

Whole-bone scaling of the avian pelvic limb

Michael Doube,¹ Stephanie C. W. Yen,¹ Michał M. Kłosowski,¹ Andrew A. Farke,² John R. Hutchinson³ and Sandra J. Shefelbine¹

¹Department of Bioengineering, Imperial College London, London SW7 2AZ, UK

²Raymond M. Alf Museum of Paleontology, Claremont, CA, USA

³Structure and Motion Laboratory, The Royal Veterinary College, North Mymms, Hatfield, Hertfordshire AL9 7DY, UK

Abstract

Birds form the largest extant group of bipedal animals and occupy a broad range of body masses, from grams to hundreds of kilograms. Additionally, birds occupy distinct niches of locomotor behaviour, from totally flightless strong runners such as the ratites (moa, kiwi, ostrich) to birds that may walk, dabble on water or fly. We apply a whole-bone approach to investigate allometric scaling trends in the pelvic limb bones (femur, tibio-tarsus, tarsometatarsus) from extant and recently extinct birds of greatly different size, and compare scaling between birds in four locomotor groups; flightless, burst-flying, dabbling and flying. We also compare scaling of birds' femoral cross-sectional properties to data previously collected from cats. Scaling exponents were not significantly different between the different locomotor style groups, but elevations of the scaling relationships revealed that dabblers (ducks, geese, swans) have particularly short and slender femora compared with other birds of similar body mass. In common with cats, but less pronounced in birds, the proximal and distal extrema of the bones scaled more strongly than the diaphysis, and in larger birds the diaphysis occupied a smaller proportion of bone length than in smaller birds. Cats and birds have similar femoral cross-sectional area (CSA) for the same body mass, yet birds' bone material is located further from the bone's long axis, leading to higher second and polar moments of area and a greater inferred resistance to bending and twisting. The discrepancy in the relationship between outer diameter to CSA may underlie birds' reputation for having 'light' bones.

Key words: allometry; bird; bone; scaling; walking.

Introduction

Aves constitute the largest group of extant bipeds (~10 000 species). Bipedalism evolved in dinosaurs and has been maintained by their flying avian descendants. The ancestral (and predominant) condition is for extant birds to be facultatively cursorial – that they walk or run when not flying, dabbling or employing other types of locomotion. Several groups of contemporary birds, notably the ratites (ostriches, emus, etc.), are flightless and rely solely on striding bipedal locomotion. Flightlessness evolved multiple times in birds (Phillips et al. 2010), which raises intriguing functional questions, such as whether similar musculoskeletal forms (e.g. scaling of bone shape with body size) evolved in these independently flightless lineages.

Perhaps due to many birds' prodigious flight capability, the majority of scaling, skeletal and locomotor research

interest has focussed on the mechanics of flying. The mechanics of the underwater locomotor style of penguins has also gained some attention recently (Habib, 2010). However, a few studies have investigated birds' pelvic limb bones' structural scaling (Prange et al. 1979; Alexander, 1983a; Cubo and Casinos, 1994, 1998a, 1998b), mechanical scaling (Bou et al. 1991; Cubo & Casinos, 2000a) and size-related gait alterations (Gatesy & Biewener, 1991).

Scaling studies typically use the general form of a power curve, $y = ax^b$, where x is a variable representing size, y is the variable of interest, b is the scaling exponent and a is a normalization constant. However, a is usually ignored, leaving the relationship as $y \propto x^b$. Log-transforming the original equation simplifies the problem so that it is readily analysed with linear regression, giving the relationship $\log(y) = b \times \log(x) + \log(a)$. The slope of the line is the scaling exponent b , which represents how rapidly the variable of interest changes with respect to size, and the y -intercept (or 'elevation') is $\log(a)$. If two groups have equal b but different elevations, $\log(a_1)$ and $\log(a_2)$, the log rule $\log(a_1) - \log(a_2) = \log(a_1/a_2)$ means that there is a constant ratio between their respective non-transformed values of y (because $y_1 = a_1x^b$ and $y_2 = a_2x^b$).

Correspondence

Sandra J. Shefelbine, Department of Bioengineering, Imperial College London, London SW7 2AZ, UK. E: s.shefelbine@imperial.ac.uk

Accepted for publication 17 April 2012

Article published online 21 May 2012

In general, pelvic limb bone mass scales with positive allometry (Prange et al. 1979), but there is taxon-dependent variation. For example, Galliformes (chickens and kin) and Columbiformes (pigeons and kin) show a tendency towards lighter skeletal structures, which manifests as lower scaling elevation than other birds (Cubo & Casinos, 1994). Although birds' femora have a lower physical density than bats' and rodents' femora (Dumont, 2010), birds show similar allometry in skeletal mass to mammals, and in birds skeletal mass scaling is consistent across flying and flightless clades (Prange et al. 1979).

In the avian pelvic limb, the femur constitutes a relatively smaller proportion of total limb length in larger birds than smaller birds (Gatesy & Biewener, 1991). Mediolateral and craniocaudal diameters scale with similar slopes, indicating that circularity does not change (Cubo & Casinos, 1998a). Thoracic limb bones scale in length (L), with stronger positive allometry (becoming longer with increasing size) than pelvic limb bones, which scale isometrically with body mass (M_b ; $L \propto M_b^{1/3}$). Pelvic limb bones tend to become more robust because femoral and tibiotarsal diameter (d) scale with positive allometry, at a rate conforming to the elastic similarity model ($d \propto M_b^{3/8}$; Cubo & Casinos, 1998b).

Larger birds tend to stand with a more straightened limb posture, measured by the ratio of hip height to total limb length (Gatesy & Biewener, 1991), which mitigates the need for increased joint moments to maintain limb extension. There are also substantial differences in gait kinematics as a function of body size, with small birds favouring stride frequency changes to increase speed, and larger birds favouring stride length changes. Smaller birds' relative step length (normalized to hip height) is greater than in larger birds, due to larger limb excursion angles. Birds display a wide diversity of species-specific postures and gaits, and size-normalized data show kinematic variation due to interspecific variation in posture and relative limb segment lengths (Gatesy & Biewener, 1991; Gatesy & Pollard, 2011).

In a previous study, we developed a novel three-dimensional approach to study how long bones scale, effectively sampling the entire bone rather than the traditional focus on the scaling of midshaft dimensions (Doube et al. 2009). We applied this analysis to felid (cat) limb bones, and found that the scaling of cross-sectional parameters is dependent on the proximodistal location in the bone, with epiphyses and some muscle attachment sites scaling more strongly than the midshaft, most likely due to cats' conservation of limb posture with increases in size (Day & Jayne, 2007; Wiktorowicz-Conroy AM, Pickering P, Shine C, Greaves E, Schmitt DO, Doube M, Shefelbine SJ, Hutchinson JR, in review). Because all studies of non-felid taxa to date have only measured cross-sections at the mid-diaphysis (except for occasionally sampling a few other regions), it is unknown whether other animals' bones show a similar variation in scaling along their length. Birds display a range of terrestrial locomotor styles, from completely flightless (kiwi,

moa, ostrich, dodo), to burst flight (Galliformes), perching (passerines) and dabbling (ducks, swans). Because previous studies have used restricted clades (e.g. moa as the sole flightless group; Alexander, 1983a) it is unknown whether these locomotor styles influence skeletal scaling. Here, we use computed tomographic scans of complete bones from a selection of extant and recently extinct birds from four broad locomotor groups (flightless, dabblers, burst fliers and fliers), to relate scaling trends of avian pelvic limb bones to birds' locomotor habits, and to investigate whether scaling varies along the length of each bone.

Materials and methods

Limb bones including femora ($n = 57$), tibiotarsi ($n = 19$) and tarsometatarsi ($n = 19$) bones were selected from a single, skeletally sound adult limb from 48 species of bird in four locomotor groups, ranging from 91 g to 250 kg M_b (see Table S1 in Data S1 for full list). Swimming (e.g. penguins) and soaring birds were excluded, to avoid birds that are specialized away from cursorial locomotion. Our focus was intentionally on birds that are either phylogenetically close to the base of the avian crown clade (Phillips et al. 2010) or with locomotor styles presumably similar to the ancestral condition, which was quite terrestrially specialized (e.g. Hutchinson, 2004a); or in a few cases bearing unusual terrestrial specializations (e.g. *Pachyornis*, *Geococcyx*, *Sagittarius*). Specimens were classified according to their predominant locomotor mode, as observed empirically and from textbook description (del Hoyo et al. 1992): flightless birds were those that locomote exclusively by walking or running (i.e. avoiding aquatic flightless taxa); dabbling birds were waterfowl that paddle with the feet or locomote on the shore, whose primary escape behaviour is to run into water; burst fliers were those able to fly quite powerfully over short distances as an escape mechanism; flying birds were those with a strong flight ability but which nevertheless spend a sizeable proportion of their time locomoting on the ground or perching.

Bones were imaged in an X-ray microtomographic scanner (μ CT: HMX ST 225; Nikon Metrology, Tring, UK) or, for the largest specimens, clinical computed tomography (CT: GE LightSpeed16; GE HiSpeed CT/i; GE LightSpeed Ultra; GE Healthcare, Little Chalfont, UK). Within-slice resolution and slice spacing varied depending on the data set. Specimens were scanned so that as far as possible, each image contained only the bone of interest and low attenuating material such as air or supporting foam, facilitating later segmentation by thresholding. Image stacks of up to 2000 slices were reduced by decimation without averaging to 65–225 slices for analysis, and high-value pixels from objects other than the bone of interest were manually given a value corresponding to air. Cross-sectional area (CSA), second moments of area (I_{max} , I_{min}) and polar moment of inertia (J_z) were measured on every slice in each stack with Slice Geometry in BONEJ (Doube et al. 2010). All image processing was performed with IMAGEJ version 1.43 (Rasband, 2009).

Scaling exponents and elevations were calculated with the reduced (standardized) major axis method ('Model II') in *SMATR* (version 2.1) for R (version 2.12.1; Warton et al. 2006; Warton & Ormerod, 2007; R Development Core Team, 2009) against measured bone length, or M_b , which was known for five specimens and otherwise estimated as means calculated from literature values (Dunning, 2008; Farke & Alicea, 2009). Linear regression

was performed 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 (PIC; Felsenstein, 1985), which showed only trivial influences of phylogeny on the overall scaling trends across all clades (see supplementary material in Data S1 for details of this phylogenetically corrected analysis). All specimens were included in calculations without consideration for multiple individuals of some species, as PIC showed minimal effect of duplicates on scaling trends. Birds were grouped by locomotor style for comparative analysis of scaling relationships. Exponents and elevations of the scaling equations were also compared between birds and data previously collected from cats (Doube et al. 2009) using the slope.com and elev.com comparisons in SMATR. An alpha-level of $P = 0.05$ was taken as the cutoff for statistical significance. Normalized cross-sectional variables were generated by taking the n th root (from the unit's exponent) and dividing by bone length (e.g. I_{\max} in mm^4 , normalizes as $I_{\max}^{1/4}/L$). The normalized value is a dimensionless, size-independent measure of shape.

Results

Femoral length scales against M_b isometrically ($L \propto M_b^{1/3}$), but tibiotarsus and tarsometatarsus lengths scale positively, becoming relatively longer in more massive birds. Statistically robust scaling relationships were found with high R^2 and $P < 0.001$ for most comparisons (Table 1).

Femoral length $\propto M_b$ slopes were not significantly different between dabbling and burst flight ($P = 0.43$), yet dabblers appeared to group below the general trend (Fig. 1). Comparison of the elevations of the two gait groups showed that dabblers' femora are about 30% shorter than burst fliers for the same body mass ($P < 0.001$; see Equation S12 in Data S1 for calculation and Table 1 for elevations). Femoral length elevation was not significantly different

between the other groups. A similar trend is seen in mid-diaphyseal CSA vs. M_b (Fig. 1) – dabblers have 16% smaller CSA ($P < 0.05$) than burst fliers with similar M_b , but the same scaling exponent ($P = 0.17$).

Normalized I_{\max} plots showed distinctive trends in the maximum second moment of area for each of femur, tibiotarsus and tarsometatarsus (Fig. 2). In general, the heavier birds have greater normalized I_{\max} values, indicating increasing robustness and positive allometry (in the isometric case, all lines would overlie each other). Thus, as birds increase in size, the diaphysis becomes relatively shorter as the proximal and distal ends of the bones begin to broaden progressively closer to the midshaft.

Scaling exponents do not differ significantly between locomotor groups for I_{\max} vs. length and I_{\max} vs. M_b . When all locomotor groups are pooled, I_{\max} scales with strong positive allometry in the femur, but only moderately so in the tibiotarsus and tarsometatarsus with respect to length, but with respect to M_b , femoral, tibiotarsal and tarsometatarsal I_{\max} scale moderately positively (Fig. 3). This is a side-effect of positive allometry of tarsometatarsal and tibiotarsal length vs. M_b . The isometric scaling of tarsometatarsal I_{\max} vs. length is partly due to larger birds' gracile tarsometatarsus, for example the ostrich (*Struthio camelus*), which has a normalized I_{\max} far below birds of similar size (Fig. 2).

Polar moment of inertia (J_z) scaling at the femoral mid-diaphysis, $J_z = a\text{CSA}^b$, showed no significant difference ($P = 0.293$) in slopes between cats ($b = 2.02 \pm 0.13$) and birds ($b = 1.95 \pm 0.06$), and neither differed from isometry ($b = 2$), indicating that both clades retain proportional cross-sectional geometry as their femora increase in size. However, there is a significant difference ($P < 0.001$) in ele-

Table 1 Differences in length vs. mass scaling depending on gait group: significant differences can be inferred by non-overlapping 95% confidence intervals (CI).

Bone	Gait group	<i>n</i>	Slope $L \propto M_b^b$	Slope –CI	Slope +CI	Elev.	Elev. –CI	Elev. +CI	R^2
Femur	All	57	0.312	0.291	0.334	1.85	1.83	1.87	0.928
	Flightless	20	0.304	0.273	0.339	1.86	1.81	1.91	0.944
	Dabbling	7	0.295	0.233	0.375	1.73	1.70	1.77	0.947
	Burst flight	24	0.327	0.275	0.390	1.88	1.86	1.91	0.838
	Flying	6	0.344	0.288	0.412	1.84	1.81	1.87	0.979
Tibiotarsus	All	19	0.385	0.351	0.424	2.01	1.97	2.04	0.964
	Flightless	7	0.366	0.288	0.466	2.05	1.91	2.18	0.947
	Dabbling	5	0.335	0.222	0.505	1.98	1.91	2.05	0.929**
	Burst flight	5	0.421	0.281	0.630	2.02	1.92	2.12	0.932**
	Flying	2	–	–	–	–	–	–	–
Tarsometatarsus	All	19	0.413	0.356	0.478	1.79	1.73	1.85	0.912
	Flightless	7	0.377	0.258	0.550	1.87	1.64	2.09	0.864**
	Dabbling	5	0.333	0.201	0.552	1.72	1.63	1.81	0.891*
	Burst flight	5	0.464	0.329	0.655	1.82	1.73	1.91	0.951**
	Flying	2	–	–	–	–	–	–	–

Elev., log-transformed elevations; * $P < 0.05$ and ** $P < 0.01$, otherwise $P < 0.001$.

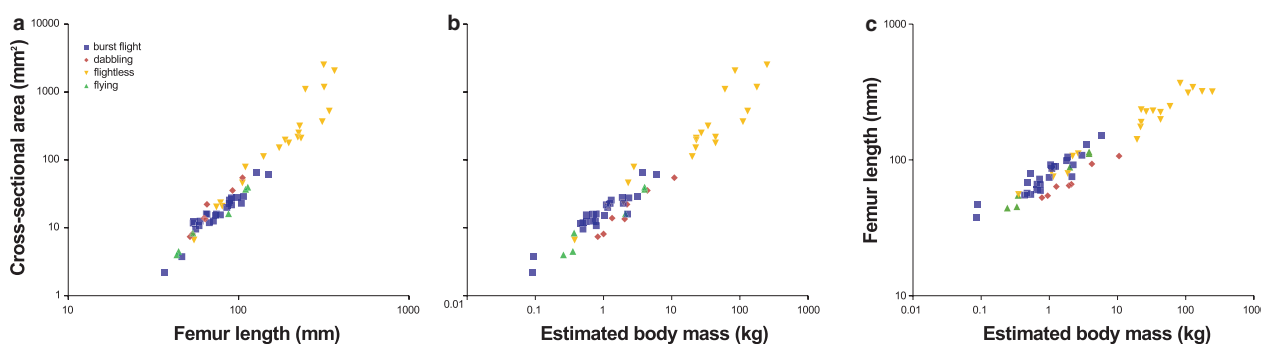


Fig. 1 Cross-sectional area (CSA) at the femoral mid-diaphysis and femur length, vs. either femur length or body mass as animal size variables. Note the change in position of the four locomotor groups between (a) CSA vs. length and (b) CSA vs. body mass plots, and the consistent slope between the four groups. Dabblers' femoral length and CSA have a lower elevation than burst flying birds (b,c), meaning that their femora are on average 30% shorter and have 16% less CSA for the same body mass.

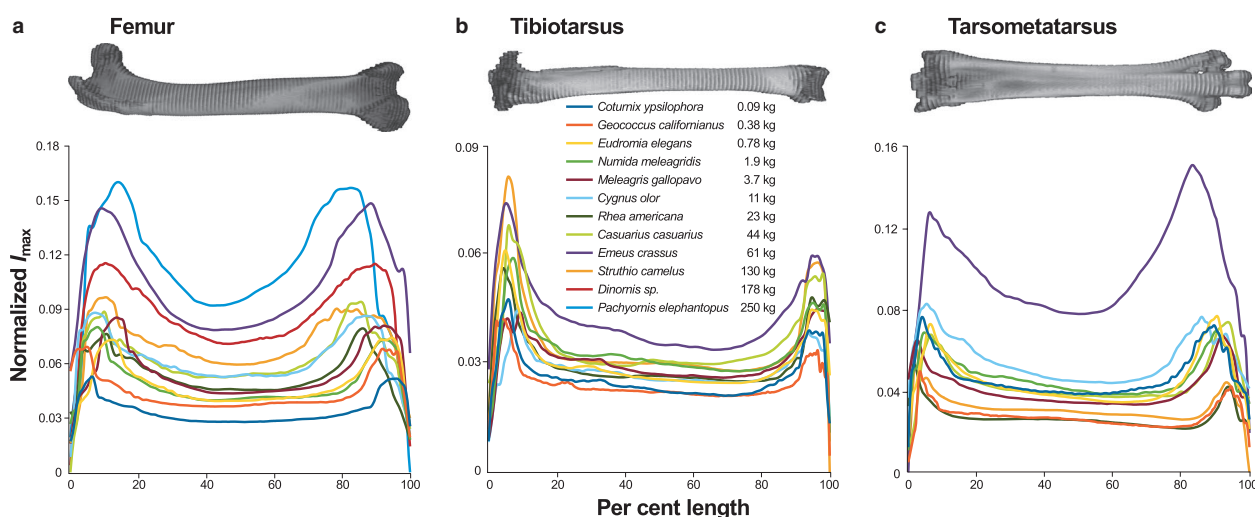


Fig. 2 Normalized I_{\max} ($I_{\max}^{1/4}/L$) for femur (a), tibiotarsus (b) and tarsometatarsus (c) from birds selected for even coverage of the whole body mass range of 0.09–250 kg. There is a general trend towards increased robustness with increasing body mass, visible as an upward displacement in normalized I_{\max} in larger birds. Note the relative shortening of the diaphysis and disproportionately increasing size of the proximal and distal bone ends in more massive birds, especially in the femur. Also note the gracile tarsometatarsus of the ostrich (*Struthio camelus*) and peculiarly robust bones in the stocky moa *Emeus crassus* and *Pachyornis elephantopus*. Reconstructed scans of helmeted guineafowl (*Numida meleagris*) are displayed above each plot, scaled to 100% length, with proximal at 0% length and distal at 100% length.

variation between the two groups (Fig. 4), with cats $a = -0.402$ and birds $a = -0.231$. Substituting these values into Equation S12 (Data S1) shows that for the same CSA, birds have on average 1.5 times greater J_z than felids.

Discussion of results

In agreement with most prior work, we find taxon- and locomotor group-specific scaling in the pelvic limb bones of birds. However, the most interesting locomotor group comparisons come from differing elevations of the scaling relationships and not the scaling exponents, which were similar between locomotor groups. Whereas Cubo & Casinos (1997) found that the second moment of area of palaeog-

nath (burst-flying tinamous and flightless ratites) pelvic limb bones scaled with strong allometry (Cubo & Casinos, 1997), we found that our flightless locomotor group, primarily composed of palaeognaths (17/20 specimens), scaled allometrically, but no different from the general trend.

By comparing the elevation of scaling relationships that have similar slopes, it was possible to determine that dabblers have relatively short and slender femora compared with other birds of the same M_b . In isolation, it might seem that dabbler's femora are just short, or just slender, but once the CSA vs. length and length vs. M_b relationships (Fig. 1a,c) are interpreted together, dabblers' femora are actually not unusually slender; they are unusually short and have similar CSA to femora of similar length from other

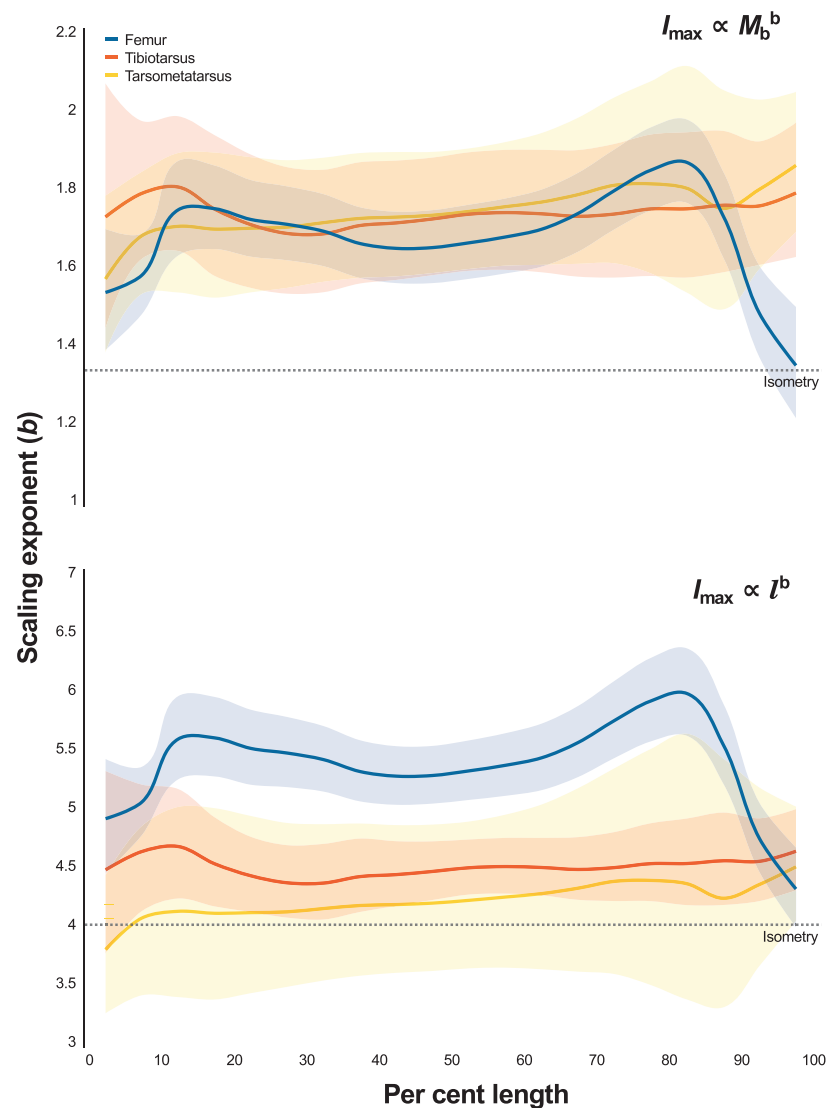


Fig. 3 Scaling exponents for second moment of area (I_{\max}) vs. body mass (M_b) and bone length (L) for all positions along the bone length from proximal (0% length) to distal (100% length). Dashed lines indicate isometric scaling exponents, solid lines the calculated scaling exponent for each position across all birds in all locomotor groups, coloured areas indicate the 95% confidence interval of the estimated scaling exponent. Tibiotarsal and tarsometatarsal I_{\max} scale less strongly against L than against M_b , but the femur scales similarly positively in both comparisons, because its length is isometric against body mass, and tibiotarsal and tarsometatarsal lengths scale with positive allometry against body mass. Peaks at either end of the femoral plot show disproportionate increases in I_{\max} around the femoral head, greater trochanter and condyles.

birds. Unfortunately, the selection of dabblers was exclusively from Anatidae, so it is impossible to separate phylogenetic from biomechanical influences on femur shape. It is interesting that Bou et al. (1991) noted that ducks' femora had particularly low bending and torsional moments at breaking for their M_b (Bou et al. 1991), which likely relates to the smaller femora measured here. So how reliable is the practice of using M_b as the sole independent, animal size variable? Mass is meant to represent axial load on the bone. But avian bones are also loaded in bending, where their length influences the bending moment, and in torsion, where their diameter influences the torsional moment. As the dabblers illustrate, using scaling relationships with both body mass and bone length can help complete the analysis of shape change with respect to size.

Birds are often considered to have light skeletons as an adaptation for flight, but our cross-sectional data show no evidence that volant birds have saved mass over flightless

birds by, for example, reducing their CSA; CSA vs. M_b relations are roughly collinear for all gait groups (Fig. 1b). On the other hand, birds may use bone material more efficiently than mammals, by arranging the same CSA further from a bone's axis, as evidenced by the difference in elevation between cats and birds in the J_z vs. CSA relationship. The higher second (I_{\max} , I_{\min}) and polar (J_z) moments of area relate to greater resistance to bending and torsion, respectively. In addition, avian trabecular bone (at least in the femur) has much less calcified tissue per unit volume (bone volume fraction, BV/TV) than in mammals (Doube et al. 2011), further reducing the comparative volumetric density of avian bone organs. Dumont (2010) found that dry bone material density was less in birds' femora than in bats or rodents (Dumont, 2010), but this accounts for only about 10% of the mass difference between bones of the same shape from these animals and is inconsistent throughout the skeleton, so that on average birds' bone material could

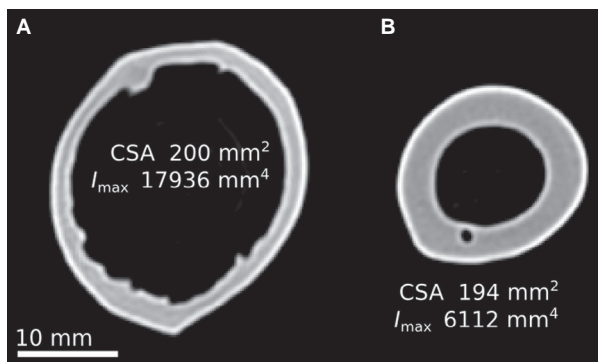


Fig. 4 CT scans from the midshaft of (A) cassowary (*Casuarius casuarius*; UMCZ 372.a) and (B) leopard (*Panthera pardus*; NHM 1880.2.16.1) femora, with estimated body masses of 44 and 50 kg, respectively. Although the leopard's CSA is only 3% smaller than the cassowary, its I_{\max} is little over a third of the bird's. Under the same bending moment M and assuming identical elastic moduli E , the cassowary femur (c) would have half as much strain as the leopard femur (l_e), because $\varepsilon = My/EI$ and substituting $I_{le} = I_c/3$ and $y_{le} = \frac{2}{3}y_c$ gives $\varepsilon_{le} = 2 \frac{My_c}{EI_c}$.

be more dense than bats and rodents. In contrast, a 1.25-fold increase in outer diameter without an increase in CSA (Fig. 4) results in a 36% reduction in the bone volume fraction of the organ. It seems that human experience of judging expected mass from visualized volume is the source of bird bones' reputation for lightness. Greater outer diameter of avian bones creates an expectation that the bone should have a mass equal to its volume \times bone density, but because of the large medullary cavity (which in some avian bones is filled with air and not marrow) the bone is emptier than expected and the organ's overall density is less than expected, so it feels lighter than expected.

Avian pneumatized bones are more robust than predicted if they were optimized for minimal mass. The ratio of outer to inner diameter (K) is lower than expected, perhaps as an adaptation to 'the rough-and-tumble lives that most birds lead' (Currey & Alexander, 1985). Birds' pelvic limb bone scaling might then be determined directly by hindlimb use, incorporating safety factors as seen in limb bones of other clades (Biewener, 1982), rather than strictly mass-sparing for flight. The current comparison of birds to felids is broadly in line with Currey & Alexander's finding that birds tend to have thin-walled bones (Currey & Alexander, 1985), and we extend the concept to encompass the elevation value of the I vs. CSA scaling relationship. CSA and I scaling relationships reveal not only trends similar to K , but also include geometric data with implications for twisting and bending strength that are not captured by K .

In three-point bending tests on a selection of flying birds, pelvic limb bones' maximum load (P_{\max}) did not show a significant difference from isometric scaling ($P_{\max} \propto M_b^1$; Cubo & Casinos, 2000a). In torsion, femoral breaking moment $\propto M_b^{1.16}$ and tibiotarsal breaking moment $\propto M_b^{1.05}$,

so that geometric similarity was the most likely explanation, although elastic similarity was not an impossibility for femur torsional strength scaling (Bou et al. 1991). Isometric scaling against M_b implies that the maximum load that birds' legs might endure is well matched to loading due to accelerating and decelerating body mass, for example during take-off and landing. These mechanical data agree most closely with the length vs. M_b relations in Table 1, and with the I_{\max} vs. length relation, rather than the I_{\max} vs. M_b relation that shows significant positive allometry in all three bones. By plotting scaling exponents as a function of per cent length (Fig. 3), we show that I_{\max} of the tarsometatarsus and tibiotarsus does not change considerably with respect to bone length but, in relation to M_b , these bones' I_{\max} increases faster than predicted by a simple mass-supporting role for the cross-section. In other words, the increased length of these bones also acts on the cross-section by increasing the bending moment so we see isometry in I_{\max} vs. length, as predicted to maintain a near-constant functional strain in bending. Because length increases allometrically vs. M_b , I_{\max} , which is isometric with length, must also increase allometrically with increasing M_b .

Our sample characterizes the scaling pattern that is ancestral for neornithine birds, which could shed light on locomotor evolution in the more basal, extinct clades. Very large (several tonne) non-avian theropods remained bipedal (Farlow et al. 2000), yet the largest recently extinct birds were no larger than 500 kg, possibly due to the modern avian requirement for contact incubation of eggs (Deeming & Birkhead, 2009) or torsional constraints (Gatesy & Biewener, 1991). Within this mass range, cursorial birds show distinct scaling trends in their pelvic limb bones that relate to the characteristic gaits of birds. The femur is loaded in torsion (Carrano & Biewener, 1999; de Margerie et al. 2005) and bending, depending on the erectness of the bird's limb posture. The ostrich pelvic limb during walking is characterized by internal-external rotation and abduction-adduction, with little hip flexion-extension, and an almost horizontal femur posture, but with increasing limb flexion during running (Rubenson et al. 2007) as in other birds (e.g. Gatesy, 1999). The tibiotarsus and tarsometatarsus seem to be loaded primarily in bending in most birds. Our data show that the avian femoral condyle scales more strongly than the proximal femur and femoral diaphysis, suggesting high loads of muscular and ligamentous insertions and joint pressure. Birds lack true epiphyses, rendering them less able to increase bone girth, which is manifested as less pronounced scaling of proximal and distal extrema than in cats. Furthermore, cats do not change posture with increasing M_b (Day & Jayne, 2007; Wiktorowicz-Conroy et al. in review), possibly requiring larger joint moment arms to compensate for larger externally imposed joint moments, whereas birds have a diminished requirement for longer joint moment arms due to their posture becoming more erect with increasing M_b (Gatesy & Biewener, 1991),

although this may still be greater than antelopes whose moment arms scale closer to isometry than birds (Alexander, 1977; Maloij et al. 1979).

Unfortunately, it has proven difficult to predict posture from cross-sectional geometry (Farke & Alicea, 2009), which would be useful for reconstruction of palaeontological specimens. Carrano argued that mammals may be better models for interpreting non-avian theropod locomotion than birds (Carrano, 1998), and Shaw and Ryan recently showed characteristic differences in primates' humeral and femoral cross-sectional properties that related to locomotor behaviour (Shaw & Ryan, 2012), but the entire practice of seeking correspondence between locomotor style and pelvic limb morphology may be questionable at best (Olmos et al. 1996). For example, attempting to predict loading from bone diameter could be challenged because differences in diameter do not correspond to differences in bending or twisting stresses (Bou et al. 1991).

Moa have a cortical diameter/thickness ratio similar to mammals, but the also flightless ostrich (*S. camelus*) has a ratio more similar to flighted birds (Currey & Alexander, 1985), suggesting that this could be an adaptation to high-speed running or might represent a more recent evolutionary loss of flight. Our data show that the ostrich tarsometatarsus is particularly gracile when normalized against its length. On the other hand, moa (*Pachyornis*, *Emeus*) femora and tibiotarsals can be especially stout (Fig. 2), which led Alexander to conclude that giant moa were rapid runners (Alexander, 1983b, 1985), although no further evidence supports this (except for *Dinornis*; Hutchinson, 2004b). It is also possible that *Pachyornis*' stout skeleton evolved without a biomechanical or adaptive explanation as a developmental anomaly or side-effect of heterochronic changes, such as in island gigantism, or to cope with strenuous fighting behaviour, or digging during foraging or burrowing. Although none of these explanations can be tested directly, more detailed functional analyses may be enlightening.

Our locomotor classification was based on direct observation and textbook description, and not quantitative behavioural data. The flightless birds are absolutely flightless so there is little ambiguity in their classification. The dabblers were all from the same taxonomic group, and showed distinctive scaling trends, suggesting that this classification was appropriate. The fliers and burst fliers were more difficult to separate, and neither group was outstanding in the analysis, but also no cluster appeared in our results that we could use for reclassification. We acknowledge that several of the species in the study typically undertake periodic migration, the phase of which was unknown for our specimens. Nor was the sex known, or the egg-laying status; however, we excluded bones that contained medullary bone tissue (Dacke et al. 1993) that might have interfered with our measurements. Strictly, J_z is only mechanically accurate for circular cross-sections; however, it

is convenient for comparative use as the sum of I_{\max} and I_{\min} , which tend to be closely related. In most cases the cross-section of the mid-shaft in particular is nearly circular, so J_z is a reasonable approximation (Cubo & Casinos, 1998b). Finally, we did not distinguish between marrow-filled or pneumatized bones, although pneumatized femora tend to be more gracile (relatively thin cortex) than marrow-filled femora (Cubo & Casinos, 2000b).

Concluding remarks

Comparison between locomotor groups reveals similar scaling exponents in all groups, but differing elevations that highlight particularly small femora in dabbling birds (Anatidae) compared with other birds of similar M_b . Calculated scaling trends depend on the relation between the chosen independent 'size' variable and the dependent variable measure, which may not be as mechanically simple as assumed. We confirm the previous finding that the femur comprises a smaller percentage of pelvic limb length in larger birds than in smaller birds. Except in the femoral condyle, proximal and distal extrema do not scale with substantially stronger allometry than at the midshaft, unlike in cats. This is perhaps due to larger birds having a straightened limb posture, reducing joint moment, and larger cats remaining crouched, increasing joint moment. Birds have similar CSA for M_b as cats. However, the bone material is arranged further from the centre of the cross-section, resulting in increased stiffness in torsion and bending, presumably reflecting the dominance of these loading modes in avian locomotion.

Acknowledgements

The authors are grateful to Mathew Lowe and Michael Brook at the University Museum of Zoology, Cambridge, and to Kristof Zyskowski at the Yale Peabody Museum of Natural History, New Haven, Connecticut, for assistance with specimen loans. We thank Richie Abel for assistance with μ CT scanning at the Natural History Museum, London, and the Department of Radiology at Stony Brook University Hospital for access to facilities there. We thank the two anonymous reviewers for their comments, which helped us to improve the manuscript. This research was funded by the UK Biotechnology and Biological Sciences Research Council.

References

- Alexander RM (1977) Allometry of the limbs of antelopes (Bovidae). *J Zool* **183**, 125–146. doi:10.1111/j.1469-7998.1977.tb04177.x.
- Alexander RM (1983a) Allometry of the leg bones of moas (*Dinornithes*) and other birds. *J Zool* **200**, 215–231. doi:10.1111/j.1469-7998.1983.tb05785.x.
- Alexander RM (1983b) On the massive legs of a moa (*Pachyornis elephantopus*, *Dinornithes*). *J Zool* **201**, 363–376. doi:10.1111/j.1469-7998.1983.tb04282.x.

- Alexander RM (1985) The legs of ostriches (*Struthio*) and moas (*Pachyornis*). *Acta Biotheor* **34**, 165–174. doi:10.1007/BF00046782.
- Biewener AA (1982) Bone strength in small mammals and bipedal birds: do safety factors change with body size? *J Exp Biol* **98**, 289–301.
- Bou J, Olmos M, Casinos A (1991) Strengths of the limb bones of birds and small mammals in bending and twisting. *Ann Sci Nat Zool* **12**, 197–207.
- Carrano MT (1998) Locomotion in non-avian dinosaurs: integrating data from hindlimb kinematics, in vivo strains, and bone morphology. *Palaeobiology* **24**, 450–469.
- Carrano MT, Biewener AA (1999) Experimental alteration of limb posture in the chicken (*Gallus gallus*) and its bearing on the use of birds as analogs for dinosaur locomotion. *J Morphol* **240**, 237–249. doi:10.1002/(SICI)1097-4687(199906)240:3 < 237::AID-JMOR3 > 3.0.CO;2-N.
- Cubo J, Casinos A (1994) Scaling of skeletal element mass in birds. *Belg J Zool* **124**, 127–137.
- Cubo J, Casinos A (1997) Flightlessness and long bone allometry in Palaeognathiformes and Sphenisciformes. *Netherlands J Zool* **47**, 209–226.
- Cubo J, Casinos A (1998a) The variation of the cross-sectional shape in the long bones of birds and mammals. *Ann Sci Nat Zool* **19**, 51–62. doi:10.1016/S0003-4339(98)80134-2.
- Cubo J, Casinos A (1998b) Biomechanical significance of cross-sectional geometry of avian long bones. *Eur J Morphol* **36**, 19–28.
- Cubo J, Casinos A (2000a) Mechanical properties and chemical composition of avian long bones. *Eur J Morphol* **38**, 112–121.
- Cubo J, Casinos A (2000b) Incidence and mechanical significance of pneumatization in the long bones of birds. *Zool J Linn Soc* **130**, 499–510. doi:10.1006/zjls.2000.0238.
- Currey JD, Alexander RM (1985) The thickness of the walls of tubular bones. *J Zool* **206**, 453–468. doi:10.1111/j.1469-7998.1985.tb03551.x.
- Dacke CG, Arkle S, Cook DJ, et al. (1993) Medullary bone and avian calcium regulation. *J Exp Biol* **184**, 63–88.
- Day LM, Jayne BC (2007) Interspecific scaling of the morphology and posture of the limbs during the locomotion of cats (Felidae). *J Exp Biol* **210**, 642–654. doi:10.1242/jeb.02703.
- Deeming DC, Birchard GF (2009) Why were extinct gigantic birds so small? *Avian Biol Res* **1**, 187–194. doi:10.3184/175815508X402482.
- Doube M, Wiktorowicz-Conroy AM, Christiansen P, et al. (2009) Three-dimensional geometric analysis of felid limb bone allometry. *PLoS ONE* **4**, e4742. doi:10.1371/journal.pone.0004742.
- Doube M, Kłosowski MM, Arganda-Carreras I, et al. (2010) BoneJ: free and extensible bone image analysis in ImageJ. *Bone* **47**, 1076–1079. doi:10.1016/j.bone.2010.08.023.
- Doube M, Kłosowski MM, Wiktorowicz-Conroy AM, et al. (2011) Trabecular bone scales allometrically in mammals and birds. *Proc R Soc B* **278**, 3067–3073. doi:10.1098/rspb.2011.0069.
- Dumont ER (2010) Bone density and the lightweight skeletons of birds. *Proc R Soc B* **277**, 2193–2198. doi:10.1098/rspb.2010.0117.
- Dunning JB (2008) *CRC Handbook of Avian Body Masses*, 2nd edn. Boca Raton: CRC Press.
- Farke AA, Alicea J (2009) Femoral strength and posture in terrestrial birds and non-avian theropods. *Anat Rec* **292**, 1406–1411. doi:10.1002/ar.20963.
- Farlow JO, Gatesy SM, Holtz TR, et al. (2000) Theropod locomotion. *Am Zool* **40**, 640–663. doi:10.1093/icb/40.4.640.
- Felsenstein J (1985) Phylogenies and the comparative method. *Am Nat* **125**, 1–15.
- Gatesy SM (1999) Guineafowl hind limb function. I: cineradiographic analysis and speed effects. *J Morphol* **240**, 115–125. doi:10.1002/(SICI)1097-4687(199905)240:2 < 115::AID-JMOR3 > 3.0.CO;2-Y.
- Gatesy SM, Biewener AA (1991) Bipedal locomotion: effects of speed, size and limb posture in birds and humans. *J Zool* **224**, 127–147. doi:10.1111/j.1469-7998.1991.tb04794.x.
- Gatesy SM, Pollard NS (2011) Apples, oranges, and angles: comparative kinematic analysis of disparate limbs. *J Theor Biol* **282**, 7–13. doi:10.1016/j.jtbi.2011.05.009.
- Habib M (2010) The structural mechanics and evolution of aquaflying birds. *Biol J Linn Soc* **99**, 687–698. doi:10.1111/j.1095-8312.2010.01372.x.
- del Hoyo J, Elliott A, Sargatal J, et al. (1992) *Handbook of the Birds of the World*. Barcelona: Lynx Edicions.
- Hutchinson JR (2004a) Biomechanical modeling and sensitivity analysis of bipedal running ability. I. Extant taxa. *J Morphol* **262**, 421–440.
- Hutchinson JR (2004b) Biomechanical modeling and sensitivity analysis of bipedal running ability. II. Extinct taxa. *J Morphol* **262**, 441–461.
- Maloij GMO, Alexander RM, Njau R, et al. (1979) Allometry of the legs of running birds. *J Zool* **187**, 161–167. doi:10.1111/j.1469-7998.1979.tb03940.x.
- de Margerie E, Sanchez S, Cubo J, et al. (2005) Torsional resistance as a principal component of the structural design of long bones: comparative multivariate evidence in birds. *Anat Rec A Discov Mol Cell Evol Biol* **282**, 49–66. doi:10.1002/ar.a.20141.
- Olmos M, Casinos A, Cubo J (1996) Limb allometry in birds. *Ann Sci Nat Zool* **17**, 39–49.
- Phillips MJ, Gibb GC, Crimp EA, et al. (2010) Tinamous and moa flock together: mitochondrial genome sequence analysis reveals independent losses of flight among ratites. *Syst Biol* **59**, 90–107. doi:10.1093/sysbio/syp079.
- Prange HD, Anderson JF, Rahn H (1979) Scaling of skeletal mass to body mass in birds and mammals. *Am Nat* **113**, 103. doi:10.1086/283367.
- R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available: <http://www.R-project.org>.
- Rasband WS (2009) *ImageJ*. Bethesda, Maryland, USA: U.S. National Institutes of Health. Available: <http://rsb.info.nih.gov/ij/>.
- Rubenson J, Lloyd DG, Besier TF, et al. (2007) Running in ostriches (*Struthio camelus*): three-dimensional joint axes alignment and joint kinematics. *J Exp Biol* **210**, 2548–2562. doi:10.1242/jeb.02792.
- Shaw CN, Ryan TM (2012) Does skeletal anatomy reflect adaptation to locomotor patterns? Cortical and trabecular architecture in human and nonhuman anthropoids. *Am J Phys Anthropol* **147**, 187–200. doi:10.1002/ajpa.21635.
- Warton DI, Ormerod J (2007) *smatr: (Standardised) Major Axis Estimation and Testing Routines*. Available: <http://web.maths.unsw.edu.au/~dwarton>. Accessed 30 Sep 2008.
- Warton DI, Wright IJ, Falster DS, et al. (2006) Bivariate line-fitting methods for allometry. *Biol Rev* **81**, 259–291. doi:10.1017/S1464793106007007.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1. Table S1. List of all birds, the length of bones they contributed, the source museum and accession number, and body mass. Supporting information. PIC analysis. Figure S1. Cladogram generated for PIC. Table S2. Summary of PIC results, where $\log_{10}(B) \propto b \log_{10}(M_b)$ for birds' femora.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.