Trabecular bone scales allometrically in mammals and birds

Michael Doube¹, Michał M. Kłosowski¹, Alexis M. Wiktorowicz-Conroy², John R. Hutchinson² and Sandra J. Shefelbine¹,*

¹Department of Bioengineering, Imperial College London, South Kensington, London SW7 2AZ, UK
²Structure and Motion Laboratory, The Royal Veterinary College, North Mymms, Hatfield, Hertfordshire AL9 7TA, UK

Many bones are supported internally by a latticework of trabeculae. Scaling of whole bone length and diameter has been extensively investigated, but scaling of the trabecular network is not well characterized. We analysed trabecular geometry in the femora of 90 terrestrial mammalian and avian species with body masses ranging from 3 to 3400 kg. We found that bone volume fraction does not scale substantially with animal size, while trabeculae in larger animals’ femora are thicker, further apart and fewer per unit volume than in smaller animals. Finite element modelling indicates that trabecular scaling does not alter the bulk stiffness of trabecular bone, but does alter strain within trabecular bone under equal applied loads. Allometry of bone’s trabecular tissue may contribute to the skeleton’s ability to withstand load, without incurring the physiological or mechanical costs of increasing bone mass.

Keywords: trabecula; bone; allometry; scaling

1. INTRODUCTION

Many bones contain lightweight internal lattices of trabeculae (Latin, ‘little beams’) that provide structural support, particularly near joints. Trabeculae have long been recognized as important contributors to bone strength [1,2], yet in contrast to whole bones [3–6], little is known about how trabecular scale in relation to animal size, or if they scale at all [7,8]. As animals increase in size, their bones must sustain higher loads. Whole bones become relatively more robust as they become longer (diameter ∝ bone length¹.03–1.20 [4,6]). To accommodate increased load in large animals, trabecular bone could increase stiffness by increasing the amount of bone per unit volume or by altering the geometry and the arrangement of individual trabeculae as body size and bone loading increase. In the only previous broad comparative study of trabecular scaling, Swartz et al. [8] found little dependence of trabecular length and width on body mass (M₀) in the species they sampled. When they considered only bats’ trabecular bone, trabecular length and width scaled close to isometry (trabecular length and trabecular width ∝ M₀¹/3 : M₀ range 4.6 g – 0.7 kg). In five laboratory and domestic mammals (rat, rabbit, rhesus monkey, pig and cow; M₀ range approx. 0.4–400 kg), Mullender et al. [7] found significant differences in trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) and trabecular number, but concluded that trabeculae did not scale because Tb.Th varied by less than one order of magnitude.

Using a broad sample of 90 species of terrestrial birds and mammals, we asked whether trabecular bone displays allometric scaling of its foam-like structure (such as bone volume fraction, trabecular number and Tb.Th) and if so, what the mechanical consequences of geometric changes are to bones and bone tissue. We made X-ray microtomographic (μCT) scans of trabecular bone from two sites in each animal’s femur, applied three-dimensional image analysis techniques to determine standardized measurements of trabecular bone structure [9,10], and calculated scaling exponents from these measurements. We then created finite element (FE) models from representative individuals’ μCT scans and assessed how bone mechanics change in relation to scaling of trabecular geometry.

2. MATERIAL AND METHODS

(a) Specimen selection

To provide a comprehensively comparative study of trabecular scaling in terrestrial vertebrates, we quantified trabecular bone geometry in the femora of 72 terrestrial mammalian, 18 avian and 1 crocodilian species covering a six order of magnitude range in body mass. We chose species with walking and running/hopping locomotor habits across the size range of terrestrial vertebrates (M₀ = 3.0 × 10⁻³ to 3.4 × 10⁻¹ kg; electronic supplementary material, table S1). A broad range of size within clades was selected, so that clades were represented across their size range as much as possible. Most small specimens were borrowed from museums (University Museum of Zoology, Cambridge and the Natural History Museum, London, UK), while larger specimens that required trimming for microtomography were donated from personal collections. A full list of specimens is included in the electronic supplementary material, table S1. Most (85/91) specimens were skeletally mature adults. Epiphyseal growth plates were present in several sub-adult specimens but data from these specimens did not fall outside the overall trends, so were included in the final analyses. We did not preferentially select either sex because sex was unknown for most of the specimens.


Received 12 January 2011
Accepted 15 February 2011

* Author for correspondence (s.shefelbine@imperial.ac.uk).

Published online
We collected X-ray microtomographic (X-Tek HMX ST 225, Nikon Metrology Ltd, Tring, UK) images of trabecular bone in the head and condyle of a single femur from each specimen (figure 1a–d). Maximum possible resolution, dependent on specimen size, was used up to a maximum isotropic voxel size of 15 μm (range 3.4–15 μm voxel$^{-1}$) to prevent undersampling of trabeculae, but this resolution criterion limited maximum specimen size to 30.7 mm owing to the scanner’s 2000 × 2000 pixel detector panel. Two regions

(b) X-ray microtomography

Figure 1. Trabeculae scale with increasing animal size. X-ray microtomograms of the femoral heads from four terrestrial mammalian species show trabecular bone geometry across a six order of magnitude range of body mass ($M_b$): (a) *Suncus varilla*, lesser dwarf shrew, 0.005 kg $M_b$; (b) *Vulpes lagopus*, Arctic fox, 3.5 kg $M_b$; (c) *Equus caballus*, Przewalski’s horse, 202 kg $M_b$; (d) *Elephas maximus*, Asian elephant, 3400 kg $M_b$. Strong, significant scaling relationships with $r$ exist for (f) Tb.Th, (g) Tb.Sp and (h) Conn.D but not for (e) BV/TV, where weak allometry is present in avian trabeculae only. Regression lines are plotted for trabecular parameters where $R^2 > 0.3$ and $p < 0.05$. Slopes, $R^2$ and $p$-values are shown in table 1. Scale bar 1.0 mm; note increased magnification in (a). Triangles, mammal femoral head; squares, mammal femoral condyle; crosses, bird femoral head; pluses, bird femoral condyle; diamonds, crocodile femoral head; circles, crocodile femoral condyle.
of interest (ROI) were defined: a cube in the centre of the femoral head and a cube in the centre of the lateral femoral condyle (see electronic supplementary material, figure S1). For large specimens, in which the total width including greater trochanter or medial condyle was greater than 30 mm (femoral head radius, $r > 8$ mm), 1 cm cubes were cut from the centre of the femoral head and the lateral condyle for scanning. Specimens were mounted in polyurethane foam or florists’ foam (OASIS, Smithers-Oasis UK Ltd, Washington, UK) and centred. Low noise, high contrast and high-resolution projections were obtained by selecting target metal (Mo or W), beam current (50–190 mA), beam voltage (45–180 kV) and frame collection rate (0.5–4 fps) according to the radioopacity of the specimen. Three thousand one hundred and forty two projections were taken at 0.115° intervals, resulting in an exposure time of between 14 min and 3 h 20 min. Projections were reconstructed into tomographic slices using a modified Feldkamp cone-beam algorithm (CT Pro v. 2.0, Nikon Metrology Ltd, Tring, UK) and exported in 16-bit DICOM format (VG Studio Max v. 2.0, Volume Graphics, Heidelberg, Germany). A total of 73 mammal, 24 bird and eight reptile femora were scanned, resulting in 204 scans. Six bird femora and all but one reptile femur (Crocodylus niloticus, Nile crocodile) contained calcified cartilage or woven bone rather than trabeculae in our ROI; these specimens and specimens with medullary bone [11], pathology or post-mortem decay were excluded from later analysis.

(c) Image analysis
Image stacks were cropped to remove cortical bone, empty background and cutting swarf, resulting in images filled with only trabecular bone and narrow space. Images were thresholded with ImageJ’s [12] isodata algorithm applied to a histogram of all voxels in the stack, puriﬁed to remove background cavities and small foreground particles [13], eroded, puriﬁed again and dilated (see electronic supplementary material for validation studies). We implemented standard three-dimensional measures of trabecular architecture [9,10] as a plug-in, BoneJ [14], for ImageJ. We measured bone volume fraction (BV/TV; bone volume/total volume) as the proportion of ROI volume comprising mineralized bone. We determined Tb.Th and Tb.Sp (thickness of the narrow space) with the local thickness method, which deﬁnes the thickness at a point as the diameter of the largest sphere that ﬁts within the structure and which contains the point [15,16]. We measured connectivity density (Conn.D; number of trabeculae per unit volume) using the Euler characteristic, which is essentially a count of topological holes in the structure [13,17]. Degree of anisotropy (DA) was estimated with the mean intercept length method [10,18], which counts object—background boundaries along line probes in different three-dimensional directions and summarizes the boundary-counts’ orientation dependence as the ratio of the best-ﬁt ellipsoid’s minor and major axes. Bone surface area per unit volume (BS/TV) was measured by constructing a triangular surface mesh of the foreground (bone) by marching cubes [19,20], summing the areas of the surface triangles to calculate bone surface area (BS), and dividing by the stack volume (TV).

(d) Scaling exponent calculation
True body masses were unknown for most of our specimens, so we used femoral head radius ($r$) as a measure of animal size. Femoral head radii, measured using least-squares sphere-fitting, varied from 0.335 ($Suncus etruscus$) to 64.1 mm ($Elephas maximus$). Scaling exponents ($a$, where $B \propto r^a$) were computed for log$_{10}$transformed variables ($B_i$), such that log$_{10}$($B_i$) $\propto$ a log$_{10}$($r$), with the reduced (standardized) major axis method in SMATR for R [21,22], which is robust to arbitrary scale differences that occur when comparing dimensions such as $r$, measured in millimetres and Conn.D, measured in cubic millimetres. We used linear regression to show the strength of correlation of the variables ($R^2$) and the probability that the observed correlation was due to chance ($p$). We conducted an analysis of phylogenetically independent contrasts [23], which showed only minor inﬂuences of phylogeny on the overall scaling trends across all clades, but some clades (especially birds versus mammals) showed divergent scaling (see electronic supplementary material for details of the phylogenetic analysis). To enable body mass-based comparisons between mammals and birds, we used known-mass specimens and body mass estimates from literature values [24,25] and calculated scaling exponents for $r$ and $B$ against $M_b$.

(e) Finite element analysis
To explore the mechanical effects of trabecular scaling, we constructed FE models from µCT images to test the hypothesis that increased scale of a trabecular network results in an increased apparent Young’s modulus ($E_{app}$) and thus greater stiffness of the overall structure under loading. We created tetrahedral FE meshes (MIMICS v. 12.3, Materialise, Leuven, Belgium) from eight µCT scans, selected from mammals representing a wide range of body masses, taxa and trabecular dimensions (table 2). Elements were assigned isotropic, linear elastic material properties with an elastic modulus ($E_{elas}$) of 20 GPa and Poisson’s ratio of 0.3 [26]. We determined the apparent elastic modulus ($E_{app}$), which is the modulus of the cube as a whole, by applying a constant compressive strain to one face, fixing the opposite face and calculating the reaction force/area of the cube face (ABAQUS v. 6.6-1, Simulia Ltd, Warrington, UK). We found $E_{app}$ in all three axial directions for each cube. To investigate the effects of trabecular architecture on strain within trabeculae, we applied equal apparent stress ($\sigma_{app}$, force/cube edge length$^3$) to each model in the direction corresponding to greatest $E_{app}$ and computed strain in each element.

3. RESULTS
(a) Structural scaling of trabeculae
BV/TV remained relatively constant across the range of animal sizes, but showed weak, significant positive scaling in avian femoral condyles (table 1, figure 1e and see electronic supplementary material, table S1 for individual measurements). Birds had lower BV/TV than mammals ($0.19 \pm 0.10$ versus $0.37 \pm 0.10$, Welch $t$-test $p < 0.001$) and the femoral head had higher BV/TV than the condyle (mammals: $0.42 \pm 0.08$ versus $0.31 \pm 0.08$, Welch paired $t$-test $p < 0.001$; birds: $0.27 \pm 0.09$ versus $0.13 \pm 0.05$, Welch paired $t$-test $p < 0.001$).

Tb.Th and Tb.Sp showed positive allometric scaling with increasing $r$ (table 1 and figure 1f,g), indicating that larger animals have thicker trabeculae that are further apart. Asian elephant trabeculae (0.511 mm Tb.Th) were as thick as the diaphysial width of the smallest animals’ femora (0.4–0.5 mm).
Conn.D showed strong negative allometric scaling with \( r \) (table 1 and figure 1b) indicating that larger animals have fewer trabeculae per unit volume than smaller animals, consistent with thicker, sparser trabeculae and no change in BV/TV. BS/TV showed negative allometric scaling with \( r \) (table 1), consistent with decreasing Conn.D and increasing Tb.Th. DA did not scale significantly with increasing \( r \) (table 1), showing that larger animals’ trabeculae are not more aligned, for example, in the axial direction.

Calculation of scaling exponents against \( M_b \) emphasized the generally stronger scaling of avian trabeculae compared with mammalian trabeculae (table 1), and differing scaling of femoral head radius between the clades (\( r \propto M_b^{0.36} \) for mammals and \( r \propto M_b^{0.42} \) for birds).

(b) Mechanical consequences of trabecular scaling 

\( E_{\text{app}} \) was not substantially different between models with differing trabecular structures (table 2), suggesting that scaling of individual trabeculae may not have a direct influence on the stiffness of the bone as a whole. Modal element strain tended to decrease with increasing Tb.Th (Spearman’s \( \rho = -0.524, \ p = 0.197 \)) and was particularly low in the most massive animals’ models (table 2 and figure 2).

(c) Morphology

We observed that very thick trabeculae tended to be penetrated by osteonal canals. While intra-trabecular osteons appeared more commonly in larger animals, osteonal tunnelling was not limited to large animals as vascularized trabeculae were present in several small mammals including mouse lemur (Microcebus murinus, 70 g \( M_b \)), black lemur (Eulemur macaco, 3.0 kg \( M_b \)) and ruffed lemur (Varecia variegata, 2.04 kg \( M_b \)).

4. DISCUSSION

Our data show that trabecular bone architecture varies as a function of animal size. Trabeculae in larger land animals are thicker, further apart, and less densely connected than those in smaller animals. Although BV/TV is a major determinant of apparent modulus [27–30], larger animals do not have substantially more trabecular bone mass per unit volume to support increased loads, which may be an adaptation that limits the physiological cost of producing, maintaining and moving more tissue. Because the cost of mass in flight is much greater than in terrestrial locomotion, decreased flight habits typical of large birds might be the dominant influence on avian BV/TV allometry, rather than body mass. The flightless kiwi (Apteryx oweni and Apteryx haastii), though only 1–2 kg \( M_b \), had the greatest BV/TV (0.396 and 0.393) of the birds in this study. Trabecular bone geometry relates to ‘prevailing mechanical conditions’ [31], so significant differences in trabecular geometry between the femoral head and condyle probably reflect the differing load environments of the coxofemoral and femorotibial joints.

Swartz et al. [8] proposed two models of trabecular scaling, constant trabecular size (CTS) and constant trabecular geometry (CTG), in which the number of trabeculae in each hemispherical femoral head (Tb) would be proportional to its volume (CTS; Tb \( \propto r^3 \))
Table 2. Summary of finite elements findings. Modal strains were calculated in the same direction as $E_{\text{app}1}$. See figure 2 for element strain frequency distributions. $M_b$, body mass; BV/TV bone volume fraction; Tb.Th, trabecular thickness; $E_{\text{app}}$, apparent elastic modulus in each of three axial directions, ordered by magnitude; asterisk denotes known body mass.

<table>
<thead>
<tr>
<th>specimen</th>
<th>$M_b$ (kg)</th>
<th>BV/TV</th>
<th>Tb.Th (mm) (mean ± s.d.)</th>
<th>$E_{\text{app}1}$ (GPa)</th>
<th>$E_{\text{app}2}$ (GPa)</th>
<th>$E_{\text{app}3}$ (GPa)</th>
<th>modal strain ($\mu$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>long-eared hedgehog, <em>Hemiechinus auritus</em></td>
<td>0.22</td>
<td>0.52</td>
<td>0.138 ± 0.038</td>
<td>6.55</td>
<td>6.30</td>
<td>5.24</td>
<td>−37.5</td>
</tr>
<tr>
<td>polecat, <em>Mustela putorius</em></td>
<td>1.16</td>
<td>0.40</td>
<td>0.129 ± 0.039</td>
<td>7.90</td>
<td>7.53</td>
<td>6.58</td>
<td>−48.5</td>
</tr>
<tr>
<td>coypus, <em>Myocastor cuppus</em></td>
<td>7.5</td>
<td>0.44</td>
<td>0.195 ± 0.061</td>
<td>7.82</td>
<td>7.53</td>
<td>5.70</td>
<td>−22.5</td>
</tr>
<tr>
<td>grey wolf, <em>Canis lupus</em></td>
<td>*35</td>
<td>0.43</td>
<td>0.230 ± 0.094</td>
<td>5.40</td>
<td>5.08</td>
<td>4.39</td>
<td>−25</td>
</tr>
<tr>
<td>Siberian tiger, <em>Panthera tigris</em></td>
<td>*130</td>
<td>0.46</td>
<td>0.365 ± 0.096</td>
<td>5.81</td>
<td>5.34</td>
<td>4.69</td>
<td>−43.5</td>
</tr>
<tr>
<td>cattle, <em>Bos taurus</em></td>
<td>*500</td>
<td>0.50</td>
<td>0.340 ± 0.121</td>
<td>6.74</td>
<td>4.88</td>
<td>3.90</td>
<td>−23</td>
</tr>
<tr>
<td>white rhinoceros, <em>Ceratherium simum</em></td>
<td>3000</td>
<td>0.42</td>
<td>0.247 ± 0.067</td>
<td>6.40</td>
<td>4.94</td>
<td>4.43</td>
<td>−15.5</td>
</tr>
<tr>
<td>Asian elephant, <em>Elephas maximus</em></td>
<td>*3400</td>
<td>0.48</td>
<td>0.511 ± 0.151</td>
<td>7.77</td>
<td>5.01</td>
<td>4.86</td>
<td>−12.5</td>
</tr>
</tbody>
</table>

Figure 2. Trabeculae in larger animals have higher elastic moduli than in small animals. Relative frequency distributions of element strains in finite element models of trabecular bone show that under the same apparent stress, thicker, sparser trabeculae from larger animals had a greater proportion of their bone tissue experiencing less compressive strain than trabeculae from smaller animals. Results from only the two largest and two smallest animals are illustrated for clarity. Dashed line, polecat (*Mustela putorius*); dotted line, long-eared hedgehog (*Hemiechinus auritus*); long dashed line, white rhinoceros (*Ceratherium simum*); short dashed line, Asian elephant (*E. maximus*).

or constant (CTG; $Tb \propto r^0$). We estimated Tb as $Tb = \text{Conn.D} \times 2/3m^3$, and calculated that $Tb \propto r^{1.99}$, which lies between the CTS and CTG predictions. Tb ranged from 20 to 30 trabeculae in very small shrews to 102 trabeculae in an Asian elephant. Although Conn.D reduces rapidly with increasing $r$, femoral head volume increases faster, resulting in greater Tb. Each trabecula then supports a reduced proportion of the femoral head's load, increasing redundancy in the face of single element failure.

There are limits to the range that each trabecular dimension can take, and therefore, limited potential scaling exponents. In particular, a trabecula cannot be thicker than the diameter of the bone that contains it. This creates a natural upper limit of $a = 1$, where $Tb.Th \propto r^a$ (i.e. isometry, Swartz et al.'s CTG model), because if trabeculae increased in thickness faster than the femoral head increased in radius ($a > 1$), trabeculae would outgrow the femoral head's narrow cavity. Furthermore, osteocytes within human trabeculae are never more than 230 $\mu$m from the bone surface, probably owing to diffusion limiting cellular metabolism [32, 33]. This leads to an approximate upper limit for Tb.Th of about 0.46 mm, preventing isometric scaling of trabeculae ($Tb.Th \propto r^1$, CTG). Isometrically scaling shrew-sized trabeculae (mean Tb.Th = 0.052 mm) to the elephant, a 200-fold change in $r$, would result in trabeculae approximately 10 mm thick, and scaling down elephant-sized trabeculae (mean Tb.Th = 0.511 mm, close to the aforementioned upper limit) to the shrew would result in trabeculae less than 3 $\mu$m wide, about half the width of an osteocyte. We found that $Tb.Th \propto r^{0.43–0.66}$, which lies between the CTS and CTG predictions. While Mulhender et al. [7] were correct in stating that Tb.Th has a range of approximately one order of magnitude, this does not preclude the allometric relationship that we show.

Our FE models show that under equal apparent stress ($E_{\text{app}}$), strain within trabeculae from different species varies with trabecular geometry (figure 2) so that thicker trabeculae have less strain. It must be emphasized that this effect is seen at the scale of tens of micrometres, about the size of osteocytes, and not at the scale of the overall bone. It must be noted that by applying equal compressive strain and apparent stress to each linear-elastic mesh, we could determine the effects of trabecular geometry alone on $E_{\text{app}}$ and element-level strain. In selecting equal, non-physiological, loading conditions we purposefully ignored the complicated variations that must occur during physiological loading in these animals of greatly different $M_b$, so the calculated results should not be extrapolated to stresses and strains in real, living tissue. Accurate data on joint stresses are scarce, but if we assume that larger animals have greater joint stress than smaller animals, we could apply greater apparent stress to bone cubes from bigger animals, and smaller apparent stress to cubes from smaller animals. Owing to the linear elastic nature of our models, this difference in loading would shift modal element strains towards a common value by increasing the low strains in larger animals and reducing the high strains in smaller animals. If apparent stress scales with body size as we assume, scaling...
of trabecular geometry might therefore act to moderate trabecular strain. Bone tissue is known to model in response to its changing mechanical environment [34–36] and remodel to repair microdamage [37]. In general terms, bone is added when dynamic strain is high [36] and removed when dynamic strain is low [35], which is hypothesized to maintain strain in a safe range [34,38]. Mitigation of high strain reduces the rate of microdamage accumulation [39] and the associated metabolic cost of tissue repair, while the avoidance of low strains prevents bone mass being added where it is mechanically unnecessary. Though the actual stimulus may not be strain, it is likely to be a derivative of strain (such as fluid shear), which is most conveniently estimated by strain in this experimental model. Structural allometry of trabecular dimensions might, therefore, be an interspecific manifestation of bone tissue’s drive to maintain mechanical homeostasis. It appears that changes in geometry are preferred over increased bone mass, because BV/TV does not scale substantially with animal size.

Larger and fewer trabeculae may be a result of selectively increasing the thickness of overstrained trabeculae and removing understrained trabeculae [34–36], but the relative contributions of mechanics and genetics to trabecular ontogeny are almost unknown [40,41]. Structural scaling of trabecular bone might result from phenotypic adaptation, in which bone tissue is identical in different animals and adapts to changing external factors such as mechanical load. Genotypic adaptation may have occurred if having larger, fewer trabeculae conferred an advantage to larger animals, and large trabeculae could arise in the relatively unloaded fetus. Larger animals tend to live longer than smaller animals, so it is possible that longevity may allow more time for a drift towards thicker, fewer trabeculae.

We observed intra-trabecular osteon formation in the thick trabeculae of large animals and unexpectedly, in thinner trabeculae of smaller mammals such as cheetah (Acinonyx jubatus) and lemurs (M. murinus, E. macaco and V. variegata). Intra-trabecular osteons, previously identified in human trabeculae [32,33], may act to ensure adequate diffusion of nutrients to, and waste products from, osteocytes by limiting the maximum distance between osteocytes and the bone surface to approximately 230 μm, and therefore maximum Tb.Th to 460 μm. None of the animals listed above have Tb.Th approaching this upper limit, so they might require increased vascular perfusion of trabeculae owing to athletic specialization, or some other metabolic demand that reduces blood’s oxygen saturation before it reaches deep bone tissues. Vascularization by tunnelling osteons also changes trabecular geometry from solid to tube-like, which might result in increased bending stiffness of individual trabeculae for the same mass while maintaining blood supply to deeply embedded osteocytes. While the latter explanation was thought unlikely by Lozupone & Favia [33], it does illustrate an important limitation to current concepts of trabeculae, which treat each lattice element as a solid rod or plate. The thickness of each element is intuitively its external diameter, but if the element contains a hole, the measured thickness (Tb.Th) is the wall thickness and not the external diameter.

5. CONCLUSIONS

Trabecular bone scales allometrically, within physiological limits to trabecular size. Reorganization of bones’ internal structure might protect trabeculae from increased strains owing to large body size, representing a mass-efficient strategy for maintaining bone strain in a safe range at the trabecular scale. This may represent a new approach to designing cellular solids for engineered structures of differing scale.

The authors are grateful to Matthew Lowe of the University Museum of Zoology, Cambridge and to Louise Tomsett and Roberto Portela-Miguez of the Natural History Museum, London for assistance with specimen loans. Material was kindly donated by Whipsnade Zoo and Robert Ker. We thank Richard Abel for assistance with μCT scanning. M.D. thanks the ImageJ community for programming advice. John Currey, Dennis Carter, Elizabeth Loboa, Joanna Laurson, Olga Panagiotopoulou and Victor Seidel critically read the manuscript. Comments from three anonymous reviewers improved the presentation of this text. This research was funded by the UK Biotechnology and Biological Sciences Research Council.

REFERENCES


