Massive, solidified bone in the wing of a volant courting bird

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One pervasive morphological feature of tetrapods is the pipe-like, often marrow-filled, structure of the limb or long bones. This ‘hollow’ form maximizes flexural strength and stiffness with the minimum amount of bony material, and is exemplified by truly hollow (air-filled), or pneumatic, humeri in many modern birds. High-resolution microCT scans of the wings of two male club-winged manakins (*Machaeropterus deliciosus*) uncovered a notable exception to the hollow-tube rule in terrestrial vertebrates; males exhibited solidified ulnae more than three times the volume of birds of comparable body size, with significantly higher tissue mineral densities. The humeri exhibited similar (but less extreme) modifications. Each of the observed osteological modifications increases the overall mass of the bone, running counter to pervasive weight-reducing optimizations for flight in birds. The club-winged manakin is named for a pair of unique wing feathers found in adult males; these enlarged feathers attach directly to the ulna and resonate to produce a distinctive sound used in courtship displays. Given that the observed modifications probably assist in sound production, the club-winged manakin represents a case in which sexual selection by female choice has generated an ecologically ‘costly’ forelimb morphology, unique in being specialized for sound production at a presumed cost in flight efficiency.

Keywords: sexual selection; female choice; sonation; vertebrate forelimb; *Machaeropterus deliciosus*; long bone

1. INTRODUCTION

Bone structure represents a balance between flexural strength on the one hand, and tissue economy on the other. One result is tetrapod limb bones that can be characterized as hollow, tubular structures; dense compact bone bears bending or torsional stresses on the periphery of the shaft, whereas less dense internal supportive tissues lend secondary support, or are lacking entirely, with a marrow-filled cavity lending haematopoietic function [1].

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While nearly all terrestrial vertebrates maintain this hollow-tube form, the most extreme manifestation of this strength-to-weight trade-off is the pneumatized long bones of birds [2]. Cranial and cervical pneumatization appears widespread in non-avian dinosaurs [3] and probably expanded during the evolution of birds to include pneumatization of the appendicular skeleton [4], ultimately including the proximal long bone of the wing, the humerus. This trend towards increased skeletal pneumatization is commonly attributed to the need to increasingly lighten the avian skeleton for flight, while maintaining the ability of these long bones to resist bending and breaking.

Pneumatization of the humerus is common in birds and widespread taxonomically [5]. Known exceptions to the hollow-tube rule in birds are relatively rare. These exceptions include species in which flightlessness, and/or the need to reduce buoyancy to accommodate diving habits, has resulted in increased ossification of wing and leg bones (i.e. ratites, loons, penguins; [5]).

Here, we report the first fully flighted bird identified with a massive and fully mineralized wing bone. *Machaeropterus deliciosus* (Pipridae) is a sexually dimorphic, lek-breeding passerine found in the Ecuadorian/Colombian Andes. Male club-winged manakins exhibit typical flight habits, but additionally have a highly unusual wing function; wing-produced sonations (non-vocal acoustic signals) as the primary means of acoustic communication [6]. These wing-produced sounds are mechanistically unique among birds [7]. As the common name alludes to, the males’ wings have a pair of grossly enlarged secondary feathers that have strong resonant qualities enabling them to produce a harmonic, tonal *ting* sound used in courtship [8]. Here, we report that males have wing bones whose volume is substantially greater than for any other bird in its size class, and whose mineral density is exceptionally high throughout its uniquely solid interior.

2. MATERIAL AND METHODS

For the two main long bones of the wing, the humerus and ulna, we examined four features: shape, volume, solidness (% volume mineralized) and tissue mineral density (TMD) of the bone’s shaft (a common estimate of relative mineralization calculated from microCT scans). Comparisons were made between *M. deliciosus* and a set of seven other piprids: *Machaeropterus deliciosus* (UMMZ 255055); *M. regulus* (KUNHM 114486); *Pipra mentalis* (CU 44248); *P. erythrocephala* (KUNHM 89141); *P. pipra* (KUNHM 87699); *P. filicauda* (KUNHM 73336); *Lepidothrix coronata* (KUNHM 66851); *Chloropipo holochlora* (KUNHM 87655) (UMMZ, University of Michigan Museum of Zoology; KUNHM, University of Kansas Natural History Museum; CU, Cornell University Museum of Vertebrates). These species were chosen as conservative phylogenetic and functional controls; five of the species are representatives of the nearest relatives of the club-winged manakin, which exhibit variation in their wing-sound production from secondarily lost to quite complex, and two are more distantly related, non-sonating, piprid species.

One wing from each specimen was disarticulated, pinned into an extended position, allowed to dehydrate for two days, mounted between layers of styrofoam for scanning, scanned, digitally ‘dissected’ and measured.

Samples were scanned at Cornell University’s MicroCT imaging facility. A single scan was made with a GE CT120 microCT scanner (GE Healthcare, London, Ontario, Canada). The scan obtained 1200 projections at 0.3° intervals over 360° using 80 keV, 32 mA, 100 ms exposure time and 25 µm x–y–z voxels. The digitized projections were used to reconstruct a three-dimensional dataset using a convolution back-projection approach, giving a 80 × 80 × 50 mm³ volume of image data with 25 µm isotropic voxels.

Image datasets were calibrated to the conventional scale of Hounsfield radiodensity units (HU) using a water/bone phantom (SB3, a proprietary ‘synthetic bone’ sample of 1073 mg ml^{−1})

scanned with the samples. It has been shown that TMD estimates from microCT scans are 'underestimated but well-correlated with synchrotron radiation CT and gravimetric methods (such as ashing)'. Thus, comparisons between measurement methods are difficult to interpret, but are reasonable within method type [9]. An example of typical microCT TMD estimates can be found in Main *et al.* [10] where a TMD of $930 \text{ mg HA ml}^{-1}$ is reported for tibial cortical bone in mature mice. We herein avoid conclusions drawn from cross-study comparisons of 'density' and rely on the control species to indicate the difference between 'normal', or expected, values for small-flighted birds, and those of *M. deliciosus*.

Additional, higher resolution whole body scans of *M. deliciosus* (UMMZ 255254), and *L. coronata* (CU 44196) were made at Digi-morph (Digimorph, University of Texas, Austin; www.digimorph.org) and used for visualization purposes.

MICROVIEW and OSIRIX software (OSIRIX v. 3.7.1; PIXMEO, Geneva, Switzerland, MICROVIEW v. 2.2: GE Healthcare) were used to (i) isolate individual bones or subsamples thereof from the scan background, (ii) visualize overall bone shape and internal structure and (iii) calculate bone volumes and mineral densities. Each bone was characterized as follows:

(a) Shape

OSIRIX visualization functions were used to create various three-dimensional visualizations of the overall bone shape, bone surfaces and internal structure. These qualitative visualizations enabled characterization of easily observable deviations from the smooth columnar shape typical of passerine long bones (no quantitative metrics were used).

(b) Total bone volume

Segmentation—defining the region of interest (ROI) circumscribing each humerus and ulna—was performed using MICROVIEW's polygon selection tool, advanced ROI tools and manual editing. Final ROIs completely circumscribed the external perimeter of the bone and excluded adjacent non-bony tissues. MICROVIEW produces a volume calculation as the number of voxels defined by an ROI $\times$ voxel volume. The pneumatic fossa and foramen of the humerus (a depression with pores that penetrate the humeral shaft) were 'capped' manually to capture the internal volume (i.e. pneumatized lumen) as part of the overall humeral volume. An index of total bone volume was created by dividing total bone volume by the length of the bone to account for variation in overall body size among species.

(c) Solidness

The volume of the total bone volume that was above a mineral density threshold of 156 mg ml^{-1} (determined manually using MICROVIEW's density threshold tools) was considered ossified. Solidness was calculated as ossified volume/total bone volume.

(d) Tissue mineral density

A virtual thin slice (3.5 mm) of cortical bone was taken mid-shaft for each bone. The TMD was estimated using MICROVIEW's bone analysis calculation. TMD is an average value calculated from all tissues within the slice over the 156 mg ml^{-1} threshold, and therefore does not include the unossified lumen of the shaft, but only the density of the cortical bone or ossified tissues. Note that porosity within scan voxels reduce the attenuation value, and thus the inferred density, resulting in the markedly lower TMD estimates from microCT scans than density calculations derived from gravimetric methods.

Significance was represented as the number of standard deviations the club-winged manakin's values fell outside the mean values among the control species ($n = 7$) for volume index, solidness (%) and TMD. Data was deposited in the Dryad repository [11].

3. RESULTS

Machaeropterus deliciosus exhibits a highly derived ulna, and a similarly but less modified humerus. Specifically, the ulna is uniquely shaped, being relatively triangular in cross section and highly sculpted on one surface with a series of ridges and valleys where the secondary (inner wing) feathers insert (see figure 1a and the electronic supplementary material, movies S1 and S2). The bone is also large, being more than three times the average volume of the control species (figure 2a). The most striking modification is the

Figure 1. Visualized comparison of *Machaeropterus deliciosus* ulna to control species. External shape (a) and cross-section of ulnar shaft segment (b) for *Lepidothrix coronata*, *Pipra pipra*, *Pipra mentalis* and *Machaeropterus deliciosus*, respectively. Cross-section coloration indicates TMD with blue representing lower densities typical of the controls and red indicating relatively higher densities. (c) Cross-sectional slice through whole body scan of *L. coronata* (left) showing bird-typical pneumatic/hollow humeri (h) and marrow-filled ulnae (u) and *M. deliciosus* (right) showing unique solid, highly mineralized and enlarged ulna and humerus.

solid nature of this bone. While nearly half of the volume of the control species' ulnae were unossified (probably marrow-filled, consistent with the registered attenuation values and typical of passerine ulnae), essentially the entire volume of *M. deliciosus*' ulna was mineralized, or solid (figures 1b,c and 2b) and was significantly more dense than any tissue comprising the control bones (see figures 1b and 2c, and electronic supplementary material, movie S3).

4. DISCUSSION

Together, the modifications observed in *M. deliciosus*—greater volume, complete ossification and increased TMD—all contribute to a significantly more massive

Figure 2. Histograms of relative bone (a) volume, (b) solidity/ossification and (c) tissue mineral densities, for humerus (blue) and ulna (yellow) of each species (cholo, *Cholopipo holochlora*; lcoro, *Lepidothrix coronata*; pery, *Pipra erythrocephala*; ppip, *Pipra pipra*; pfli, *Pipra filicauda*; pment, *Pipra mentalis*; mreg, *Machaeropterus regulus*; mdel, *Machaeropterus deliciosus*). Single asterisk and triple asterisks indicate more than 2 and more than 4 s.d. above control species means, respectively.

bone. The massive nature of the *M. deliciosus* ulna contrasts sharply with the 'lightweight skeleton' paradigm of birds, which posits that flying birds have especially thin-walled and even pneumatic long bones to lighten their skeleton for flight [2]. Thus, it appears that nearly omnipresent skeletal adaptations for flight in birds have been abandoned by the club-winged manakin, and an entirely new morphospace (represented by the unique combination of shape, size and bone composition) has been explored in this species.

The sound-producing display in which the wing is used requires flipping the dorsal surface of the wing up over the male's back, and knocking the enlarged tips of the modified feathers together across the back over and over again in a series of approximately 36 knocks that

occur at a rate of approximately 107 Hz [7]. These knocks help stimulate resonance in the modified feathers and cause the wing feathers to vibrate [8]. In the course of this display, the ulna is subject to at least four categories of novel forces or functions: (i) mechanical (shearing, torsional and/or bending) forces transmitted through the attachment of the enlarged feather bases on the ulna, (ii) vibrational/acoustic interactions at the feather–bone interface that can affect transmission of the sound, (iii) atypical forces along the bone's shaft from the odd set of wing motions and position used and the resultant modified musculature [12] and (iv) potential resonant qualities of the bone itself that relate to the fast cyclic motion of the wing during sound production, and/or the phase relationship between the vibrating feathers.

While we cannot determine the exact or most critical functions of the modified bones based on this study alone, we can deduce two basic biomechanical consequences of the modified morphology. The ridged and sculpted shape of the bone is perhaps most unambiguously interpreted: similar to a chopstick cradled among fingers in a firm grasp that allows control of the instrument, the bases of the enlarged resonating feathers nestle among the deep ridges on the ulna, locking them firmly in place compared with the relatively loose, ulna-to-secondary 'point' attachments found in other birds and improving the birds' ability to control the exact placement and motion of the heavy distal feather tips.

Acoustically, sound energy generated by the resonating feathers should be more efficiently emanated if the bases of the feathers are securely attached to a solid, stiff mass. The observed modifications of the material and geometric properties of the ulna are consistent with this functionality: the higher mineral density, and thus modulus, is a property shared with other bones with auditory function [13], and here probably creates a greater impedance mis-match of the bone to the feather which should aid in maximizing the amount of the feather's vibrational energy that is reflected back out the shaft of the feather as sound (rather than lost through transmission through the bone). Likewise, the overall geometrical changes of increased diameter and cortical thickness (to the point of solidity), increase both mass per unit length and the stiffness of the bone.

Finally, mechanically, male club-winged manakins must both enact the sound-producing behaviours themselves, as well as transport and manipulate the enlarged acoustically adapted feathers and presumably bones. Determining the exact nature of any acoustic adaptations and teasing these apart from the resultant mechanical ones is likely to be a significant challenge, but one rewarded by the generation of insights into the trade-offs that adapt this bone for sound production in order to accommodate the signalling need of males in this lek-breeding species.

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