (Fig. 1) may be thick (chicken and ostrich) or quite thin (emu) at midshaft; and it may be separated by a distinct layer of endosteal laminar bone.

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(ELB) as described by Chinsamy et al. (19) (chicken and emu), or not (ostrich). The innermost layer of MB in the ostrich [adjacent to the endosteal surface of cortical bone (CB)] appears to arise from dense sheets to form tubular structures that parallel the long axis of the bone (Fig. 1). Thin hairlike spicules (Fig. 1C) of mineralized bone protrude from the tubes and may be intimately involved in their formation from the basal layer. Mineralized spicules were also noted arising from emu MB (Fig. S2), but the tubelike structures were not so apparent or distinct. The MB tissues are morphologically distinct from overlying CB and are similarly distributed in both dinosaur (Fig. 1D) and ratite (Fig. 1, E and F) samples. Higher magnifications of T. rex (Fig. 1G) and ratite (Fig. 1, H and I) tissues show the open, crystalline, and fibrous structure of these highly vascular tissues, in contrast to the denser CB.

In a fresh fracture, dense and relatively homogenous dinosaur CB is distinct from the loosely organized and highly vascular MB internal to it (Fig. 2A). A distinct layer corresponding to ELB (19) separates the two bone types. A large erosion room is visible at this boundary, lined with laminar tissue. An emu bone fragment (Fig. 2B) in similar orientation shows MB tissues with a distinctive, less organized and “crumby” texture relative to overlying CB. It is interspersed with or laid down between large erosion rooms within the deep cortex and ELB of the tibial shaft. The dense cortex and laminar structure of the ELB are easily distinguished from surrounding MB. The ostrich MB (Fig. 2C) differs in both texture and orientation, with open cavities that are bordered by tubelike bone spicules.

The lacy vascularity of the T. rex tissues (Fig. 2D) is consistent with the larger vascular canals and whorled pattern of the emu (Fig. 2E) and especially with the ostrich (Fig. 2F), where wide blood-filled sinuses separate forming bone spicules. In ground section (Fig. 2G), MOR 1125 femur cortical bone is characterized by well-developed multigeneration Haversian systems with obvious and defined cement lines, supporting a mature status for this dinosaur (2). A region of decreased vascularity and laminar structure marks the ELB. In contrast, the medullary tissues arising from the ELB are densely vascularized but show no evidence of Haversian remodeling or cement lines, indicating that this tissue is newly deposited, or younger bone. A fresh cut section of emu bone (Fig. 2H) in comparable orientation shows dense CB and vascular, crystalline, and loosely organized MB, separated by a thin, dense, and less vascular ELB. MB in the ostrich (Fig. 2I) is more extensive than in the emu samples, most likely because shell ing had not yet begun (14). No distinct ELB is visible. MB appears laminar rather than spiculated in Fig. 2I, because the tubules formed by bony spicules are oriented longitudinally rather than in cross section as in Fig. 2F, but it is the same tissue.

Additional pattern similarities are seen in demineralized (14) ratite (Fig. 3, B and C) and T. rex (Fig. 3A) medullary tissues. In all cases, the matrix is fibrous and randomly organized. The reddish color in extant tissues is due to blood retained in sinuses that separate the bone spicules. The T. rex tissues are similarly pigmented, due either to diagenetic alteration or to close association of bony tissues with blood-producing marrow during the life of the dinosaur.

In all MB tissues shown, large vascular sinuses are easily discerned (Fig. 3, D to F), but in the T. rex, vascular openings are surrounded by circumferentially oriented matrix
fibers (Fig. 3D) that are less apparent in extant bone. The ostrich medullary tissues are denser than either the emu (Fig. 3E) or T. rex samples, particularly closer to the cortex, but the variation in the size and density of vascular sinuses (Fig. 3F) is similar to that seen in the T. rex tissues. In planar view, MOR 1125 undemineralized tissues show a random orientation of fibers, and vascular openings penetrate deep into the tissues (Fig. 3G, inset) and exhibit an unusual doublet or triplet pattern, where multiple vessels penetrate an osteonlike core (arrows), also seen in the emu (Fig. 3H, arrows). The ostrich medullary tissues (Fig. 3I) are more variable, denser, and less random in appearance than those of the emu, but the morphology changes as the tissues extend into the medullary cavity. Close to the cortex (Fig. 3I, inset, and fig. S3), the bone is sheetlike, relatively dense, and punctured by vascular sinuses exhibiting the doublet pattern (arrows) noted above. As tissues extend into the medullary cavity (Fig. 3I), this pattern becomes obscured. Inset bone has been stained (14) for better contrast.

In regions of MOR 1125 bone where most of the medullary tissues have eroded (Fig. 3J), patches of denser CB can be seen, emphasizing the random mazelike pattern and large vascular sinuses of medullary tissues, a pattern also seen in the emu bone (Fig. 3K). The ostrich MB shows a similar pattern of bony spicules surrounding large and small blood sinuses (Fig. 3L).

Scanning electron micrographs (14) reveal the distinctive grainy texture and disorganized morphology of demineralized T. rex and avian MB (Fig. 4). This contrasts with the smooth and fibrous texture of demineralized CB from the same specimens (Fig. 4, E to H). Higher magnifications of demineralized CB (Fig. 4, I to K) emphasize the smooth, fibrous, and more ordered nature of all specimens, although in MOR 1125 (Fig. 4I), degradation is apparent.

MB occurs naturally only in extant female birds, although it varies in amount and distribution among taxa and with ovulatory phase (5, 20). It is chemically, functionally, and structurally distinct from both overlying CB and internal trabecular bone (21, 22). Although “medullary” and “trabecular” bone are terms often used interchangeably in the literature, MB has a larger surface area and is more vascular than other bone types, allowing rapid calcium mobilization (5). It is more highly mineralized, with a greater apatite-to-collagen ratio (5, 7, 20–22), and incorporates acidic mucopolysaccharides and glycosaminoglycans that are not present in CB (5, 11). Additionally, the matrix of MB is higher in noncollagenous proteins and lower in collagen, and has a higher collagen III-to-collagen I ratio (22) relative to other bone types. If preservation allows, these characteristics will be used as part of ongoing research to chemically distinguish the two bone types in this dinosaur.

The existence of avian-type MB in dinosaurs has been hypothesized (9, 23) but not identified. In part, this could be because of taphonomic bias, because the death and fossilization of an ovulating dinosaur would be comparatively rare. Additionally, MB in extant birds is fragile, the spicules separating easily from the originating layer (fig. S1). Dinosaur MB may separate and be lost from overlying CB in a similar manner during diagenesis.

The location, origin, morphology, and microstructure of the new T. rex tissues support homology with ratite MB. The T. rex tissues line the medullary cavities of both femora of MOR 1125, suggesting an organismal response. The tissues are similar in distribution to those of extant ratites, being more extensive in proximal regions of the bone. They are clearly endosteal in origin, and the microstructure with large vascular sinuses is consistent with the function of MB as a rapidly deposited and easily mobilized calcium source. The random, woven character indicates rapidly deposited, younger bone. Finally, the robustly supported relationship between theropods and extant birds (15–18, 24, 25) permits the application of phylogenetic inference to support the identification of these tissues (26, 27).

The morphology of these dinosaur tissues is not identical to that of extant neognaths...
A relatively thicker tyrannosaur would be expected. Fore, slight variations in bone and tissue types distinct in size, biomechanical constraints, finally, resorption. These factors may also contribute birds, resulting in less demand for bone calcium for rapid mobilization of skeletal calcium for reproduction. In extant birds, the retention of a primitive trait. This hypothesis may be tested by examination of the limb bones of the recently reported oviraptor containing eggs in the reproductive tract. The existence of MB in crocodiles has been referred to anecdotally, although phylogenetically, varying with both, sex steroid levels, and its presence in MOR 1125 may reflect the retention of a primitive trait. This hypothesis may be tested by examination of the limb bones of the recently reported oviraptor containing eggs in the reproductive tract. The existence of MB in crocodiles has been referred to anecdotally, although phylogenetically, 24.

References and Notes
14. Materials and methods are available as supporting material on Science Online.
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![Fig. 4. Scanning electron microscope images of demineralized MB (A) to (D)](Image 36x480 to 469x725) MB [A] to [D] and CB [E] to [K]. Demineralized, aldehyde-fixed MB tissues from (A) MOR 1125, (B) CB layering hen, (C) ostrich, and (D) ostrich show random, crumbly texture. Organized collagen fiber bundles are not distinct in any sample because of rapid deposition and woven character. Scale bars for (A) and (B), 40 μm; for (C) and (D), 20 μm. Demineralized fragments of cortical bone from (E) MOR 1125, (F) chicken, (G) ostrich, and (H) ostrich are shown. A fibrous character dominates all samples. Scale bars for (E), (F), (G), and (H), 30 μm; for (G), 10 μm. Higher magnification of demineralized CB from (I) MOR 1125, (J) ostrich, and (K) ostrich CB demonstrates the structural similarity between samples, although the MOR 1125 matrix is highly degraded. Scale bars for (I) and (K), 6 μm; for (J), 5 μm.

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Ivory-billed Woodpecker (Campephilus principalis) Persists in Continental North America

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The ivory-billed woodpecker (Campephilus principalis), long suspected to be extinct, has been rediscovered in the Big Woods region of eastern Arkansas. Visual encounters during 2004 and 2005, and analysis of a video clip from April 2004, confirm the existence of at least one male. Acoustic signatures consistent with Campephilus display drums also have been heard from the region. Extensive efforts to find birds away from the primary encounter site remain unsuccessful, but potential habitat for a thinly distributed source population is vast (over 220,000 hectares).

The ivory-billed woodpecker is one of seven North American bird species that are suspected or known to have become extinct since 1880 (1). One of the world’s largest woodpeckers, this species of considerable beauty and lore was uncommon but widespread across lowland primary forest of the southeastern United States until midway through the 19th century (2, 3). Its disappearance coincided with the systematic annihilation of virgin tall forests across the southeastern United States between 1880 and the 1940s. Relentless pursuit by professional collectors accelerated the species’ decline from 1890 to the early 1920s. The last well-documented population occupied a stand of old-growth bottomland hardwood forest in northeastern Louisiana (the Singer Tract) during the late 1930s (3–6). That population disappeared as the Singer Tract was logged amid cries for protection of both forest and bird. The final individual in the Singer Tract, an unpaired female, was last seen in cut-over forest remnants in 1944 (7).

A resident subspecies of ivory-billed woodpecker (Campephilus principalis bairdiff) occupied tall forests throughout Cuba, and a small population was mapped and photographed in eastern Cuba as late as 1956 (8). Fleeting observations of at least two individuals in 1986 and 1987 by several experts are widely accepted as valid (9), but repeated efforts to confirm the continued existence of that population have failed (10).

Anecdotal reports of ivory-billed woodpeckers in the southern United States continue to this day. Such reports are suspect because of the existence and relative abundance throughout this region of the superficially similar pileated woodpecker (Dryocopus pileatus). Three reports were accompanied by physical evidence, but their veracity continues to be questioned [supporting online material (SOM) text]. Thus, no living ivory-billed woodpecker has been conclusively documented in continental North America since 1944.

At approximately 13:30 Central Standard Time (CST) on 11 February 2004, while kayaking alone on a bayou in the Cache River National Wildlife Refuge, Monroe County, Arkansas, G. Sparling spotted an unusually large red-crested woodpecker flying toward him and landing near the base of a tree about 20 m away. Several field marks suggested that the bird was a male ivory-billed woodpecker (SOM text), and Sparling hinted at his sighting on a Web site. T. Gallagher and B. Harrison were struck by the apparent authenticity of this sighting and arranged to be guided through the region by Sparling. At 13:15 CST on 27 February 2004, within 0.5 km of the original sighting, an ivory-billed woodpecker (sex unknown) flew directly in front of their canoe with the apparent intention of landing on a tree near the canoe, thereby fully revealing its dorsal wing pattern. The bird instead veered into the forest, apparently landed briefly several times (each time blocked from the observers’ sight by trees), and then flew off (SOM text and fig. S1). Efforts to locate the bird over the next several days failed, but subsequent surveys by teams of experienced observers yielded a minimum of five additional visual encounters between 5 April 2004 and 15 February 2005 (SOM text). All seven convincing sightings were within 3 km of one another.

At 15:42 Central Daylight Time on 25 April 2004, M. D. Luneau secured a brief but crucial video of a very large woodpecker perched on the trunk of a water tupelo (Nyssa aquatica), then fleeting from the approaching canoe (fig. S2 and movie S1). The woodpecker remains in the video frame for a total of 4 s as it flies rapidly away. Even at its closest point, the woodpecker occupies only a small fraction of the video. Its images are blurred and pixillated owing to rapid motion, slow shutter speed, video interlacing artifacts, and the bird’s distance beyond the video camera’s focal plane. Despite these imperfections, crucial field marks are evident both on the original and on deinterlaced and magnified video fields (11) (fig. S3). At least five diagnostic features allow us to identify the subject as an ivory-billed woodpecker.

1) Size. When the woodpecker first begins to take flight from the left side of a tupelo trunk, two video fields reveal the dorsal surface of the right wing and a large black tail (Fig. 1). The minimum distances between the “wrist” and the tip of its tail—measured independently on each of the two video fields and compared to known scales (the diameter of the tupelo trunk at two places)—are 34 to 38 cm. These values exceed comparable values for the pileated woodpecker and correspond to the upper range for the ivory-billed woodpecker (fig. S4).