The effects of locomotion on the structural characteristics of avian limb bones

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Despite the wide range of locomotor adaptations in birds, little detailed attention has been given to the relationships between the quantitative structural characteristics of avian limb bones and bird behaviour. Possible differences in forelimb relative to hindlimb strength across species have been especially neglected. We generated cross-sectional, geometric data from peripheral quantitative computed tomography scans of the humerus and femur of 127 avian skeletons, representing 15 species of extant birds in 13 families. The sample includes terrestrial runners, arboreal perchers, hindlimb-propelled divers, forelimb-propelled divers and dynamic soarers. The hindlimb-propelled diving class includes a recently flightless island form. Our results demonstrate that locomotor dynamics can be differentiated in most cases based on cross-sectional properties, and that structural proportions are often more informative than bone length proportions for determining behaviour and locomotion. Recently flightless forms, for example, are more easily distinguished using structural ratios than using length ratios. A proper phylogenetic context is important for correctly interpreting structural characteristics, especially for recently flightless forms. Some of the most extreme adaptations to mechanical loading are seen in aquatic forms. Penguins have forelimbs adapted to very high loads. Aquatic species differ from non-aquatic species on the basis of relative cortical thickness. The combination of bone structural strength and relative cortical area of the humerus successfully differentiates all of our locomotor groups. The methods used in this study are highly applicable to fossil taxa, for which morphology is known but behaviour is not. The use of bone structural characteristics is particularly useful in palaeontology not only because it generates strong signals for many locomotor guilds, but also because analysing such traits does not require knowledge of body mass, which can be difficult to estimate reliably for fossil taxa. © 2008 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2008, **153**, 601–624.

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INTRODUCTION

Mechanical loading during life is an important stimulus for the development of adult skeletal morphology (Carrier & Leon, 1990; van der Meulen, Beaupré, & Carter, 1993; Carter, van der Meulen, & Beaupré, 1996; Cubo & Casinos, 1998; Lanyon & Skerry, 2001; Ruff, 2003a, b). However, different skeletal features appear to exhibit different sensitivities to applied mechanical loading during growth. For example, in both birds and humans, changes in forelimb to hindlimb skeletal proportions at the initiation of flying or

bipedal locomotion, respectively, have a greater effect on bone strength parameters (such as section modulus) than on bone lengths (Carrier & Leon, 1990; Ruff, 2003a, b). Such observations raise questions regarding the relationships between length and cross-sectional geometry of limb bones and locomotor behaviour across vertebrates in general. Those taxa that experience unusual loading situations or undergo drastic changes in load during growth may be especially informative.

Birds represent an interesting group with which to study the structure and geometry of appendicular elements because avian flight exerts unique stresses on skeletal elements (Biewener & Dial, 1995). The forelimb skeleton of volant birds must resist loads

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from the proportionately large flight muscles and loads from aerodynamic forces, while the hindlimbs are involved in initiating launch. These mechanical loads begin near the ontogenetic onset of flight (via wing exercise), and result in specific and rapid changes in bone strength (Carrier & Leon, 1990). Because wing inertia, muscle power and flight gait all vary among bird species, it is expected that birds exhibiting different flight behaviours will subject their forelimb bones to varying types and magnitudes of mechanical load. Different bird taxa should thus develop differing resistances to mechanical loading of bone.

Relative wingspan also varies according to flight mode, and there is functional importance of aspect ratio for efficient production of lift (Pennycuick, 1989; Norberg, 1990; Alexander, 2002). As a result, forelimb bone lengths are also related to locomotor behaviour in birds. However, forelimb bone lengths may not be not as finely 'tuned' because of the lower developmental plasticity of bone length. In addition, span is only one component of aspect ratio [which is generally calculated as the square of the span divided by the wing area, or span over average chord length (Norberg, 1990; Alexander, 2002)]. Variation in chord also produces variation in wing loading between species with similar wingspan and body mass. 'Wing loading' is defined as body weight divided by total wing area, and is different from the mechanical loads on bones. The wing loading of similar sized bird species may differ by as much as a factor of two, even if span is identical, as a result of vastly different wing chords (Pennycuick, 1989).

In addition to variation in flight dynamics, birds vary greatly in the extent to which they use their hindlimbs for locomotion, ranging from exclusive use (flightless forms) to very limited use (e.g. cliff-nesting 'hyperaerial' forms). Furthermore, specific locomotor behaviours involving the hindlimbs are quite diverse: many birds are not only accomplished flyers, but also strong runners, swimmers or climbers. Hindlimb bone mechanical loads, and thus strength relative to body size, should also vary greatly among birds.

Despite this behavioural and morphological diversity, few studies have attempted to address and quantify the relationships between skeletal structural characteristics of avian limbs and bird behaviour comprehensively, especially in a comparative context. There have been a few analyses of the mechanical properties of bird long bones across several clades (Cubo & Casinos, 1998, 2000; Casinos & Cubo, 2001; de Margerie et al., 2005), but these have not included the full range of locomotor behaviour represented among birds, and have used only one or two specimens to represent most species (owing, in part, to destructive sampling techniques). These investiga-

tions have helped to elucidate the importance of torsional versus bending loads in avian limb bones (also see Biewener & Dial, 1995). However, they have not expressly examined relationships between forelimb and hindlimb strength. Furthermore, previous studies have not included lineages demonstrating very recent flight loss. As we show here, comparing the structural characters of closely related volant and flightless bird species yields some unique insights into the evolution of the avian skeleton.

In this study we demonstrate strong functional relationships between internal structural characteristics of the limb bones and behaviour in a diverse locomotor sampling of birds. As in some other vertebrate taxa (Schaffler et al., 1985; Ruff, 2002), we show that relatively simple comparisons of hindlimb to forelimb bone structure can distinguish between most locomotor groups. The technique is easily applicable to many fossil specimens, and has the potential to shed new light on the locomotor behaviour of extinct taxa.

MATERIAL AND METHODS

SAMPLE

A total of 126 individuals representing 15 species were included in the study sample. Species, together with their locomotor classifications, are detailed in Table 1. All specimens were received on loan from the Bird Division at the Smithsonian Museum of Natural History, Washington, DC. We obtained complete specimens from between four and ten individuals per species, depending on availability. The humerus and femur were taken from one side of each specimen (left and right sides were chosen randomly). The specimens had all been collected as wild animals, except for a juvenile specimen of *Rhea americana* (which was not used in statistical analyses, but is plotted as a visual marker) and one specimen of *Spheniscus magellanicus* (whose parameters did not differ significantly from the wild individuals). Specimens were chosen based on completeness (presence of proper elements) and condition of long bone elements. Both females and males were used for each species, although the specimens were chosen at random with respect to sex. All animals were physically mature osteologically, although some animals were recorded as having plumage consistent with their first year of life.

DESCRIPTION OF LOCOMOTOR GROUPS

Species were selected for this study to represent most of the major forms of avian locomotion. We have included terrestrial runners (both volant and flightless), arboreal perchers, hindlimb-propelled divers

Table 1. Summary of species included, in order of expected hindlimb/forelimb mechanical loadings

Taxon	Common name	Wing function			Leg function				
		Swimming	Flight	Reduced	Terrestrial	Perching	Swimming	Prey capture	Reduced
Rhea americanus	Rhea			X	X				
Phalacrocorax harrisi	Galapagos cormorant			X	X		X		
Aechmophorus occidentalis	Western grebe		X				X		
Phalacrocorax auritus	Double crested cormorant		X			X*	X		
Geococcyx californianus	Roadrunner		X		X				
Tyto alba	Barn owl		X			X		X	
Aquila chrysaetos	Golden eagle		X			X		X	
Falco tinnunculus	Eurasian kestrel		X			X		X	
Puffinus griseus	Sooty shearwater	X	X				X†		
Diomedea exulans	Wandering albatross		X				X†		
Corvus corax	Common raven		X		X	X			
Uria aalge	Common murre	X	X				X†		
Cerorhinca monocerata	Rhinoceros auklet	X	X				X†		
Spheniscus magellanicus	Magellanic penguin	X			X				
Phaethon rubricauda	Tropicbird		X						X

*Volant cormorants will perch on trees, posts and wires, but they do not perch in the same way as passerines or raptorial birds. Namely, they do not tightly grip perches with a reversed hallux, but simply wrap the forward digits over the support.
†Swimming by use of the hindlimbs applies only to surface swimming in the Alcidae and Procellariiforms. Diving is accomplished by use of the wings in *Puffinus* and Alcids.

(both lift- and drag-based swimmers), forelimb-propelled divers (both amphibious and fully aquatic species) and dynamic soarers. The hindlimb-propelled diving class includes a species representing recent, insular flight loss. The basic locomotor distinctions, in terms of forelimb and hindlimb use, are summarized in Table 1. Based on these distinctions, the species can be broken down into three general locomotor categories: flightless, bimodal and 'hyperaerial'. Flightless species have reduced the forelimbs and do not use them for locomotion. Bimodal species utilize two major modes of locomotion, with the hindlimbs active in one mode and the forelimbs active in another. 'Hyperaerial' species have greatly reduced (though not eliminated) the function of the hindlimbs, and travel almost exclusively on the wing. The bimodal category can be broken into a number of subcategories, which are listed below.

These distinctions are somewhat simplified, and mostly rather qualitative, but are useful as a general framework for interpretations, and to demonstrate the variety of behaviours represented in the study. We used species-level comparisons as the basis for our statistical analyses. More detailed discussions of specific flight characteristics (gait, endurance capacity, etc.) are included in the discussion of results. Behavioural categories are presented to facilitate discussion, and to help outline the range of ecologies represented in our data set. The categories are listed below to reflect progressively decreasing use of the hindlimb and increasing use of the forelimb during locomotion.

(1) Flightless species

Loss of flight is common in birds, and has occurred throughout the history of avian evolution (Feduccia, 1999, 2003; Paul, 2002). Secondary flightlessness is not only interesting in that it represents a locomotor transition, but also because comparisons of flightless taxa to volant species shed further light on flight-specific characteristics. Our use of the term 'flightless' applies only to those taxa that do not use the wings for any type of locomotion. We analysed two secondarily flightless species.

1a. Ancient flightless lineage (Rhea americana): Rheas are large-bodied ratites that represent a lineage of birds that have been flightless for a long period of time: the lineage leading to modern ratites probably lost flight in the Late Cretaceous (Cracraft, 2001) or the Early Tertiary (Feduccia, 2003).

1b. Recent flightless lineage (Phalacrocorax harrisi): In contrast to ratites, the Galapagos cormorant is a recently flightless species whose immediate sister taxa are volant. There are no fossil or subfossil

records of *P. harrisi*, but given the relatively young age of the islands on which this species is endemic (probably less than 700 000 years, Ciofi *et al.*, 2006) it is likely that the apomorphic loss of flight in this lineage occurred in the Pleistocene or Recent. This taxon makes an especially interesting case study because of the strong behavioural contrast with its closely related, volant relatives (represented in our study by *Phalacrocorax auritus*). *P. harrisi* is a drag-based hindlimb-propelled diver, like *P. auritus*.

(2) Bimodal species

2a. Lift-based hindlimb divers/'endurance' flyers (Aechmophorus occidentalis): Grebes are diving birds that use their hindlimbs for underwater propulsion. Interestingly, unlike most hindlimb-propelled swimming birds, grebes utilize a stroke that generates lift-based thrust with the feet (Johansson & Norberg, 2001). They have high wing loadings, and are slow to take off (Feduccia, 1999). Feduccia (1999) refers to grebes as being generally weak flyers. We refer to grebes as 'endurance' flyers, rather than as weak flyers, because they have strong endurance flight ability, but are slow to launch and cannot make tight maneuvers. The high average wing loading of grebes seems to predispose them somewhat to generate flightless forms (McCall, Nee & Harvey, 1998), with at least three of the extant 21 species being flightless or nearly so (Livezey, 1989). The fast, sustained, low-manoeuverability flight of grebes is probably mostly used for migrations and long-distance feeding trips; species that move to locations without seasonally limited food supplies have a tendency to lose flight (McCall *et al.*, 1998).

2b. Drag-based hindlimb divers (Phalacrocorax auritus): Drag-based hindlimb-propelled divers appear twice in our data set; both are species of *Phalacrocorax*. The flightless cormorant, *P. harrisi*, falls in the neoflightless category (see above). To represent bimodal (i.e. volant) drag-based divers we have included *P. auritus*, the double-crested cormorant. This species acts not only as an example of a common drag-based diving bird, but also as an important contrast with the closely related *P. harrisi*.

2c. Cursorial species (Geococcyx californianus): We have included a single species, the greater roadrunner, which is truly adapted to rapid running, and is also able to fly. Roadrunners have somewhat reduced sterna (Paul, 2002), and forage primarily on the ground. They still maintain reasonable flight ability, however.

2d. Raptors and owls (Aquila chrysaetos, Falco tinnunculus, Tyto alba): This includes raptors (represented by eagles and falcons) and owls. Hindlimb loading is probably dominated by predatory behaviour in raptors and owls, and thus we hypothesized that the hindlimb to forelimb strength ratios might be higher in raptorial birds than in other arboreal birds (such as passerines). We have placed raptors and owls in their own group for the sake of clarity, and to help examine this hypothesis.

2e. Perching birds (Corvus corax): We place passerine birds (represented in our study by ravens) in a distinct group because their relative hindlimb loads are expected to be lower than in raptors or owls (as ravens do not kill prey with the hindlimbs), but greater than in soaring specialists (as ravens do walk and run on the ground for significant periods while foraging). Without experimental evidence comparing the loads for perching and prey capture, the separation of ravens from birds of prey is largely subjective. This does not affect statistical analyses, because we utilized species pair comparisons when analysing the data set (and not functional groups).

2f. Marine soaring specialists (Diomedea exulans, Puffinus griseus): We place albatrosses (*Diomedea*) and shearwaters (*Puffinus*) in the same category, as they are both specialized for marine soaring. Despite spending vast spans of time on the wing, the lack of heavy flapping in dynamic soaring should reduce forelimb loading. It is notable, however, that shearwaters utilize forelimb-propelled diving, in addition to soaring flight. Due to the use of 'aquaflying' they may have similarities to the species placed in the 'forelimb swimming' group (see below). We maintain albatrosses and shearwaters as a functional group (rather than placing *Puffinus* with alcids and penguins) because the flight performances of the two taxa are extremely similar (both being dynamic soarers), but are very different from those of alcids or penguins.

2g. Forelimb swimmers (Cerorhinca monocerata, Uria aalge, Spheniscus magellanicus): Forelimb-propelled diving has evolved at least five times in birds, specifically in the penguins (Spheniscidae), auks (Alcidae), diving-petrels (Pelecanoididae), dippers (Cinclidae) and the extinct Plotopteridae (Raikow, Bicanovsky, & Bledsoe, 1988). Of these, those represented here are two auks (the rhinoceros auklet and common murre) and the magellanic penguin. Wing-propelled diving uses the forelimbs as lift-generating hydrofoils (Lovvorn & Liggins, 2002) and represents a form of aquatic flight. For this reason, we do not consider penguins to be 'flightless' birds in this study.

Aquatic flight (or 'aquaflying') has many similarities to aerial flight, but the two differ in some important respects.

Aquatic flight is the most efficient form of aquatic locomotion utilized by birds; lift-based aquaflying imparts thrust to the water with a propulsive efficiency of over 79%, as compared to 33% or less for drag-based paddling (Lovvorn & Liggins, 2002). Aquatic flight allows for thrust generation on both upstroke and downstroke, essentially eliminating a true 'recovery' phase (Clark & Bemis, 1979; Lovvorn & Liggins, 2002). As such, penguins should have effective power stroke frequencies nearly double that of aerial flyers using the same wing-beat frequency (and amphibious flyers such as auks should have effective power stroke frequencies somewhere between one and two per wing-beat cycle). The forelimb loadings during wing-propelled swimming should be greater than those generated by aerial flight, because the fluid forces are much greater in water than in air (fluid forces are directly proportional to the density of the fluid medium (Vogel, 2003)). The lift and drag forces in the water are four times those experienced in the air at the same Reynolds number (Johansson & Aldrin, 2002).

(3) 'Hyperaerial species': Reduced hindlimb function (Phaethon rubricauda)

Tropicbirds (*Phaethon rubricauda*) are oceanic soaring birds with reduced hindlimb function (Clark, Ricklefs, & Schreiber, 1983). We have described this species as being 'hyperaerial' to indicate that it is more reliant on aerial locomotion (and less reliant on walking or perching) than most birds. Tropicbirds rely largely on elevated perches (cliffs, trees) when roosting or nesting, and walk very poorly. Tropicbirds forage by plunge diving, and are still capable of launching from the water surface (Pennycuick et al., 1990; Le Corre, 1997), despite their reduced hindlimbs.

CT SCANNING: ORIENTATION OF HUMERI

Non-spheresciform taxa

For most bird taxa, the humeri were orientated such that the tricipitalis fossa pointed upwards away from the supporting surface, with the major proximal crests (crista deltopectoralis and crista bicipitalis) roughly parallel to the surface. In this position, the surface of the humerus that was anterior during life faces downwards, towards the platform. This does not represent a natural position of the humerus in life, but it is the most feasible way to align the humerus easily without need for excessive supporting material. The humeri were supported by clay such that the midshaft (where scans were taken) was as close to

horizontal (parallel to the plane of the support surface) as possible. Scans were taken perpendicular to the long axis of the diaphysis.

Sphenisciformes

The highly derived humeri of penguins had to be aligned differently from those of the other taxa. The humeri of penguins are extremely flat dorsoventrally, and were aligned such that the wide plane of the humerus was parallel to the supporting surface. This placed the penguin humeri in an alignment that was nearly perpendicular to the alignment used for all other humeri, although the tricipitalis fossa still pointed largely upwards (away from the surface) due to the derived morphology of penguin humeri. Scans were taken perpendicular to the long axis of the diaphysis.

CT scanning: orientation of femora (all taxa)

Femora were aligned so that the midshaft was as close to horizontal (parallel to the plane of the support surface) as possible. The femora were aligned with the dorsal surface of the bone (equivalent to the anterior surface in mammals) facing upwards away from the supporting surface. Scans were taken perpendicular to the long axis of the diaphysis.

Acquisition of cross-sections

We utilized peripheral quantitative computed tomography (pQCT) scans at the midshaft of each humerus and femur to obtain cross-sectional images and to acquire geometric and density data for the cross-sections. An XCT Research SA machine was used for the pQCT scanning, and software distributed with the machine was used to calculate bone densities and cross-sectional properties. Bone structural parameters derived using pQCT have been shown to be highly correlated with actual bone rigidity and strength measured directly (Ferretti, Capozza, & Zanchetta, 1996; Martin, Severns, & Kabo, 2004). A scan resolution (pixel edge length) of 0.1–0.2 mm was employed, except for rheas, where a resolution of 0.4 mm was used. (Scan width is a constant 1 mm by machine default.) This was sufficient to resolve all long bone cortices of the specimens included in this study.

Parameters for pQCT

Bending and torsional loads predominate in vertebrate limb bones (Carter, 1978; Rubin & Lanyon, 1982; Swartz, Bennett, & Carrier, 1992; Biewener & Dial, 1995; Blob & Biewener, 1999; Carrano & Biewener, 1999). Thus, we evaluated structural charac-

teristics related to bending and torsional strength in our comparative analyses. Bone strength is inversely related to maximum stress under loading. Using a beam model of the femoral and humeral diaphyses, maximum stress in bending is given by My/I (where M is the bending moment, I is the second moment of area about the neutral axis and y is the maximum distance from the neutral axis to the edge of the section); maximum stress in torsion is given by Tr/J (where T is the torsional moment, J is the polar second moment of area about the centroid and r is the maximum radial distance from the centroid to the edge of the section) (Gere & Timoshenko, 1990). The section modulus, Z, in bending is defined as I/y, and in torsion as J/r. We considered M and T to be proportional to the product of body mass (B) and bone length (L) (femoral or humeral) (Selker & Carter, 1989; Polk et al., 2000; Ruff, 2000). Thus, bone structural strength $\propto Z/(B*L)$. Because body mass is a constant within individuals, relative femoral to humeral structural strength can be assessed as $(Z_{fem}/L_{fem})/(Z_{hum}/L_{hum})$.

The pQCT software allows incorporation of bone density as well as geometry in evaluating bone structure (bone density, in this context, refers to the actual measured density of the tissue, and not relative cortical area). This procedure has been shown empirically to improve estimates of bone rigidity and strength (as variation in both material properties and geometry are included) (Ferretti et al., 1996; Martin et al., 2004). However, we focused primarily on geometric parameters, in part because we obtained very similar results to those obtained using a combination of geometry and bone density, but also because we wished to make our methods applicable to fossil specimens (for which bone material properties cannot be assessed). In addition, it has been shown by several researchers (Biewener, 1982; McAlister & Moyle, 1983; Currey, 1987; Erickson, Catanese, & Keaveny, 2002) that compact bone material properties are relatively consistent across different vertebrate groups, including birds, with major locomotor adaptations occurring primarily through geometric structural alterations. For convenience when presenting results, we use the term 'strength' or 'structural strength' to refer to the geometric component of bone strength, assuming constant bone material properties across elements and taxa. As noted above, the inclusion of bone density data (from pQCT scans) made no appreciable difference in any of our results, supporting this approach.

We used the polar section modulus (Zp) in comparative analyses because it is related to both torsional and (twice) average bending strength in any two perpendicular planes (Ruff, 2002). As described above, the orientation of bones for scanning was standard-
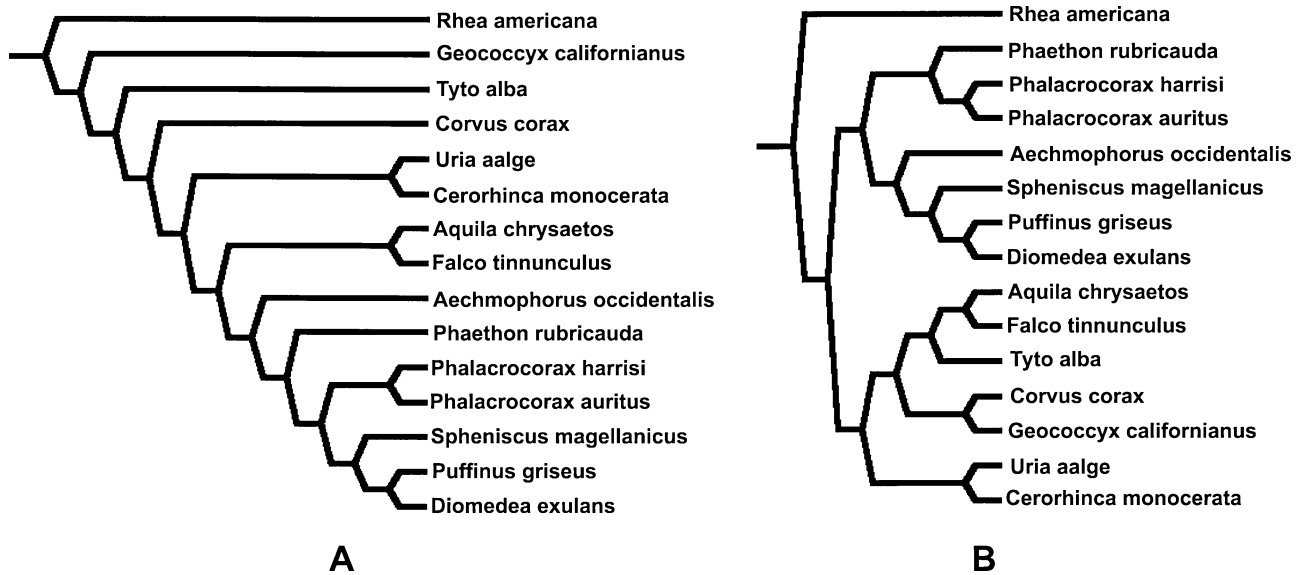
Figure 1. Avian phylogenies used for tests of phylogenetic independence. A, the phylogeny based on the topology presented by Sibley & Ahlquist (1990). B, the phylogeny based on the topology presented by Livezey & Zusi (2007).

ized but somewhat arbitrary relative to *in-vivo* orientations; thus, calculation of structural properties in any particular plane relative to the scanner is of minor interest. Also, torsional strength of the humerus has been shown to be crucial in flying birds (Biewener & Dial, 1995). Torsional strength also appears to be important for avian femora (Carrano & Biewener, 1999). To evaluate relative structural strength of the femur and humerus, following the reasoning presented above, we compared the Zp/L ratios for the two bones. We also compared femoral to humeral lengths directly.

Relative cortical thickness of long bone diaphyses shows contrasts between flying and non-flying vertebrates, as well as aquatic and non-aquatic species (Currey & Alexander, 1985; Swartz *et al*., 1992). Thus, we also included a comparison of relative cortical areas (cortical area divided by total periosteal area) of the femur and humerus among our study species.

PHYLOGENETIC INDEPENDENCE

Because of shared ancestry, species used in a comparative analysis may not be statistically independent (Felsenstein, 1985; Harvey & Pagel, 1991). Therefore, we utilized the program Phylogenetic Independence (PI) (Reeve & Abouheif, 2003) to test for phylogenetic non-independence in structural characteristics between our study species. A phylogenetic topology is required to test for phylogenetic non-independence, and we chose to utilize two different topologies and test the phylogenetic signal as predicted by each topology. The first topology is that

presented in Sibley & Ahlquist (1990) (see Fig. 1A), and the other is from the phylogeny presented in Livezey & Zusi (2007) (see Fig. 1B).

Using the phylogeny of Sibley & Ahlquist (1990) the level of phylogenetic signal was found to be non-significant ($P > 0.250$) for our section modulus and bone length data. This phylogeny predicts a high degree of functional homoplasy in the locomotor dynamics of the bird groups we included, and is based entirely on DNA–DNA hybridization data. The second phylogeny, as taken from Livezey & Zusi (2007), predicts a high degree of functional homology with regard to avian locomotor dynamics. Functionally similar groups tend to be recovered as sister taxa in the Livezey & Zusi (2007) topology. This may mean that some of the characters used in the Livezey & Zusi matrix, all of which are morphological, have functional significance. For example, owls and raptors are recovered as sister taxa, as are grebes and loons (both of which are hindlimb-propelled swimmers). Using the topology of Livezey & Zusi (2007), we find that there is a significant influence of phylogeny on the data patterning ($P < 0.025$).

For our particular data set, most of the phylogenetic signal is reliant on the base node, which is the comparison between *Rhea americana* (our only included palaeognathous bird) and all other species in the data set. As a member of an ancient flightless lineage, rheas have bone strength ratios vastly different from all other species in our data set. Because rheas are both phylogenetically distant and functionally distinct from the in-group taxa, the rheas may have a disproportionate effect on the phylogenetic

signal, which can lead to inappropriate conclusions about phylogenetic structuring of functional characters in the in-group. Setting the value for the structural strength ratio in rheas to the average for the in-group removes the effect of the rheas on phylogenetic patterning, and simulates the situation wherein the out-group taxon is functionally similar to the in-group. In this case, the procedure simulates a case in which the out-group is a volant species, rather than a large-bodied, flightless taxon. When the value for average structural strength in rheas is set to the average for all other birds in the data set (making phylogenetic signal at the base node zero), the test for phylogenetic autocorrelation fails to find a significant result using either topology [$P > 0.263$ using the Livezey & Zusi (2007) topology; $P > 0.371$ using the Sibley & Ahlquist (1990) topology].

When phylogenetic similarity does not predict functional similarity, conventional methods are often preferable (Gittleman & Kot, 1990). Phylogenetic patterning in our data set is overwhelmingly the result of the ancient difference between the neognathan in-group and the flightless palaeognath out-group taxon. This comparison is functionally trivial, and phylogenetic similarity does not generally predict functional similarity within the in-group clade. Given these considerations, we have decided to use raw data values without generating independent contrasts for this analysis. The decision to utilize species values is also driven by the type of analysis we chose to use in this study. We utilized species-to-species comparisons (via *post-hoc* Tukey–Kramer tests, see below) for our analysis, and our conclusions are driven by these species-to-species comparisons. Phylogenetically controlled data, such as independent contrasts, are awkward for this type of comparison because the generated contrasts do not correspond to individual species.

BONE STRUCTURAL PARAMETERS

Bivariate plots of femoral to humeral strength and length ratios, natural-log transformed, were used visually to assess proportional differences between species. We also carried out ANOVAs with *post-hoc* Tukey–Kramer multiple comparison tests between log ratios of femoral to humeral strength and length of each species. This technique is justified when relationships between variables are isometric within similar locomotor groups (Ruff, 2002), which is the case within virtually all of the species examined here (confirmed using reduced major axis analysis).

RESULTS

Figure 2 is a plot of femoral versus humeral structural strength (polar section moduli/length, log-transformed) for all individuals in the sample. Species are delimited by ovals with their long axes orientated isometrically; different symbols distinguish the locomotor categories described above. Mean log ratios of femoral to humeral strength for each taxon are shown in Figure 3, arranged in the same order as predicted earlier based on locomotor distinctions (Table 1). Results of the pairwise Tukey–Kramer tests for log strength ratios between species are shown in Table 2.

Variation in relative femoral to humeral structural strength among the species in our sample generally shows good correspondence to expectations based on limb use during locomotion. The flightless rheas show the relatively highest femoral to humeral strength, followed by the recently flightless Galapagos cormorants (*P. harrisi*) (Figs 2, 3). The lift-based hindlimb diving grebes (*Aechmophorus*) are next in femoral/humeral strength, followed by the other hindlimb diver, the double-crested cormorant (*P. auritus*). All of these species are significantly different from each other, and from all other species, in structural strength proportions (Table 2). The species with the next highest average femoral/humeral strength are roadrunners (*Geococcyx*), overlapping with eagles (*Aquila*). The strength ratio of *Aquila* also overlaps with the ratios of marine soaring birds (*Diomedea* and *Puffinus*). The other two raptors in our sample, falcons (*Falco*) and owls (*Tyto*), are more variable, with falcons especially showing unexpectedly low femoral/humeral strength ratios. Ravens (*Corvus*) also have low femoral/humeral strength, similar to that of falcons. Our forelimb swimmers (*Uria*, *Cerorhinca* and *Spheniscus*) have uniformly low (and statistically equivalent) femoral/humeral strength ratios, i.e. relatively strong humeri. The 'hyperaerial' tropicbird (*Phaethon*) has a significantly lower femoral/humeral strength ratio than any other species in the sample. As a visual reference for the range of geometries encompassed by our species, we have included images of paired femoral and humeral cross-sections for several representative individuals in Figure 4. The taxa depicted in Figure 4 all demonstrate extremes of morphology, and were chosen to demonstrate the full range of variation included in the data set. The greatest differences between femoral and humeral structural strength are demonstrated by *Rhea americana* and *Phaethon rubricauda*, while *Falco tinnunculus* and *Spheniscus magellanicus* cross-sections demonstrate extremes in relative cortical bone area. The two cormorants, *Phalacrocorax auritus* and *P. harrisi*, are included to demonstrate visually the marked difference in cross-sectional morphology between these two closely related species.

In contrast to femoral/humeral structural strength comparisons, variation in relative femoral to humeral length among our species bears little consistent rela-
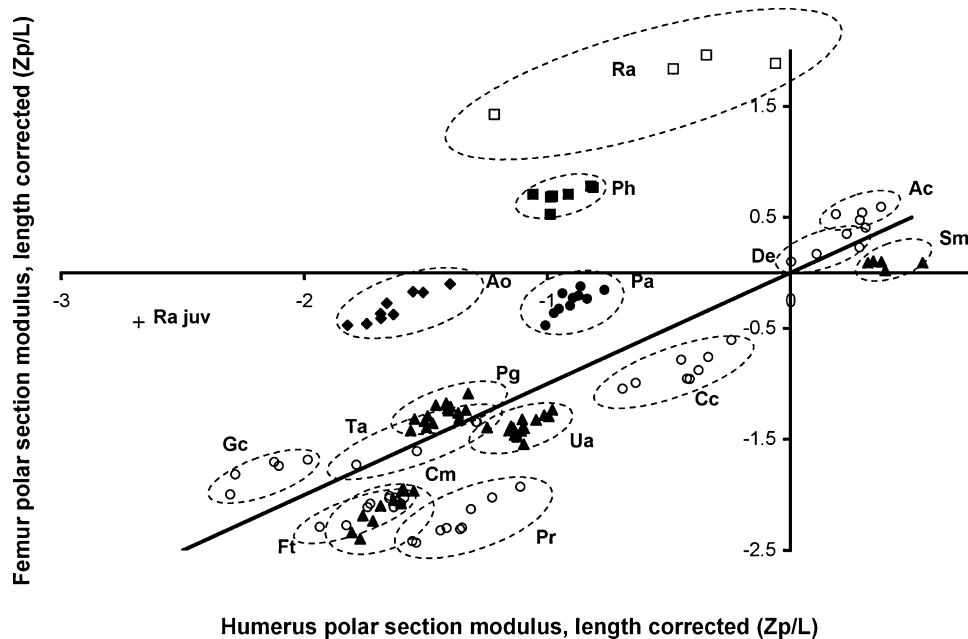
Figure 2. Polar section moduli of femora versus section moduli of humeri for all individuals, standardized for bone length (see text). *Zp* is used here as an estimate of strength in torsion and average strength in bending. The solid line represents equal strength ($y = x$). Data are presented natural log transformed, and symbols correspond to locomotor categories in Table 1. De, *Diomedea exulans*; Ac, *Aquila chrysaetos*; Sm, *Spheniscus magellanicus*; Ph, *Phalacrocorax harrisi*; Pa, *P. auritus*; Ao, *Aechmophorus occidentalis*; Pg, *Puffinus griseus*; Ua, *Uria aalge*; Cc, *Corvus corax*; Cm, *Cerorhinca monocerata*; Ta, *Tyto alba*; Ft, *Falco tinnunculus*; Gc, *Geococcyx californianus*; Pr, *Phaethon rubricauda*; Ra, *Rhea americana*. The single juvenile rhea was not used in statistical analyses. The long axis of ovals delimiting species indicate isometry. Symbols are as follows, filled symbols, diving birds (those with subaqueous locomotion); triangles (▲), forelimb-propelled diving; squares (■,□), flightless taxa. Grebes are indicated by diamonds (◆) and volant cormorants are shown with filled circles (●). The single juvenile rhea is indicated by a cross (+). All other species are delimited by open circles (○).

tionship to locomotor differences. Ratio comparisons of femoral to humeral length are shown in Figures 5 and 6, and Table 3. The most 'hindlimb-dominant' taxon, *Rhea*, shows femoral/humeral length proportions indistinguishable from those of falcons, and smaller than those of roadrunners and penguins. The recently flightless Galapagos cormorants are very similar in length proportions to eagles and have slightly lower ratios than ravens. The two hindlimb divers – grebes and double-crested cormorants – have among the lowest femoral/humeral length proportions, most similar to those of the marine soaring shearwaters and 'hyperarial' tropicbirds. The three forelimb swimmers – penguins, auks and murres – have widely divergent length proportions overlapping those of several other locomotor categories. There are a few instances where femoral/humeral length proportions may have a straightforward locomotor interpretation; for example, the relatively long femur of roadrunners may be related to their cursoriality, and the relatively long humerus of albatrosses (and to a lesser extent shearwaters) to their extreme adapta-

tions to marine soaring. However, in most cases, a direct locomotor inference is difficult to draw from bone length proportions.

Figure 7 shows cortical area percentage in the femora plotted against that of the humeri for all individuals (of all species). This plot quickly summarizes a measure of cortical thickness (or, considered in reverse, a measure of 'hollowness'). Species that swim underwater in search of food generally have bones with thick cortices. Penguins possess the thickest cortices (in both the humerus and the femur, also see Fig. 4). Flightless cormorants show marked thickening of the cortices in the femur, compared with volant cormorants, while the relative cortical areas in the humerus of the two species are similar. All diving species have relative cortical areas above 0.50 (i.e. 50% cortical bone) in the humerus. The only non-diving species to fall above this threshold are rheas and a few tropicbird individuals. The shearwaters (*Puffinus grisesus*), which are both dynamic soarers and aquaflyers, group with other aquaflyers based on relative cortical area of the humerus.
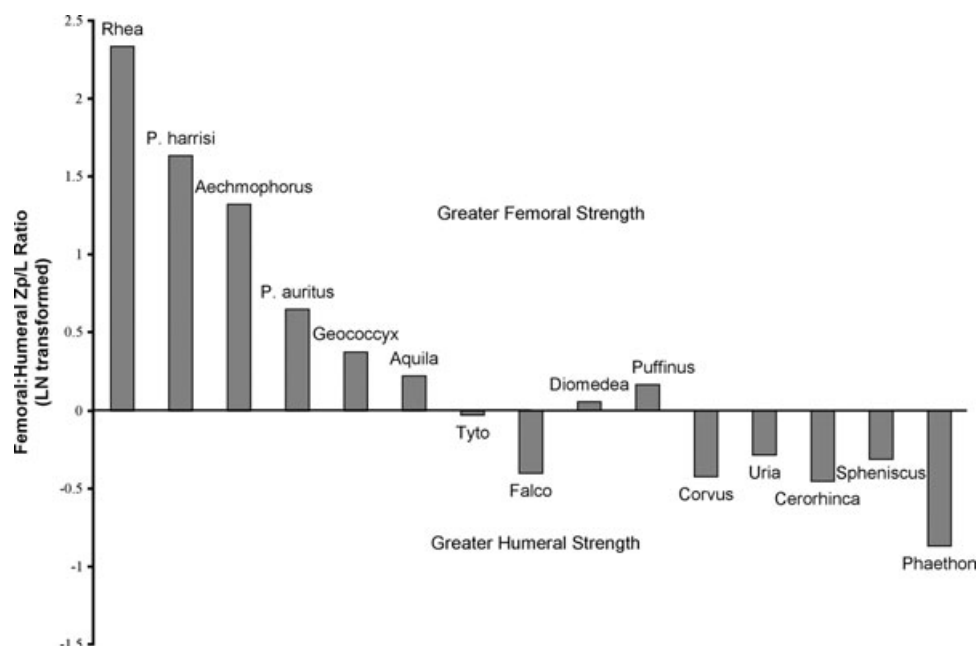
Figure 3. Mean natural log-ransformed ratios of femoral to humeral section modulus. Taxa are ordered according to the expected functional trend (see Table 1).

Because humeral cortical thickness shows utility in differentiating aquatic and non-aquatic taxa, we combined our cortical thickness measures with hindlimb/forelimb strength proportions to provide a better separation of functional groups. Figure 8 shows the section modulus ratio plotted against the percentage of cortical area of the humerus, for all individuals across all species. This plot produces a separation between all locomotor groups, even those that appear similar according to the femoral to humeral strength ratio alone (e.g. it separates wing-propelled divers from ravens and falcons, Fig. 3). A summary of the strength ratio parameters that distinguish functional groups is given in Table 4, and a summary of the relative humeral cortical area parameters that distinguish functional groups is provided in Table 5.

DISCUSSION

Overall, hindlimb to forelimb bone structural strength ratios are very good osteological indicators of locomotor function in birds, and appear to contain greater behavioural signal than hindlimb to forelimb bone length proportions in avian taxa. Structural ratios clearly predict which taxa are neoflightless within this sample. Length ratios, taken alone, do not accurately predict flightlessness. The ratio of femur length to humeral length in the recent flightless species *Phalacrocorax harrisi* overlaps tightly with the same ratio in the volant species *Corvus corax* (ravens). Even the length ratios in *Rhea americana* do

not give an unambiguous result. Both roadrunners (*Geococcyx*) and penguins (*Spheniscus*) have relatively shorter humeri than *Rhea* individuals.

The most obvious message taken from plotting femoral length against humeral length is that such length ratios are relatively inaccurate, on their own, for making functional comparisons between species. The element lengths are quite important for their use in including moment arm distances in the bending strength calculations, and length ratios are also helpful in identifying marine soaring birds (albatrosses especially). Element lengths can also be used in certain functional comparisons, if sufficient data are available. Middleton & Gatesy (2000) found that relative element lengths within the forelimb of birds could be used to infer volancy versus flightlessness in some lineages. However, this approach requires material from both the proximal and the distal forelimb, and the results do not give the same resolution of behavioural inferences as are achieved from structural ratios. Overall, structural ratios are considerably more useful for understanding functional aspects of locomotion in birds than are length ratios alone. There are several reasons why this could be the case. One possibility is that element lengths might be largely genetically determined (and more phylogenetically constrained). This hypothesis is supported by numerous experimental and observational studies (Lanyon, 1980; Biewener & Bertram, 1993, 1994; Trinkaus, Churchill & Ruff, 1994). It is also possible that the manner in which bone element length relates

Table 2. Results of ANOVA with *post-hoc* Tukey–Kramer multiple comparison tests between log ratios of femoral to humeral strength of each species

	Aechmop	Aquila	Cero	Corv	Diom	Falco	Geoc	P. aur	P. har	Phaet	Puff	Rhea	Sphen	Tyto
Aechmophorus	—													
Aquila	0.0001	—												
Cerorhinca	0.0001	0.0001	—											
Corvus	0.0001	0.0001	**0.3050**	—										
Diomedea	0.0001	**0.1722**	0.0001	0.0001	—									
Falco	0.0001	0.0001	**0.9656**	**1.0000**	0.0001	—								
Geococcyx	0.0001	**0.1848**	0.0001	0.0254	0.0001	0.0001	—							
P. auritus	0.0001	0.0001	0.0001	0.0001	0.0022	0.0001	0.0001	—						
P. harrisi	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—					
Phaethon	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—				
Puffinus	0.0001	**0.9934**	0.0001	0.0001	**0.5304**	0.0001	0.0001	0.0001	0.0001	0.0001	—			
Rhea	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—		
Spheniscus	0.0001	0.0001	0.1160	**0.4415**	0.0003	**0.7130**	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—	
Tyto	0.0001	0.0013	0.0001	0.0001	**0.9773**	0.0001	0.0295	0.0001	0.0001	0.0001	0.0040	0.0001	0.0001	—
Uria	0.0001	0.0001	0.0001	0.0043	0.0001	0.0292	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	**1.0000**	0.0001

Because the vast majority of the comparisons were statistically significant, we have indicated in bold those results that are not significant (i.e. pairings which are statistically similar).
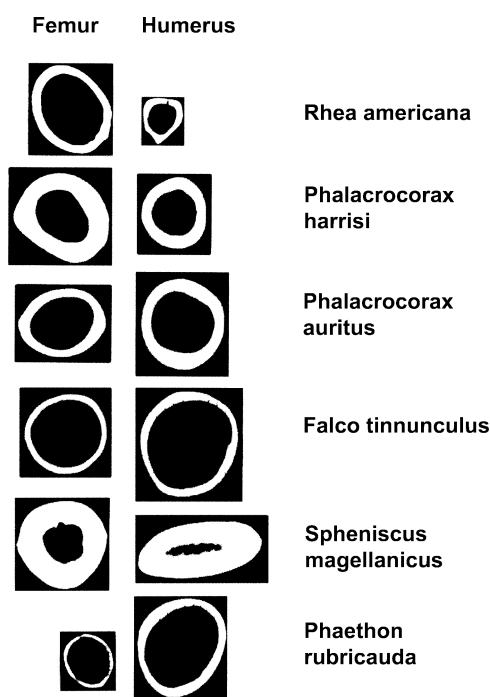
Femur   Humerus

Rhea americana

Phalacrocorax harrisi

Phalacrocorax auritus

Falco tinnunculus

Spheniscus magellanicus

Phaethon rubricauda

Figure 4. pQCT images from a subset of the study species included, representing the range of geometries in the data set. Species not shown to same scale.

to locomotion is quite complicated. Species with similar wingspans may still have vastly different aspect ratios or wing loadings (depending on the chord), as well as differing capacities for aerobic or anaerobic power. Thus, species with similar spans can have very different flight kinematics (Pennycuick, 1989; Neuweiler, 2000). Bone cross-sectional structure, because it responds to mechanical loads experienced during life (Carrier & Leon, 1990; Ruff, 2003a, b), is more easily interpreted in functional terms. Furthermore, bone strength may take into account a wider range of locomotor forces than bone length does alone, given that bone strength reflects adaptation to the full range of loads experienced by any given bone element.

Alternative methodologies exist for approximating the cross-sectional structure of long bones, in cases where CT imaging is unavailable or prohibitively expensive. Biplanar radiographs can be used to reconstruct cross-sectional contours with reasonable accuracy (Biknevicius & Ruff, 1993; Runestad et al., 1993), especially when combined with external moulding of periosteal surfaces (O'Neill & Ruff, 2004). External breadths alone give some information on cross-sectional size and shape, but can be misleading if relative cortical thickness varies significantly. As we have shown here, relative cortical breadth varies considerably among birds, and may even be very

different between closely related species. Within procellariiform birds, for example, the relative cortical areas of albatrosses and shearwaters differ markedly (presumably as a result of diving habits in shearwaters). In fossil taxa, which may have unknown structural and functional differences from living relatives, assumptions about cortical breadth can be especially problematic. Therefore, we recommend measuring the cross-sectional properties when assessing long bone diaphyseal structure. In the following sections, we summarize results for each of the major locomotor groups in our study.

FLIGHTLESS TAXA

Both rheas and flightless cormorants have very high femoral to humeral structural ratios compared with the rest of our sample. The humeri of *P. harrisi* maintain roughly the same absolute structural strength as the volant cormorant species, *P. auritus*, although the femora of *P. harrisi* are considerably stronger. Because flightless cormorants are larger-bodied than *P. auritus*, their humeri are, in fact, reduced in strength relative to body mass. However, the elevated femoral to humeral strength ratio in flightless cormorants is more a product of femoral robustness than humeral weakness.

In contrast, the humeri of *Rhea americana* individuals are very slender, and are only slightly more robust than the humeri of the much smaller *P. harrisi*. The implication is that rheas have reduced their forelimb more extensively than *P. harrisi* because ratites have been flightless for much longer than Galapagos cormorants. Additionally, *P. harrisi* seems to have developed more robust hindlimbs than its volant relatives in a very short period of time, which we suspect is largely the result of accommodation to increased walking stresses. Length ratios are a poor choice for distinguishing flightless species from volant relatives. This appears to occur because the forelimbs of flightless species initially become more gracile and weak, with length declining more slowly. As length ratios alone do not account for any kind of robustness, they cannot accurately predict the behaviour of flightless species with long, weak forelimb skeletons (or the behaviour of flying species with short, strong forelimb elements).

Individuals of *Phalacrocorax harrisi* have greatly thickened bone cortices in their femora relative to flying cormorants. These thick-walled femora have significantly greater structural strength than the femora of volant cormorants (Table 2; Fig. 2). The overall breadth of the femora is also increased in *P. harrisi*, but not to a great degree. Thus, the increase in bone strength in the hindlimb of flightless cormorants is largely a result of increased bone wall
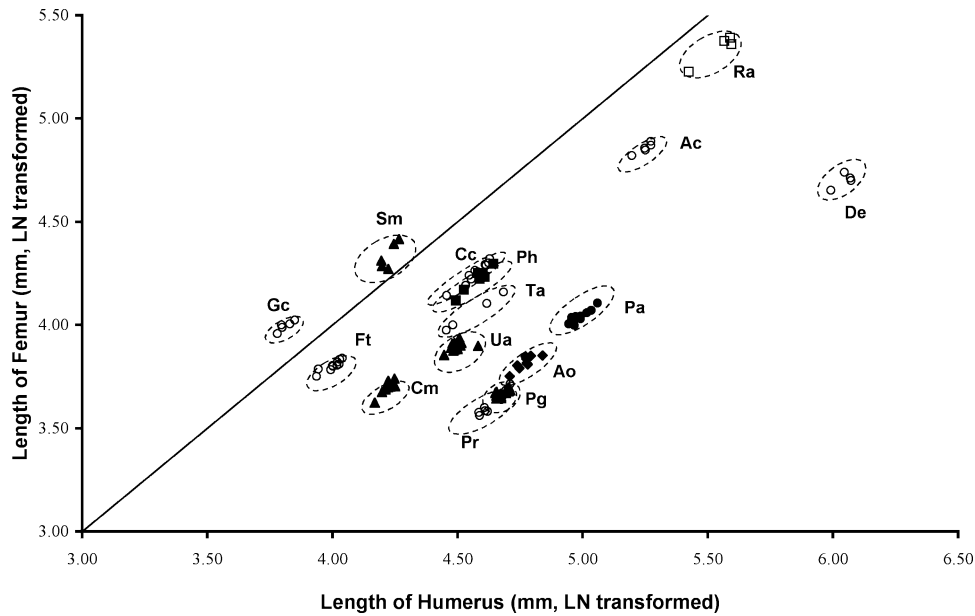
Figure 5. Total length of femora versus total length of humeri for all individuals. Data are presented natural log-transformed. The solid line represents equal length ($y = x$). Symbols and species name abbreviations follow those in Figure 2. The long axes of the ovals delimiting species indicate isometry.
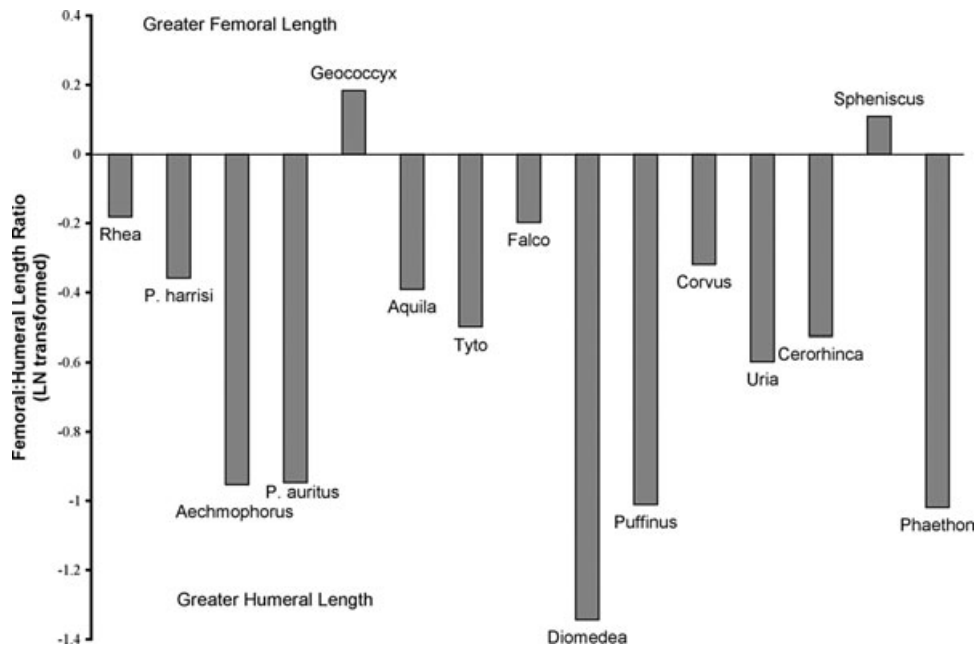

Figure 6. Mean natural log-transformed ratios of femoral to humeral length. Taxa are ordered according to the expected functional trend (see Table 1).

thickness, as opposed to an increased bone radius. It may be that greatly thickened cortical bone is a trait of more recent flightless morphs, and that such lineages will tend to develop greater bone breadths (with thinner cortices) over time. Alternatively, the thickened cortices could be an adaptation that increases density for diving (see below). We cannot determine whether either of these tentative hypotheses is accurate at this time, but we expect that future work comparing the cross-sectional properties of flightless and volant sister taxa will help to clarify the mechanical consequences of flight loss.

Table 3. Results of ANOVA with *post-hoc* Tukey–Kramer multiple comparison tests between log ratios of femoral to humeral length of each species

	Aechmop	Aquila	Cero	Corv	Diom	Falco	Geoc	P. aur	P. har	Phaet	Puff	Rhea	Sphen	Tyto
Aechmophorus	–													
Aquila	0.0001	–												
Cerorhinca	0.0001	0.0001	–											
Corvus	0.0001	0.0001	0.0001	–										
Diomedea	0.0001	0.0001	0.0001	0.0001	–									
Falco	0.0001	0.0001	0.0001	0.0001	0.0001	–								
Geococcyx	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	–							
P. auritus	**1.0000**	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	–						
P. harrisi	0.0001	**0.4886**	0.0001	0.0338	0.0001	0.0001	0.0001	0.0001	–					
Phaethon	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	–				
Puffinus	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	**0.9999**	–			
Rhea	0.0001	0.0001	0.0001	0.0001	0.0001	**0.9893**	0.0001	0.0001	0.0001	0.0001	0.0001	–		
Spheniscus	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	–	
Tyto	0.0001	0.0001	**0.8162**	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	–
Uria	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Because the vast majority of the comparisons were statistically significant, we have indicated in bold those results that are not significant (i.e. pairings which are statistically similar).
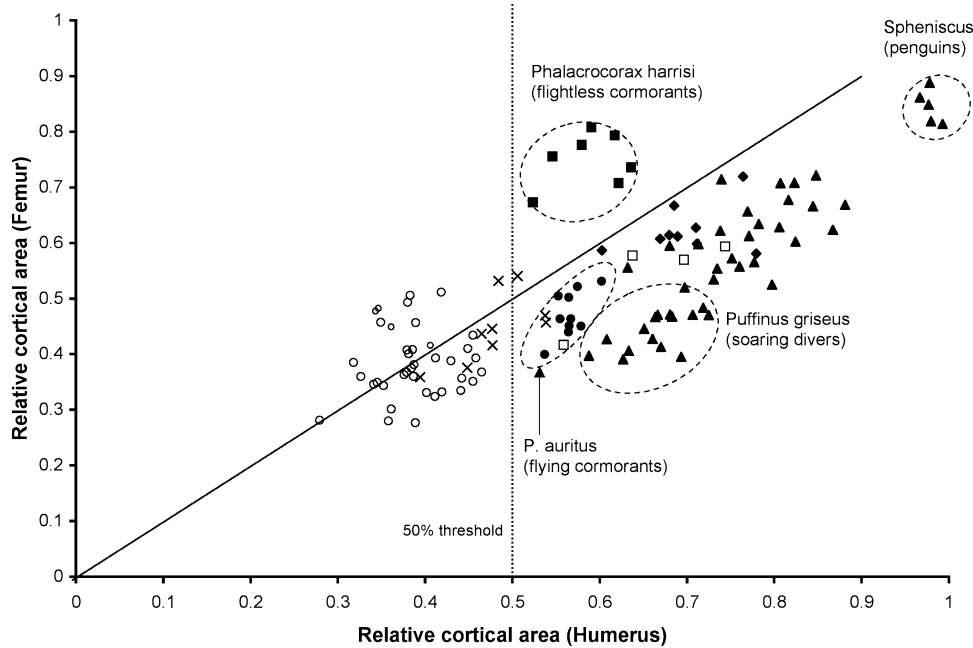
Figure 7. Percentage of bone cross-section composed of cortical bone in the femur and humerus of each individual. This represents an inverse measure of 'hollowness' in the limb bones scanned. *Phalacrocorax harrisi* and *Spheniscus magellanicus* are both highlighted for qualitative reference, as these species have the thickest-walled bones of the taxa studied. *Phalacrocorax auritus* individuals are highlighted for comparison with *P. harrisi*. *Puffinus griseus* individuals, which utilize both dynamic soaring and wing-propelled diving, are highlighted to emphasize that their cortical areas are similar to other divers. Symbols and labels are as in Figure 2, except that X's denote hyperaerials.
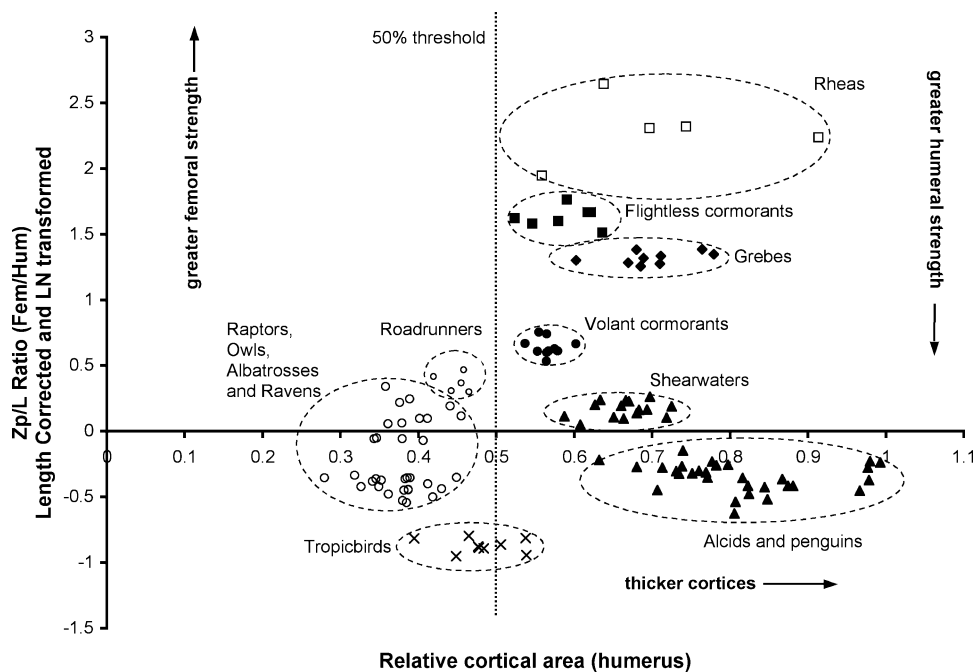

Figure 8. Section modulus ratio versus humeral cortical bone percentage in cross-section (at midshaft). Section modulus ratio is corrected for bone element length and natural log-transformed. Symbols and labels are as for Figure 2, except that X's denote hyperaerials.

Table 4. Summary of structural ratio parameters distinguishing functional groups. All are based on natural log-transformed, length-corrected structural ratios

Functional group		Mean structural ratio	SD	95% CI		Min.	Max.
Ancient flightless	1a	2.33	0.25	1.84	2.82	1.95	2.64
Recent flightless	1b	1.63	0.08	1.47	1.79	1.51	1.76
Grebes (hindlimb lift swimming)	2a	1.32	0.05	1.23	1.41	1.26	1.39
Hindlimb drag swimmers	2b	0.65	0.07	0.52	0.78	0.53	0.75
Cursorials	2c	0.38	0.07	0.24	0.52	0.30	0.47
Raptorials	2d	−0.07	0.28	−0.61	0.47	−0.48	0.34
Pelagic soarers	2e	0.11	0.06	−0.01	0.23	−0.06	0.26
Arboreals	2f	−0.42	0.08	−0.57	−0.27	−0.54	−0.33
Wing–propelled swimmers	2g	−1.05	0.11	−1.26	−0.84	−0.36	−0.15
Hyperaerials	3	−0.87	0.06	−0.98	−0.76	−0.95	−0.80

Minimum, maximum and average values of section modulus femur/humerus ratio are included, along with the 95% confidence interval about the mean.

Table 5. Summary of relative humeral cortical area parameters distinguishing functional groups

Functional group		Mean relative cortical area	SD	95% CI		Min.	Max.
Ancient flightless	1a	0.710	0.13	0.45	0.97	0.559	0.914
Recent flightless	1b	0.588	0.08	0.43	0.74	1.514	1.764
Grebes (hindlimb lift swimming)	2a	0.699	0.05	0.60	0.80	0.602	0.779
Hindlimb drag swimmers	2b	0.566	0.02	0.53	0.60	0.537	0.602
Cursorials	2c	0.448	0.02	0.41	0.48	0.419	0.465
Raptorials	2d	0.381	0.04	0.30	0.46	0.279	0.449
Pelagic soarers	2e	0.609	0.12	0.38	0.84	0.379	0.725
Arboreals	2f	0.371	0.03	0.31	0.43	0.318	0.418
Wing-propelled swimmers	2g	0.811	0.09	0.63	0.99	0.632	0.993
Hyperaerials	3	0.481	0.04	0.39	0.57	0.394	0.539

Relative cortical area is the cortical area (at midshaft) taken over the total periosteal area. Minimum, maximum and average values of the relative cortical area are included, along with the 95% confidence interval about the mean. These values are primarily presented for distinguishing aquatic and non-aquatic taxa.

HYPERAERIALS

Structural strength ratios are also informative for distinguishing species with greatly reduced hindlimb function (*Phaethon*). Length ratios inaccurately suggest that albatrosses have more reduced hindlimb function than *Phaethon*. Although albatrosses certainly do make more limited use of their hindlimb than many birds (length ratios correctly suggest they are not cursorial), the function of their hindlimbs is not as reduced as in *Phaethon*. Length ratios do not distinguish hyperaerials from other functional groups (the length ratios of *Phaethon* individuals overlap with those of cormorants and shearwaters). In contrast, within our bone strength analysis, *Phaethon* individuals fall out as significantly different from all other taxa studied, having the lowest femoral to humeral strength ratio. Although *Phaethon* has reduced the length of its femora (and hindlimb in general), it is the extreme reduction in hindlimb loading (and thus femoral strength) that really typifies its complete dedication to aerial mobility at the expense of terrestrial locomotion. Other taxa that would be expected to show similar bone strength traits are frigate birds (genus *Fregata*) and caprimulgiform birds (nightjars, whip-poor-wills and allies).

HINDLIMB-PROPELLED DIVERS

The use of structural ratios proved to be sensitive enough as a behavioural indicator to differentiate between the two groups of hindlimb-propelled divers (cormorants and grebes). Interestingly, despite the fact that cormorants and grebes utilize different swimming mechanics (drag-based paddling in cormorants and lift-based swimming in grebes), the two taxa have very similar femoral strength. However, *Aechmophorus occidentalis* individuals are smaller-bodied than *Phalacrocorax auritus* individuals. If the total forces imparted upon the water during swimming are proportional to body mass, then it is likely that the lift-based swimming performed by the grebes generates greater hindlimb stress. In other words, femoral structural strength relative to body mass is higher in *Aechmophorus occidentalis* than in *Phalacrocorax auritus*. By contrast, grebes have humeri that are proportionately weaker than those of double-crested cormorants. Overall, comparing ratios between forelimb and hindlimb cross-sectional parameters indicated that grebes sustain far greater mechanical loads on their hindlimbs than on their forelimbs, as compared with cormorants. It is not immediately apparent why the humeri of grebes are subjected to relatively low loads. It may be that endurance flight produces low loads as a result of the modest power output of aerobic muscle. The highly aerobic pectoralis muscles of mallard ducks, for example, produce an average of 175 W kg^{-1}, as compared with 390 W kg^{-1} obtained from the anaerobically dominated avian muscles in some galliform birds (Askew & Marsh, 2002).

PELAGIC SOARING BIRDS

Two of the species included in our analysis, *Puffinus griseus* and *Diomedea exulans*, are pelagic seabirds that rely greatly on soaring. Albatrosses (*Diomedea*) are particularly specialized for soaring, and are generally considered to rely primarily on high-speed dynamic soaring, by using either wind gradients (Tickell, 2000) or gust soaring (Pennycuick, 2002). The length of the forelimbs vastly exceeds that of the hindlimbs in these animals, which spend vast amounts of time in the air (often without flapping for long periods). However, our results indicate that the forelimbs in these two species are not particularly strong compared with the hindlimbs (Fig. 2), but instead that the strength of the femora and humeri are roughly equal (assuming similar material properties). The most likely explanation is that rather little muscle power is exerted on the humeri during flight in these animals, as they rarely flap their wings during steady flight. As gliding requires no thrust production, aerodynamic forces on the wings should

also be reduced during soaring flight. By contrast, although the hindlimbs are short, they must be strong enough to accelerate the animals to their launch window during a running start. Albatrosses, in particular, are large-bodied, and will require significant force output from the hindlimbs during launch runs. It appears that launch loads on the humeri are relatively modest in albatrosses (judging by their relatively low humeral structural strength). This may be a result of the running launch dynamic, but we have only modest support for this explanation at this time.

In addition, high-aspect-ratio soaring birds (especially those of large size) probably utilize a continuous vortex gait, primarily (rather than a vortex ring gait), which utilizes a passive upstroke via aerodynamic lift (Rayner, 1988, 1991, 2001). Because the upstroke is passive, the work done by the supracoracoideus muscle is rather minimal. Using the continuous vortex gait avoids the additional costs of cutting off lift production at the end of every downstroke (Rayner, 2001). In general, being able to utilize a continuous vortex gait probably limits required muscle power and associated bone stress and strain. Comparing bone strength across species may broadly estimate relative power requirement differences between taxa.

Puffinus is more closely related to albatrosses than to alcids, and shares specializations for prolonged pelagic soaring with albatrosses. However, because *Puffinus* dives and uses the forelimbs during subaqueous locomotion, it shares some behavioural characteristics with alcids. Our bone strength ratio results indicate that *Puffinus griseus* is more similar to albatrosses, from a structural standpoint, than it is to alcids. This implies, first and foremost, that the functional signal from marine soaring specialization is strong. It suggests, additionally, that forelimb-propelled swimming does not always result in low hindlimb/forelimb *Zp* ratios (i.e. much stronger bones in the forelimb than in the hindlimb). However, incorporating both relative cortical area and bone strength together visually separates shearwaters and albatrosses, and groups *Puffinus* near other forelimb-propelled diving forms (such as alcids and penguins).

Using the ratios of bone structural parameters along with length ratios provides a quantifiable metric for distinguishing pelagic soaring birds from species that use mostly flapping flight, and the approach further distinguishes them from pelagic soaring birds that rarely land at sea (tropicbirds). Given that this analysis relies only on osteological signals, there is clear utility for the same techniques in identifying the behaviour of fossil seabirds. We suspect that similar osteological signals may exist to distinguish between inland soaring birds and those

species that mostly utilize flapping flight, but we have yet to analyse birds which travel mainly by inland soaring, such as vultures. Eagles and ravens both soar, but are much less reliant on convective soaring than vultures.

FORELIMB-PROPELLED DIVERS

Structural strength ratios accurately predict that the forelimb is the primary propulsive module for penguins and alcids. Length ratios are ambiguous for these taxa, especially for penguins (which have relatively long femora and short humeri). Wing-propelled divers have interesting structural properties, especially with regard to the humerus, in several respects. All of the wing-propelled divers we studied have robust humeri with thickened cortices, and a flattened shape. Penguins, being completely committed to wing-propelled diving, demonstrate the most extreme derivations of forelimb structure of the species included in our study (Fig. 4).

Our results suggest that the forelimbs of wing-propelled divers, and especially those of penguins, are adapted to high loads. This may help to explain small wing size in many wing-propelled divers. There is some debate as to why the wings of aquatic flyers are small, especially in alcids, but also in penguins. Because water is much denser than air (about an 850-fold difference), the lift and drag forces in the water are four times that in the air at the same Reynolds number (the forces differ by the same factor as density, 850 times, if all other factors are kept constant). Combined with the increased inertia of wings in aqueous media, this tends to favour short, high-aspect-ratio wings for cost-effective high-speed swimming (Johansson & Aldrin, 2002). However, Bridge (2004) has shown that alcids with wing areas reduced by moulting do not show increased swimming performance, and in fact probably show slightly reduced performance. Bridge (2004) concluded that small wing size in alcids is not an adaptation to diving, but rather an adaptation to high-speed flight in an open environment (open ocean). Furthermore, the shearwater species included in our analysis dives effectively using a wing-propelled kinematic, and yet has long forelimbs.

Nevertheless, the thick cortices and overall high resistance to bending and/or torsion in the humeri of wing-propelled divers does suggest that the wings are subjected to high loads under water. Bone length ratios vary substantially between wing-propelled divers, but bone strength ratios are more consistent across aquaflying taxa (especially between alcids and penguins), which suggests that adaptation to load is an important constraint. As wing-propelled swimmers generate propulsive forces in both the upstroke and the downstroke (in a medium with high drag and wing inertia), muscular forces should be high in both the downstroke and the upstroke phases. Shortening the bones further increases their resistance to bending deformation, so short wings/flippers are advantageous under high loads.

Because the high density of water also increases lift, and because a propulsive upstroke is possible, small wings should manage quite well from a propulsive standpoint for aquatic locomotion. Combined with the structural problems (high bending loads) of flying underwater, this serves as a likely combination of factors to select for small wings in alcids and penguins. For alcids, stout wings are probably advantageous both for rapid flight and for resisting high forces while swimming, and may be less of a compromise than sometimes assumed. Shearwaters presumably maintain long forelimbs because they are dynamic soarers, in addition to being wing-propelled divers (dynamic soaring requires long wingspans to be effective). Shearwaters may incur a swimming cost for their retention of a longer wing, but that is currently unknown. It may be that shearwaters utilize different kinematics from alcids and penguins when swimming, or that they reduce the wingspan by a greater fraction than alcids when diving.

Interestingly, despite the use of the forelimbs for propulsion underwater, alcids and penguins do not appear to have structural strength ratios significantly different from those of all non-aquatic birds. The femoral to humeral bone strength ratios of auks and penguins are lower than the same ratios in most other birds, but ravens and falcons have bone strength ratios that are similar to those of auks and penguins. The strength ratio in *Phaethon* is even lower still, but this is primarily attributable to reduced hindlimbs (rather than especially robust forelimb elements). However, because the cross-section of the humerus is so non-circular in penguins and auks, the polar section moduli utilized in our analysis are less representative of average bending strength in those taxa (Daegling, 2002). Comparing bending moments in functionally meaningful anatomical planes may be necessary to confirm the biomechanical relationship of penguins and alcids to other birds.

RAPTORS AND OWLS

All three species of hypercarnivorous birds included in our analysis (*Aquila chrysaetos*, *Tyto alba* and *Falco tinnunculus*) demonstrate very round cross-sections with thin cortices (indicative of bending in multiple planes and/or high amounts of torsion), in both the femur and the humerus. These structural traits may be convergent between owls and raptors, but they may also be synapomorphic. The phylogeny

of Livezey & Zusi (2007), utilized in our study for tests of phylogenetic signal, suggests a close relationship between owls and raptors. Mayr (2005) also suggested that the two groups have a sister taxon relationship.

These hypercarnivorous species differ in their prey preferences and hunting behaviours. Typically, such fine-grained differences are difficult to ascertain osteologically, but these three species demonstrate differences in bone strength ratios that appear to correspond to foraging parameters. The ratio of femoral bone strength to humeral bone strength is highest in eagles (*Aquila*), which take large-bodied prey from the ground (Watson, 1997). Prey size can be very large relative to the eagle; *Aquila chrysaetos* individuals sometimes kill pronghorn juveniles as heavy as 25 kg (Tigner, 1973), which is over five times the mass of an average *A. chrysaetos* individual. High femoral structural strength in eagles may reflect these prey preferences, or it may be a scaling effect related to launching at larger body sizes. Barn owls (*Tyto alba*) take smaller terrestrial prey, especially in the size range 10–40 g (Roulin, 2002), and *Falco tinnunculus* routinely (if not primarily) also captures small terrestrial prey such as voles (Costantini *et al.*, 2005). Given that *F. tinnunculus* and *T. alba* hunt similar-sized prey, we expected the ratios of section moduli for *F. tinnunculus* to be similar to those recorded in *T. alba*. Instead, *T. alba* individuals have a structural ratio intermediate to those measured for falcons and eagles, while *F. tinnunculus* has the lowest ratio of femoral bone strength to humeral bone strength of the three raptorial species.

We are not yet certain why *T. alba* has a significantly greater ratio of femoral to humeral bone strength than *F. tinnunculus*. It may be that there are differences in the talon force generated by owls and hawks of similar size, as has been suggested by Ward, Weigl & Conroy (2002). Specifically, owls seem to generate greater forces in the distal hindlimb than hawks of similar size. Based on this finding, and combined with information on limb element lengths, Ward *et al.* (2002) concluded that raptors have hindlimbs more highly adapted to rapid deployment while owls have hindlimbs more highly derived for force production. If this difference holds for the proximal hindlimb as well, then our results may reflect differences both in diet (small prey versus large prey) and in talon use between owls and raptors. However, the precise manner in which the force exerted on prey by the distal limb translates up the limb to the femur has not been measured. Other activities of the hindlimb might therefore be involved in the signal we see regarding structural strength of the hindlimb in raptorial birds. Launch loads, for example, may differ between species and size classes.

Another explanation for the observed difference between the ratios in the falcons and owls is that the falcons may have especially strong humeri (rather than the owls having particularly strong femora). This is lent some support by the fact that *Falco tinnunculus* individuals have humeral strengths that overlap with those of *Tyto alba*, but *F. tinnunculus* is (on average) substantially smaller than *T. alba* by mass. Falcons may exert high stresses on their humeri, relative to other birds of prey, during high-speed flight (for which most species of *Falco* are adapted). Overall, it appears that both raptors and owls have high strength values in both the forelimb and the hindlimb (especially as compared with similarly sized birds), and this implies high stresses on the appendicular skeleton in predatory birds. In order to assess the validity of these two hypotheses further, and to understand stress/strain trends in predaceous birds better, a much wider range of raptors and owls will need to be analysed. Including species with more profound differences in food preference (and foraging strategy) may help us assess how prey capture affects the structural adaptations of hypercarnivorous birds. Specfically, the inclusion of species that specialize in particular prey items (such as piscivorous or ophiophagus taxa) or those taxa that hunt almost entirely on the ground (such as secretary birds, *Sagittarius serpentarius*) might broaden our perspective on raptor mechanics.

ROADRUNNERS

The position of roadrunners (*Geococcyx*) in our analysis has implications for interpreting limb proportions in running birds that fly (this is of distinct importance for interpretations of behaviour in fossil birds). Roadrunners have very short humeri relative to their femora, even more so than the two flightless species (*Rhea* and *Phalacrocorax harrisi*). Based on length values alone, one might easily suggest that *Geococcyx* was flightless. The humeri, however, are short and strong, and their bone strength is a good quantitative measure that roadrunners are actually capable of flight. There is obviously a distinct ecological difference between being largely cursorial (but volant) and being flightless. For analyses of fossil species, where a full skeleton is rarely available, the ability to make this distinction accurately and empirically, using only two elements, will be of great utility.

RELATIVE CORTICAL AREAS AND 'HOLLOWNESS'

Penguins possess the thickest walled bones of any of the birds examined. Some penguin humeri were composed of 96% cortical bone in cross-section. Auks have thick-walled bones as well, although the medullary

space is not as reduced as in penguins. Such extremely thickened cortices may have multiple adaptive advantages. It seems that the thick-walled bones of penguins act to increase resistance to stress and strain while simultaneously lowering buoyancy. Such thickening of the cortices may also be important in maintaining very stiff bones. Bone stiffness, specifically in the humeri, may be of great importance when flying underwater. The walls of penguin femora are also thickened (but somewhat less so than in their humeri). The femora may be under high loads from extended walking (with an unusual gait), although buoyancy reduction is likely to be important. Utilizing relative cortical area and section modulus ratio simultaneously clearly distinguishes penguins and auks from the few species with which they share similar hindlimb to forelimb bone strength ratios. Combining cortical area measures with section modulus ratios also predicts the subaqueous locomotion of shearwaters (which otherwise group with albatrosses). Strength ratios predict the use of forelimb propulsion in aquaflyers, while relative cortical areas clearly distinguish such animals as aquatic. Relative cortical area is generally quite effective in this regard; nearly all non-aquatic birds have less than 50% relative cortical area in their humeri, while all aquatic birds fall above that threshold (the major exception to this pattern is *Rhea americana*, which probably has thick-walled humeri as a by-product of humeral breadth reduction).

Wall (1983) and Currey & Alexander (1985) have discussed the significance of relative cortical thickness of long bones in relation to buoyancy problems among aquatic vertebrates. With the exception of some deep divers that achieve the same effect through collapse of the lungs (such as cetaceans), aquatic animals tend to have thicker cortices ('denser' bones) than non-aquatic animals, probably to reduce buoyancy. Wall (1983) gives a figure of 30% of the total bone diameter occupied by the cortices as an approximate cut-off value between terrestrial and aquatic or semi-aquatic mammals. Interestingly, when translated into percentage cortical area (cortical area/total periosteal area), this is virtually equivalent to the 50% threshold that we found here between birds with and without subaqueous locomotion (Fig. 7). This applied better to the humerus than to the femur, which also tended to be thicker-walled in aquatic birds but showed more overlap between aquatic and non-aquatic forms. Perhaps the particularly thick-walled humeri of aquatic divers are a combined solution to buoyancy problems together with the high mechanical loads on these elements and need for a streamlined limb contour (flattening or narrowing of the bone contour) in at least some taxa.

PHYLOGENETIC EFFECTS AND PHYLOGENETIC INDEPENDENCE

Species were chosen for this study based on functional characteristics of locomotion, and those with similar structural ratios are overwhelmingly convergent in our data set, according to both phylogenies utilized. This high level of convergence, combined with a strong functional signal, helped to minimize confounding influences of phylogeny. Future research, using expanded species sets, will need to include repeated tests for phylogenetic non-independence, as there is no guarantee that species-level structural parameters are statistically independent given any particular species sample. Cubo *et al.* (2005) found that some subsets of avian structural characters do maintain a signal of shared ancestry, and we have found some evidence for that here, using the topology of Livezey & Zusi (2007). Using alternative phylogenetic hypotheses and study taxa may change the phylogenetic signal of the sample.

Furthermore, characters can demonstrate functional signal and historical signal simultaneously (Desdevises *et al.*, 2003), so we cannot claim that the trends in structural characters that we have discussed here are devoid of historical influence, regardless of the results of tests for statistically significant phylogenetic signal. Nonetheless, we consider the functional conclusions drawn from our data set to be quite robust. Our particular data set includes a large number of functionally similar, but phylogenetically distant, species that demonstrate strong correlations between bone structure and locomotor function. The differences between volant and flightless cormorants are clearly a case of functional differences that have evolved in a very short time span (since the flightless Galapagos cormorants recently split from a volant ancestor very similar to *Phalacrocorax auritus*). The three groups of wing-propelled subaqueous swimming taxa in our data set (penguins, shearwaters and alcids) possess a suite of structural similarities detected in our analysis. Alcids and penguins are especially similar, structurally. None of these wing-propelled swimming taxa is considered to be a near relative of each another, however, by either of the phylogenies we utilized in our analysis. The flattened humeral cross-section, thickened cortices and high humeral bone strength seen in wing-propelled divers is clearly functional in nature, even if historical trends are also present. Similarly, the weak hindlimbs of *Phaethon rubricauda* are clearly indicative of a reduced ability to walk and utilize running launches, even if we allow for the impact of phylogeny. Interpreting the selectional implications of this reduced hindlimb function would require a more explicit phylogenetic context: *Phaethon* may be

selected for reduced hindlimb function itself, or it may have inherited weak hindlimbs and reduced walking capability from an ancestor that was under such selection (or both).

IMPLICATIONS

Given how well structural ratios predict locomotor behaviour in modern birds, there is great potential for the application of our methods to behavioural questions related to fossil animals. Structural ratios take into account actual loads experienced by animals during life, which makes them highly suited for correlating morphology and behaviour. The hindlimb to forelimb bone strength ratios of birds appear to give a good overall picture of locomotor function that length ratios miss. Accounting for differences in relative cortical area provides further distinction between aquatic and non-aquatic forms. We hope to use these same techniques in the future to understand better the origin of flight within advanced theropod dinosaurs, and the subsequent changes to (and loss of) flight behaviour in the evolution of birds. Furthermore, such analyses should be extended to other flying vertebrates, such as pterosaurs and bats (although the differences in morphology and flight behaviour between groups will necessitate subtle changes in methodology).

Bone length measurements have long been used as a source of behavioural inference, especially within the field of vertebrate palaeontology, and bone lengths continue to be used as metrics for predicting avian behaviour (Middleton & Gatesy, 2000; Zeffer, Johansson & Marmebro, 2003; Nudds, Dyke, & Rayner, 2004, 2007; Nudds, 2007).

Our observation that length proportions do not always perform reliably as behavioural indicators in living taxa is therefore perhaps the most significant result of this study. Length ratios do carry some behavioural signal, but it is limited. Nudds *et al*. (2007) tested brachial index (the ratio between humeral length and ulnar length) as an indicator of flight dynamics, and did find statistically significant signals of flight kinematics and wing shape within brachial index measurements. However, the signal related to wing shape was weak, and flight kinematics were only defined within broad groups based on span reduction during the upstroke phases of flight. Even with a very large data set, and a rigorous statistical methodology, length ratios only define locomotor ability in birds within the broadest limits. Middleton & Gatesy (2000) examined length disparity between elements within the forelimbs of theropod dinosaurs, throughout their 230-Myr existence (including modern avian diversity). The authors found notable and informative patterns (for example,

a relative lengthening of the humerus in flightless forms) but the behavioural signal was relatively weak when compared with the clear indications of locomotor ability we have found here using limb bone structural strength.

In addition to providing a strong indicator of locomotor behaviour, quantifications of bone structural strengths provide useful biomechanical information regarding avian locomotion that length measurements alone do not offer. For example, bone structural strength can provide a quantitative measure of the bone reinforcement associated with subaqueous locomotion, or of the relative reduction in bone strength required by marine soaring birds. Bone strength can address issues of mechanical limits in birds, as well. For example, some birds that manage to fly long distances with continuous flapping appear to generate only modest forelimb loads (grebes), as evidenced by low humeral structural strength, suggesting that particular morphologies and/or behaviours can make volancy possible at a relatively low magnitude of skeletal stress. Bone structural strength measures are therefore not only powerful for making behavioural inferences based on osteological information, but they also hold promise for furthering our general understanding of flight mechanics and the evolution of avian locomotion.

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REFERENCES

Alexander D. 2002. *Nature's flyers: birds, insects, and the biomechanics of flight*. Baltimore, MD: Hopkins University Press.

Askew GN, Marsh RL. 2002. Muscle designed for maximum short-term power output: quail flight muscle. *Journal of Experimental Biology* **205:** 2153–2160.

Biewener A. 1982. Bone strength in small mammals and bipedal birds: do safety factors change with body size? *Journal of Experimental Biology* **98:** 298–301.

Biewener AA, Bertram JE. 1993. Mechanical loading and bone growth *in vivo*. In: Hall BK, ed. *Bone. Vol. 7: Bone growth-B.* Boca Raton, FL: CRC Press, 1–36.

Biewener AA, Bertram JE. 1994. Structural response of growing bone to exercise and disuse. *Journal of Applied Physiology* **76:** 946–955.

Biewener AA, Dial KP. 1995. In vivo strain in the humerus of pigeons (*Columba livia*) during flight. *Journal of Morphology* **225:** 61–75.

Biknevicius AR, Ruff CB. 1993. Use of biplanar radiographs for estimating cross-sectional geometric properties of mandibles. *Anatomical Record* **232:** 157–163.

Blob RW, Biewener AA. 1999. In vivo locomotor strain in the hindlimb bones of *Alligator mississippiensis* and *Iguana iguana*: implications for the evolution of limb bone safety factor and non-sprawling limb posture. *Journal of Experimental Biology* **202:** 1023–1046.

Bridge ES. 2004. The effects of intense wing molt on diving in alcids and potential influences on the evolution of molt patterns. *Journal of Experimental Biology* **207:** 3003–3014.

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. *Journal of Morphology* **240:** 237–249.

Carrier D, Leon LR. 1990. Skeletal growth and function in the California gull (*Larus californicus*). *Journal of Zoology* **222:** 375–389.

Carter DR. 1978. Anisotropic analysis of strain rosette information from cortical bone. *Journal of Biomechanics* **11:** 199–202.

Carter DR, van der Meulen MCH, Beaupré GS. 1996. Mechanical factors in bone growth and development. *Bone* **18** (S1)**:** 5–10.

Casinos A, Cubo J. 2001. Avian long bones, flight, and bipedalism. *Comparative Biochemistry and Physiology Part A* **131:** 159–167.

Ciofi C, Wilson GA, Beheregaray LB, Marquez C, Gibbs JP, Tapia W, Snell HL, Caccone A, Powell JR. 2006. Phylogeographic history and gene flow among giant Galapagos tortoises on southern Isabela Island. *Genetics* **172:** 1727–1744.

Clark BD, Bemis W. 1979. Kinematics of swimming penguins at the Detroit Zoo. *Journal of Zoology* **188:** 411–442.

Clark L, Ricklefs RE, Schreiber RW. 1983. Nest-site selection by the red-tailed tropicbird. *Auk* **100:** 953–959.

Costantini D, Casagrande S, Di Lieto G, Fanfani A, Dell'Omo G. 2005. Consistent differences in feeding habits between neighbouring breeding kestrels. *Behaviour* **142:** 1403–1415.

Cracraft J. 2001. Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. *Proceedings of the Royal Society of London Series B* **268:** 459–469.

Cubo J, Casinos A. 1998. Biomechanical significance of cross-sectional geometry of avian long bones. *European Journal of Morphology* **36:** 19–28.

Cubo J, Casinos A. 2000. Mechanical properties and chemical composition of avian long bones. *European Journal of Morphology* **38:** 112–121.

Cubo J, Ponton F, Laurin M, de Margerie E, Castanet J. 2005. Phylogenetic signal in bone microstructure of sauropsids. *Systematic Biology* **54:** 562–574.

Currey JD. 1987. The evolution of the mechanical properties of amniote bone. *Journal of Biomechanics* **20:** 1035–1044.

Currey JD, Alexander RM. 1985. The thickness of the walls of tubular bones. *Journal of Zoology* **206:** 453–568.

Daegling DJ. 2002. Estimation of torsional rigidity in primate long bones. *Journal of Human Evolution* **43:** 229–239.

Desdevises Y, Legendre P, Azouzi L, Morand S. 2003. Quantifying phylogenetically-structured environmental variation. *Evolution* **57:** 2647–2652.

Erickson GM, Catanese J 3rd, Keaveny TM. 2002. Evolution of the biomechanical material properties of the femur. *Anatomical Record* **268:** 115–124.

Feduccia A. 1999. *The origin and evolution of birds*. New Haven, CT: Yale University Press.

Feduccia A. 2003. 'Big bang' for Tertiary birds? *Trends in Ecology and Evolution* **18:** 172–174.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* **125:** 1–15.

Ferretti JL, Capozza RF, Zanchetta JR. 1996. Mechanical validation of a tomographic (pQCT) index for noninvasive estimation of rat femur bending strength. *Bone* **18:** 97–102.

Gere JM, Timoshenko SP. 1990. *Mechanics of materials*. Boston: PWS-Kent.

Gittleman JL, Kot M. 1990. Adaptation statistics and a null model for estimating phylogenetic effects. *Systematic Zoology* **39:** 227–241.

Harvey PH, Pagel MD. 1991. *The comparative method in evolutionary biology*. Oxford: Oxford University Press.

Johansson LC, Aldrin BSW. 2002. Kinematics of diving Atlantic puffins (*Fratercula artica* L.): evidence for an active upstroke. *Journal of Experimental Biology* **205:** 371–378.

Johansson LC, Norberg UML. 2001. Lift-based paddling in diving grebe. *Journal of Experimental Biology* **204:** 1687–1696.

Lanyon LE. 1980. The influence of function on the development of bone curvature. An experimental study on the rat tibia. *Journal of Zoology (London)* **192:** 457–466.

Lanyon L, Skerry T. 2001. Postmenopausal osteoporosis as a failure of bone's adaptation to functional loading: a hypothesis. *Journal of Bone Mineral Research* **16:** 1937–1947.

Le Corre M. 1997. Diving depths of two tropical pelecaniformes: the red-tailed tropicbird and the red-footed booby. *Condor* **99:** 1004–1007.

Livezey BC. 1989. Flightlessness in grebes (Aves, podicipedidae): its independent evolution in three genera. *Evolution* **43:** 29–54.

Livezey BC, Zusi RL. 2007. Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zoological Journal of the Linnean Society* **149:** 1–95.

Lovvorn JR, Liggins GA. 2002. Interactions of body shape, body size, and stroke-acceleration patterns in costs of underwater swimming by birds. *Functional Ecology* **16:** 106–112.

McAlister GB, Moyle DD. 1983. Some mechanical properties of goose femoral cortical bone. *Journal of Biomechanics* **16:** 577–589.

McCall R, Nee S, Harvey PH. 1998. The role of wing length in the evolution of avian flightlessness. *Evolutionary Ecology* **12:** 569–580.

de Margerie E, Sanchez S, Cubo J, Castanet J. 2005. Torsional resistance as a principal component of the structural design of long bones: comparative multivariate evidence in birds. *Anatomical Record A* **282A:** 49–66.

Martin DE, Severns AE, Kabo JM. 2004. Determination of mechanical stiffness of bone by pQCT measurements: correlation with non-destructive mechanical four-point bending test data. *Journal of Biomechanics* **37:** 1289–1293.

Mayr G. 2005. The postcranial osteology and phylogenetic position of the Middle Eocene *Messelastur gratulator* (Peters, 1994): a morphological link between owls (Strigiformes) and falconiform birds? *Journal of Vertebrate Paleontology* **25:** 635–645.

van der Meulen MCH, Beaupré GS, Carter DR. 1993. Mechanobiologic influences in long bone cross-sectional growth. *Bone* **14:** 635–642.

Middleton KM, Gatesy SM. 2000. Theropod forelimb design and evolution. *Zoological Journal of the Linnean Society* **28:** 149–187.

Neuweiler G. 2000. *The biology of bats.* Translation: E. Covey. New York: Oxford University Press.

Norberg UM. 1990. *Vertebrate flight: mechanics, physiology, morphology, ecology and evolution*. Berlin: Springer-Verlag.

Nudds RL. 2007. Wing-bone length allometry in birds. *Journal of Avian Biology* **38:** 515–519.

Nudds RL, Dyke GJ, Rayner JMV. 2004. Forelimb proportions and the evolutionary radiation of Neornithes. *Proceedings of the Royal Society of London, Series B* **271** (Suppl. 5)**:** 324–327.

Nudds RL, Dyke GJ, Rayner JMV. 2007. Avian brachial index and wing-kinematics: putting movement back into bones. *Journal of Zoology* **272:** 218–226.

O'Neill MC, Ruff CB. 2004. Estimating human long bone cross-sectional geometric properties: a comparison of noninvasive methods. *Journal of Human Evolution* **47:** 221–235.

Paul GS. 2002. *Dinosaurs of the air*. Baltimore, MD: Johns Hopkins University Press.

Pennycuick CJ. 1989. *Bird flight performance: a practical calculation manual*. Oxford: Oxford University Press.

Pennycuick C. 2002. Gust soaring as a basis for the flight of petrels and albatrosses (Procellariiformes). *Avian Science* **2:** 1–12.

Pennycuick C, Schaffner FC, Fuller MR, Obrecht HHIII, **Sternberg L. 1990.** Foraging flights of the white-tailed tropicbird (Phaethon lepturus): radiotracking and doubly-labelled water. *Colonial Waterbirds* **13:** 96–102.

Polk JD, Demes B, Jungers WL, Biknevicius AR, Heinrich RE, Runestad JA. 2000. A comparison of primate, carnivoran and rodent limb bone cross-sectional properties: are primates really unique? *Journal of Human Evolution* **39:** 297–325.

Raikow RJ, Bicanovsky L, Bledsoe AH. 1988. Forelimb joint mobility and the evolution of wing-propelled diving in birds. *Auk* **105:** 446–451.

Rayner JMV. 1988. The evolution of vertebrate flight. *Biological Journal of the Linnean Society* **34:** 269–287.

Rayner JMV. 1991. Avian flight evolution and the problem of *Archaeopteryx*. In: Rayner JMV, Wootton RJ, eds. *Biomechanics in evolution*. Cambridge: Cambridge University Press, 183–212.

Rayner JMV. 2001. On the origin and evolution of flapping flight aerodynamics in birds. In: Gauthier J, Gall LF, eds. *New perspectives on the origin and evolution of birds. Special Publication of the Peabody Museum of Natural History* 363–385.

Reeve J, Abouheif E. 2003. *Phylogenetic independence*, Version 2.0. Department of Biology, McGill University. Distributed freely by the authors on request.

Roulin A. 2002. Tyto alba. *BWP-update* **4:** 115–138.

Rubin CT, Lanyon LE. 1982. Limb mechanics as a function of speed and gait: a study of functional strains in the radius and tibia of horse and dog. *Journal of Experimental Biology* **101:** 187–211.

Ruff CB. 2000. Body size, body shape, and long bone strength in modern humans. *Journal of Human Evolution* **38:** 269–290.

Ruff CB. 2002. Long bone articular and diaphyseal structure in Old World monkeys and apes, I: locomotor effects. *American Journal of Physical Anthropology* **119:** 305–342.

Ruff CB. 2003a. Ontogenetic adaptation to bipedalism: age changes in femoral to humeral length and strength proportions in humans, with a comparison to baboons. *Journal of Human Evolution* **45:** 317–349.

Ruff CB. 2003b. Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. *Bone* **33:** 317–329.

Runestad JA, Ruff CB, Nieh JC, Thorington RW, Teaford MF. 1993. Radiographic estimation of long bone cross-sectional geometric properties. *American Journal of Physical Anthropology* **90:** 207–213.

Schaffler MB, Burr DB, Jungers WL, Ruff CB. 1985. Structural and mechanical indicators of limb specialization in primates. *Folia Primatologica* **45:** 61–75.

Selker F, Carter DR. 1989. Scaling of long bone fracture strength with animal mass. *Journal of Biomechanics* **22:** 1175–1183.

Sibley CG, Ahlquist JE. 1990. *Phylogeny and classification of birds: a study in molecular evolution*. New Haven, CT: Yale University Press.

Swartz SM, Bennett MB, Carrier DR. 1992. Wing bone stresses in free flying bats and the evolution of skeletal design for flight. *Nature* **359:** 726–729.

Tickell WLN. 2000. *Albatrosses*. New Haven, CT: Yale University Press.

Tigner JR. 1973. Golden eagle predation on a pronghorn fawn. *Southwestern Naturalist* **18:** 346–348.

Trinkaus E, Churchill SE, Ruff CB. 1994. Postcranial robusticity in *Homo*, II: humeral bilateral asymmetry and bone plasticity. *American Journal of Physical Anthropology* **93:** 1–34.

Vogel S. 2003. *Comparative biomechanics: life's physical world*. Princeton, NJ: Princeton University Press.

Wall WP. 1983. The correlation between high limb-bone density and aquatic habits in recent mammals. *Journal of Paleontology* **57:** 197–207.

Ward AB, Weigl PD, Conroy RM. 2002. Functional morphology of raptor hindlimbs: implications for resource partitioning. *Auk* **119:** 1052–1063.

Watson D. 1997. *The golden eagle*. London: T and A. D. Poyser.

Zeffer L, Johansson C, Marmebro A. 2003. Functional correlation between habitat use and leg morphology in birds (*Aves*). *Biological Journal of the Linnean Society* **79:** 461–484.