the fiber and possibly fill the SWNTs, forming charge-transfer complexes with individual nanotubes. The fact that solid sulfuric acid has a density of 2.13 g/ml (35) suggests that there is a similar increase in density for the ordered layer of sulfuric acid molecules surrounding the nanotubes (Fig. 3B). XRD data further support this interpretation.

In addition to XRD performed on dry fiber samples, data were also collected on a swollen SWNT fiber sample sealed in a glass capillary with 102% sulfuric acid. The pattern no longer showed the distinct Bragg reflections associated with SWNTs (4) in the neat fibers, and the scattering intensity at small angles diminished (Fig. 4), indicating that (i) the intercalation of SWNTs by sulfuric acid smeared out the triangular lattice, and (ii) the density, and therefore the electron density, of the acid-swollen fibers has become similar to that of the superrad. Instead, the scattering profile shows only highly anisotropic scattering from the pure 102% sulfuric acid (Fig. 4D) but with about the same degree of orientation (FWHM = 32.1°) as the dry fibers. This means that some of the acid molecules must be aligned with respect to the fiber axis and that the anisotropic scattering is from a cylindrical shell of perhaps three acid monolayers in which the mass density is somewhat enhanced with respect to the bulk liquid because of interactions with the nanotubes. These results further affirm our model of acid-intercalated SWNT ropes surrounding the nanotubes (Fig. 3B). XRD data on neat fibers, and the scattering intensity at small angles diminished (Fig. 4), indicating that the nanotubes. These results further affirm our model of acid-intercalated SWNT ropes surrounding the nanotubes (Fig. 3B). XRD data on neat fibers, and the scattering intensity at small angles diminished (Fig. 4), indicating that

28. A Renishaw MicroRaman System 1000 with a 780-nm diode laser was used to collect Raman spectra. A multilayer diffractometer equipped with a Cu rotating anode, double-focusing optics, evacuated flight path, and two-dimensional wire detector was used for XRD measurements.

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External and Internal Morphology of the BAR 1002’00 Orrorin tugenensis Femur


Late Miocene fossils from the lakeino formation in Kenya’s Tugen Hills are assigned to Orrorin tugenensis. Of 20 fossils recovered there to date, 3 are proximal femurs. One of these, BAR 1002’00, preserves an intact head connected to the proximal shaft by an elongated neck. Although this fossil is comparable in size to Pan troglodytes, computerized tomography scans of the neck-shaft junction of BAR 1002’00 reveal that the cortex is markedly thinner superiorly than inferiorly, differing from the approximately equal cortical thicknesses observed in extant African apes, approaching the condition in later hominids, and indicating that O. tugenensis was bipedal.

Orrorin tugenensis is thought to represent some of the earliest known hominids, securely dated biostratigraphically, geologically, and radiometrically, as well as by paleomagnetism, to the Late Miocene, approximately 6 million years ago (Ma) (1, 2). Samples (1) are derived from four localities in the Lukeino Formation (Aragai, Cheboit, Kapcheberek, and Kapsomjin), along the eastern approach to the Tugen Hills in Baringo District, Kenya. Twenty fossils representing at least five individuals have been discovered there. Of these, three are portions of femurs critical for determining posture. BAR 1215’00 is a small fragment consisting of the proximal portion of a right femur lacking neck and head and preserving only about 20 mm of the upper shaft below the base of the greater trochanter, which also is missing. BAR 1003’00 comprises approximately half of a proximal femur, including the entire lesser trochanter but lacking both the greater trochanter and the femoral head. The third partial femur, BAR 1002’00, is more complete, including about 200 mm of shaft plus an intact head that is connected to the shaft by a somewhat elongated neck; its anatomical features have been described fully and compared in detail with extant African apes and humans, as well as with Plio-Pleistocene hominids (1, 3, 4). The Tugen Hills material apparently is younger than the Chad cranium (Sahelanthropus tchadensis) from the Toros-Menalla locality (5), the age of which is estimated to be in the range of 6 to 7 million years from faunal correlations with East African sites.
(mainly Lothagam in Kenya), moderately older than the remains from Ethiopia’s Middle Awash valley, which are dated more securely than those from Chad by techniques including biostratigraphic, paleomagnetic, and radioisotopic data to a narrower range of 5.2 to 5.8 Ma (6). Beyond the different geographic and temporal relations, comparisons among the earliest putative hominids are exacerbated by the paucity of cranial elements in the Tugen material, absence of postcrania associated with the Chad cranium (5), and the inclusion of just one proximal pedal phalanx among the Middle Awash remains, along with dental elements that are said to display a suite of hominid features (6).

Despite their temporally intermediate position between the other two early sites that have yielded putative hominids, the Tugen Hills fossils sometimes still are characterized as ambiguous in the features of lower-limb anatomy shared with later members of our lineage (7). Here, we address this phylogenetic question directly through functional morphology. With upright posture and habitual or obligate bipedal locomotion accepted as critical adaptive signatures of our lineage, documenting anatomical correlates of these behaviors in BAR 1002’00 would support its hominid status. We used computerized tomography (CT) to quantify the internal distribution of cortical bone in the most ancient femora pertinent to reconstructing hominid origins.

BAR 1002’00 has several external morphological attributes that are characteristic of Plio-Pleistocene through later hominids and that distinguish them from African apes: a shallow trochanteric fossa, an obturator externus groove, and a long femoral neck (3, 8). The trochanteric fossa demarcates chimpanzee femurs from those of most past and present hominids, although its developmental genesis and functional consequences are as yet incompletely understood. In chimpanzees, the central portion of the trochanteric fossa commonly includes a cavity that extends deeply into the diaphyseal shaft, approaching the medullary canal in some specimens (8). In BAR 1002’00 as well as the other two partial femurs attributed to *Orrorin tugenensis*, in common with South African Plio-Pleistocene hominids, there is no evidence of deep penetration by the trochanteric fossa into the shaft, in contrast with the usual morphology observed in *Pan*.

The obturator externus muscle is an adductor, flexor, and external rotator of the thigh. One of its primary functions is to shorten the distance between the pelvis and the femur, and consequently to stabilize the hip joint. The obturator externus groove, present in BAR 1002’00, is believed to result from bone remodeling to accommodate the direct, although passive, contact of the obturator

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**Fig. 1.** 3-D model of BAR 1002’00 showing probe line along which BAR 1002’00 was sectioned (left), and the anterior-posterior section itself (right).

**Fig. 2.** (A) 3-D model of BAR 1002’00 reconstructed from CT sections. Probe line shows location of more proximal section. (B) Hounsfield plot with plateau at left indicates presence of some "white overflow," which occurs when all pixels have the maximum CT number (10). (C) Outline of section at interface of air to cortical bone. (D) Outline of section at interface of cortical to trabecular bone. Inferior cortical thickness (ICT) = 8.6 mm; superior cortical thickness (SCT) = 2.8 mm. Refer to Fig. 3 for notes regarding possible complicating effects of white overflow.
External tendon with the dorsal surface of the femoral neck at full extension of the femur. A sample of 155 African hominoids did not include a single example of an obturator external groove (8), whereas this feature is present in OH 20 from Tanzania, SK 82 and SK 97 from South Africa, and AL-333-95, AL 288-1, and MAK-VP-1/1 from Ethiopia (3, 8). Femoral neck length establishes the moment arm of the anterior gluteal muscles (gluteus minimus, gluteus medius, and tensor fasciae) in the pelvic-support phase of bipedal locomotion. Therefore, a long femoral neck, which implies a reorientation of the line of action of the anterior gluteals, is strongly indicative of habitual hominid locomotor function (8). In BAR 1002’00, the femoral neck length exceeds that of Miocene hominoid fossils attributed to *Afropithecus*, *Dryopithecus*, *Kenyapithecus*, *Nacholapithecus*, *Oreopithecus*, and *Ugandapithecus* (3). Among Plio-Pleistocene hominids, a long femoral neck recently has been reconstructed for the partial MAK-VP-1/1 femur (8).

The external attributes of bipedal locomotion summarized here, and more extensively elsewhere for the Tugen Hills femurs (3), have developmentally determined internal structural correlates. In particular, femoral neck length has functional importance due to the force-transmission pattern through the hip joint resulting from the mass of the trunk and head superior to it. Biomechanically, the structure of the femoral neck approximates a cantilevered beam. Among apes that assume a variety of postures during locomotion but are primarily quadrupedal, the femoral neck is relatively short. A cross section in the region of the neck-shaft junction (which biomechanically is the most relevant section) includes a central marrow cavity surrounded by trabecular bone extending outward toward the surface, which in turn is characterized by a relatively uniform dense ring of cortical bone. The outer cortical bone is thickened inferiorly due to compressive forces. Also, because cortical bone is weaker under tension than under compression, bone in the superior margin of the femoral neck is thickened as well, attaining dimensions that actually exceed those of the inferior margin in more than half (52%) of *Pan troglodytes* observed (9).

In contrast, among past and present hominids the femoral neck exhibits cortical bone that is much thinner superiorly than inferiorly (8, 9). This reduction in superior cortical thickness is chiefly due to the altered functional demands of hominid bipedal locomotion, which over time has modified the action of the abductor muscles (gluteus medius and minimus). In hominids, these muscles are aligned approximately parallel to the femoral neck so that their contraction compresses the bone, balancing the tension that produced the thickening of the superior cortex in apes. The result is that in hominids, cortical thickening is greatest along the inferior margin of the neck-shaft junction. At this point of highest bending stress, in extant humans, superior cortical thickness approximates one-quarter of inferior cortical thickness or less; in extant African apes, superior and inferior cortical thicknesses approximate a 1:1 ratio (9, 10).

We used CT scanning to display the interior structure of the more complete Tugen left femur, BAR 1002’00, which comprises the head, neck, and proximal shaft; an anterior-posterior CT scan of this fossil is shown in Fig. 1.

Overall, despite the presence of cracks and matrix in some regions, superior cortical bone consistently appears thinner than inferior cortical bone along the entire femoral neck from the head to the neck-shaft junction. We examined slices from the base of the femoral head to the trochanteric line; two are shown here (Figs. 2 and 3), and the remaining sections are in fig. S1. Each cortical thickness was calculated by the standard approach of using two thresholds (11, 12), the first at the air-bone interface (Fig. 2C and Fig. 3C) and the second at the cortical-trabecular bone interface (Fig. 2D and Fig. 3D) (13).

For measurements of cortical bone thickness in Figs. 2 and 3, the infero-superior (IS) axis was defined conventionally as a line connecting the most inferior and superior points on the outer cortex (9), measured where the axis transected the specimen’s cortical bone boundaries. In Fig. 2, C and D, although the probe is oriented to transect the thinnest portion of the inferior cortical bone, along the IS axis the ratio of inferior to superior cortical bone thickness is 3:1. In Fig. 3, C and D, because of the complex outlines of the cross section, three lines are shown: IS to the left is as previously defined, while IS to the right gauges cortical bone thicknesses at the midline of the medullary cavity. For both, the IS ratio is approximately

![Fig. 3. (A) 3-D model of BAR 1002’00 with probe at neck-shaft junction. (B) Corresponding Hounsfield plot. (C) Outline of section at interface of air to cortical bone. Projection at upper right is superior margin of the trochanteric fossa. Mass with circular outline at lower left is the lesser trochanter. (D) Outline of section at interface of cortical to trabecular bone. As defined conventionally, ICT = 11.0 mm and SCT = 5.0 mm; in center of medullary cavity, ICT = 8.6 mm and SCT = 4.0 mm; oblique, ICT = 14.0 mm and SCT = 4.0 mm. Inferiorly there is some "black overflow," which occurs when CT numbers drop sharply and then rise again, as well as "white overflow," which could arise from mineralization of trabecular bone. One study comparing scans with and without white overflow showed an average thickness overestimation of 23% (11). Unless mineralization occurred selectively in the inferior cortex, it is unlikely that mineralized cortical bone accounts for the observed ratio of ICT to SCT, particularly because BAR 1002’00 absolute ICT values are nearly equaled by some extant (i.e., unmineralized) *Gorilla* and *Homo* specimens (9), and BAR 1002’00 absolute SCT values are less than half many SCT values in *Pan* and *Gorilla*.](https://www.sciencemag.org/content/305/5684/1452.e1)

**Fig. 3.** (A) 3-D model of BAR 1002’00 with probe at neck-shaft junction. (B) Corresponding Hounsfield plot. (C) Outline of section at interface of air to cortical bone. Projection at upper right is superior margin of the trochanteric fossa. Mass with circular outline at lower left is the lesser trochanter. (D) Outline of section at interface of cortical to trabecular bone. As defined conventionally, ICT = 11.0 mm and SCT = 5.0 mm; in center of medullary cavity, ICT = 8.6 mm and SCT = 4.0 mm; oblique, ICT = 14.0 mm and SCT = 4.0 mm. Inferiorly there is some “black overflow,” which occurs when CT numbers drop sharply and then rise again, as well as “white overflow,” which could arise from mineralization of trabecular bone. One study comparing scans with and without white overflow showed an average thickness overestimation of 23% (11). Unless mineralization occurred selectively in the inferior cortex, it is unlikely that mineralized cortical bone accounts for the observed ratio of ICT to SCT, particularly because BAR 1002’00 absolute ICT values are nearly equaled by some extant (i.e., unmineralized) *Gorilla* and *Homo* specimens (9), and BAR 1002’00 absolute SCT values are less than half many SCT values in *Pan* and *Gorilla*. **
2:1. The third line (IS oblique), connecting the minimum and maximum points, exclusive of the lesser trochanter (lower left) and superior margin of the trochanteric fossa (upper right), shows a ratio of 3:1. All of these values for both slices diverge markedly from the \( \approx 1:1 \) ratio at the femoral neck-shaft junction for chimpanzees and gorillas (9) in the direction of extant human cortical bone proportions of \( \approx 4:1 \). Although some nonhomionid primates exhibit superior cortex that is moderately thinner than the inferior cortex, published values (10) were averaged along the femoral neck and are not directly comparable to the most diagnostic point at the neck-shaft interface (8, 9) reported here, where the IS ratio for the BAR 1002’00 femur is clearly distinguished from extant apes, particularly from Pan troglodytes, which is a close match in overall size for preserved portions of the fossil.

Our results show that the internal distribution of cortical bone in its femoral neck constitutes direct evidence for frequent bipedal posture and locomotion in this Late Miocene ancestor. In known features, external and internal, BAR1002’00 exhibits a total morphological pattern distinct from African apes, diagnostic of bipedal locomotion, and appropriate for a population standing at the dawn of the human lineage.

**References and Notes**


**Testing Predator-Driven Evolution with Paleozoic Crinoid Arm Regeneration**

**Tomasz K. Baumiller**1* and Forest J. Gahn2*

Regenerating arms of crinoids represent direct evidence of nonlethal attacks by predators and provide an opportunity for exploring the importance of predation through geologic time. Analysis of 11 Paleozoic crinoid Lagerstätten revealed a significant increase in arm regeneration during the Siluro-Devonian. During this interval, referred to as the Middle Paleozoic Marine Revolution, the diversity of shell-crushing predators increased, and antipredatory morphologies among invertebrate prey, such as crinoids, became more common. Crinoid arm-regeneration data suggest an increase in nonlethal attacks at this time and represent a causal link between those patterns, which implies an important role for predator-driven evolution.

Predation has been used to explain numerous macroevolutionary trends. For example, the hypothesis of escalation posits that prey evolve escape strategies (morphological, behavioral, or otherwise) as a consequence of interactions with their enemies. Vermeij (1, 2) has argued that biological hazards, including predation, have increased through geologic time and, as a consequence, so have prey responses. This general trend has not been uniform: Several intervals of intensified escalation have been suggested, including the early Cenozoic (2), the Mesozoic (3), and the middle Paleozoic (4, 5).

During the middle Paleozoic, the diversity of shell-crushing predators, especially arthropods and fishes, increased sharply and concomitantly with defensive features among mollusks, brachiopods, and crinoids, an event known as the Middle Paleozoic Marine Revolution (MPMR) (4, 5). Crinoids, which developed more spines, thicker calyx plates, and reduced viscera through this interval (Siluro-Devonian), provide an important test of defensive features among Paleozoic crinoids. Between intervals, we recorded the number of visible arms and the number of regenerating arms. Regeneration was recognized by an abrupt change in arm diameter (Fig. 1).

Our data (Fig. 2 and table S1) show that the percentage of individuals with regenerating arms (i.e., regeneration frequency) in the early Paleozoic (Ordovician and Silurian) was \(<5\%\) but increased in the middle to late Paleozoic (Devonian to Pennsylvanian) to \(>10\%\). The increase spanning the Siluro-Devonian is the only statistically significant increase in the entire time series (\(\chi^2\), \(P < 0.001\)). The increase in regeneration frequency across the Siluro-Devonian remains statistically significant (\(\chi^2\), \(P < 0.001\)) even when (i) data are grouped by period (table S2), (ii) data are separated into two intervals by the Siluro-Devonian, and (iii) individual Lagerstätten are removed one at a time (“jackknife”) (18).

Paleozoic crinoids are a morphologically heterogeneous clade—size, arm number, branching patterns of the arms, and arm-