

RESEARCH ARTICLE

Does a suspensory lifestyle result in increased tensile strength? Organ-level material properties of sloth limb bones

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ABSTRACT

The material composition of vertebrate connective tissue is highly conserved across taxa. Existing data suggest that the compressive and tensile strength of limb bones are very similar despite marked variation in limb posture and locomotor patterns. However, the material properties of limb bone tissue from suspensory taxa have not been formally evaluated. Sloths are nearly obligatory in their use of below-branch suspensory locomotion and posture, thus placing their limb bones and associated soft tissue structures under routine tensile loading. It is possible that sloth limb bones are modified for enhanced tensile strength, perhaps at the expense of compressive strength. Forelimb and hindlimb bones of two-toed (*Choloepus hoffmanni*) and three-toed (*Bradypus variegatus*) sloths were tested in compression and bending to evaluate this hypothesis. Strength and elastic (Young's) modulus were similarly lower in sloth limb bones during both compression and bending, as compared with pronograde taxa. Ratios of peak bending strength to compressive strength additionally were elevated (sloths: 1.4–1.7; upright taxa: 0.6–1.2) for sloth limb bones. Overall, the material properties measured from the limb bones of tree sloths support our hypothesis of predicted function in a tensile limb system. Future studies should aim to directly test bones in tension to confirm indications of elevated axial tensile strength. Nevertheless, the results herein expand understanding of functional adaptation in mammalian tissue for a range of locomotor/postural behaviors that were previously unexplored.

KEY WORDS: Bending, *Bradypus*, *Choloepus*, Compression, Elasticity, Strength, Stiffness

INTRODUCTION

Long bones in limbs of vertebrates are composed of osseous connective tissue with a matrix of collagen fibers and hydroxyapatite mineral composition. The proportions of matrix material give compact bone a mechanical signature that is both tough and strong (collagen), as well as stiff and hard (hydroxyapatite) relative to other biological tissues (Currey, 1984, 2002; Erickson et al., 2002). At the cellular level, compact bone is organized as cylindrical osteons containing lacunae for strain-sensing osteocytes. The axial orientation of the osteons, comprising concentric layers of collagen fibers, effectively forms repeated vertical columns of osseous tissue that significantly add to the material strength of bone, particularly in compression. Coupling

these matrix- and cellular-level properties with both the cylindrical shape of the hollow diaphysis of long bones and the distribution of compact bone material about the neutral axis, gives bone tissue substantial weight-bearing strength as well as bending resistance from multiple orientations. Thus, the strength of long bones is dictated by the microstructure, size, shape, loading conditions and material properties of the tissue (Beaupre and Carter, 1992).

Material properties at the organ level reflect the physical performance of bone tissue and are measured independent of geometric and body size scaling influences (Keaveny and Hayes, 1993). Commonly assessed material properties such as tensile and compressive strength, bending strength, elastic (Young's) modulus, stiffness, failure/yield stress and failure/yield strain provide predictions on how bone tissue behaves when loaded during routine locomotion and support (Cochran, 1982). Because of variation in limb loading regimes, it is possible that differing locomotor patterns and support requirements shaped the biomaterial capacities of the vertebrate skeleton (Erickson et al., 2002). Nevertheless, marked similarities in skeletal attributes and material properties among avian and eutherian (placental) mammalian limb bones are well established (Burr, 1980). The correspondence in material properties across hindlimb skeletons sampled from the two crown groups was first confirmed by Sharma (1944), with slight variations among quantitative values for strength, elasticity and stiffness reflecting minor modifications for limb function based on differing terrestrial locomotor patterns observed among birds and mammals (Currey, 1987). In particular, the findings of Erickson et al. (2002) improved our understanding for how biomechanical influences have only subtly modulated the structural requirements of limb bones throughout vertebrate evolution, suggesting that bone material properties do not vary substantially across major taxa. This was true regardless of phylogeny, changes in locomotor patterns and gross morphology, thus validating that bone biomechanical performance is not solely linked to adaptations within a specific niche. However, arboreal and semi-arboreal taxa were not considered in that extensive functional–evolutionary study.

Interpretation of limb bone loading and material properties in arboreal (or semi-arboreal) taxa has come primarily from research on primates (e.g. Hunt et al., 1996; Demes et al., 2001; Wright et al., 2008). All primates have arboreal origins (Cartmill, 1972; Chester et al., 2015; Boyer et al., 2017) and several extant species employ forelimb suspensory behaviors for transient arboreal maneuvering and resource acquisition (Stern and Oxnard, 1973; Larson, 1998; Wright et al., 2008). These primates may also have skeletal modifications for joint mobility that are associated with below-branch (anti-pronograde) postures (Rein et al., 2015). Nonetheless, there exist only a few quantitative studies of bone loading documenting *in vivo* strains in arboreal primates that employ forelimb suspension (or arm-swinging brachiation) and no data are currently available on material properties or performance of limb

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bones in mammals that are specialized for suspensory habits. Thus, initial understanding of suspensory function is dependent on recorded *in vivo* data from gibbons (*Hylobates*), for example, which show peak magnitudes of tensile strain when brachiating (Swartz et al., 1989) that are in contrast with large compressive strains in the distal limb bones of upright mammals during running. Demes et al. (2001) documented bone strain during climbing behaviors in rhesus macaques (*Macaca mulatta*) by recording higher peak strain magnitudes in the tibia when individuals performed vertical climbing compared with over-ground locomotion. The same study (Demes et al., 2001) also reported that the direction of bone bending largely remained constant regardless of locomotor behavior, although greater deviations in strain patterns were observed during vertical climbing. Thus, load predictability is less consistent during climbing and arboreal maneuvering, and bone bending loads can be multi-oriented in primate limbs.

Tree sloths (Xenarthra: Pilosa) demonstrate near-obligatory suspensory habits as part of their arboreal lifestyle, suggesting that their limb bones will predominately experience tensile loading. Relative to those of pronograde (above-branch) mammals, the limb bones of sloths and suspensorial primates (e.g. atelid monkeys, apes, some lorises and extinct paleoprothecid lemurs) would in turn be expected to have relatively greater tensile strength to avoid failure and ensure adequate safety factors (ratio of failure to functional stress/strain) for suspensory habits. Nonetheless, the limited available data for tensile limb systems make such predictions for modifications to bone material properties for enhanced tensile strength and elasticity tenuous. Novel evidence obtained from sloth limb bones would provide a key example of a system loaded opposite to that of upright mammals. Moreover, such an extreme representation of limb loading diversity (i.e. suspensory locomotion) could greatly improve understanding about how routine quadrupedal suspension influences potentially different osseous tissue properties from those observed in other mammals (e.g. Currey, 1962, 1970, 1984, 1987, 1990, 2002) and, in general, across vertebrate taxa (Erickson et al., 2002). Tree sloths therefore make an ideal comparator organism to assess material properties of limb bones to further interpret the mechanics of suspensory locomotion and posture.

Because of their broad geographic distribution, population size and ease of accessibility in Costa Rica, the two species *Bradypus variegatus* (Bradypodidae: three-toed sloths) and *Choloepus hoffmanni* (Choloepodidae: two-toed sloths) were targeted for this biomechanical study of their long limb bones, with the aim to reveal possible organ-level bone specializations for suspensory support. Compared with upright (or pronograde) mammals, we predict that sloth limb bones will have low compressive strength as a result of their suspensory locomotion and posture. Specifically, this hypothesis was tested using rats and semi-arboreal primates as exemplars of upright baseline and facultative suspensory outgroups, respectively. Not only does this study provide the first

dataset of bone material properties for suspensorial animals but also these data may affect further evaluation of bone biomaterial properties in mammals, specifically those with specialized arboreal habits.

MATERIALS AND METHODS

Sampling

Testing was performed on limb bones from *Bradypus variegatus* Schinz 1825 ($N=4$) and *Choloepus hoffmanni* Peters 1858 ($N=4$), as well as those from two primate species, vervet monkeys [*Chlorocebus aethiops* (Linnaeus 1758), $N=3$] and a howler monkey [*Alouatta caraya* (Humboldt 1812), $N=1$], and common laboratory rats [*Rattus norvegicus* (Berkenhout 1769), $N=6$]. Bone specimen samples were obtained opportunistically from adult individuals that died of natural causes in zoological environments (sloths and primates) and were used in previous studies (sloths: Spainhower et al., 2018, 2021), or were killed for reasons unrelated to this study (rats). Whole cadavers were stored frozen (-20°C) until tissue collection. Long bones from the forelimbs (radius, ulna and humerus) and hindlimbs (femur and tibia) were excised from either or both the left and right sides during gross dissections, swabbed clean of soft tissue using cotton-tipped applicators wetted with phosphate buffered saline (PBS), wrapped in gauze soaked with PBS, and placed in plastic specimen bags before being stored frozen (-20°C) until testing.

For evaluating material properties in compression, a sub-sample ($n=13$) of thawed whole bones from all those excised (Table 1) were cut using a band saw to nearly a 1:1 width-to-length ratio to minimize the bending moment during compressive testing. The band saw blade was wetted with a 50/50 solution of cooking oil and water to reduce frictional heating of bone tissue during sectioning. Bone sections retained their original cross-sectional geometry but were lightly sanded with fine (600–800) grit sandpaper to smooth edges and level ends for axial testing. Prior to testing, photographs of all bone sections were taken with a digital camera (P100: Nikon, Japan) mounted to a copy stand and were used for measurement of bone cross-sectional area (CSA) in ImageJ. Length, width and depth measurements were also taken from each bone section using digital calipers (accurate to 0.01 mm: Mitutoyo), and were simply used for input of test specimen dimensions into the Instron software during material testing.

To quantify material properties in bending, whole humeri and femora from a second sub-sample ($n=24$) of all bones excised (Table 1) were thawed and the mid-shaft region of each bone was imaged using a microcomputed tomography scanner (vivaCT 75, microCT scanner, Scanco USA, Inc., Southeastern, PA, USA) to visualize bone cross-sectional geometry for size normalization (Fig. 1). Briefly, long limb bones were CT scanned at 70 kVp at a voxel size of either 20.5 or 39 μm , depending on specimen size. Stacks of reconstructed microCT slices (in Scanco software) were then imported into BoneJ software (Doube et al., 2010) to estimate the second moment of area (I) at the loading site and distance to the

Table 1. Species and number of bones used for testing in both compression and bending

Species	Common name	Humerus	Radius	Ulna	Femur	Tibia	Total
<i>Bradypus variegatus</i>	Three-toed sloth	3*	3	3	6*	4	19
<i>Choloepus hoffmanni</i>	Two-toed sloth	6*	2	2	6*	2	18
<i>Chlorocebus aethiops</i>	Vervet monkey	3*	2	3	1**	3	12
<i>Alouatta caraya</i>	Howler monkey	–	–	–	1**	1	2
<i>Rattus norvegicus</i>	Laboratory rat	6**	–	–	6**	–	12

The number of forelimb and hindlimb bones of each species (n) is listed; those used in compression only are unmarked; asterisks indicate those used for bending only (*) or for both bending and compression (**).

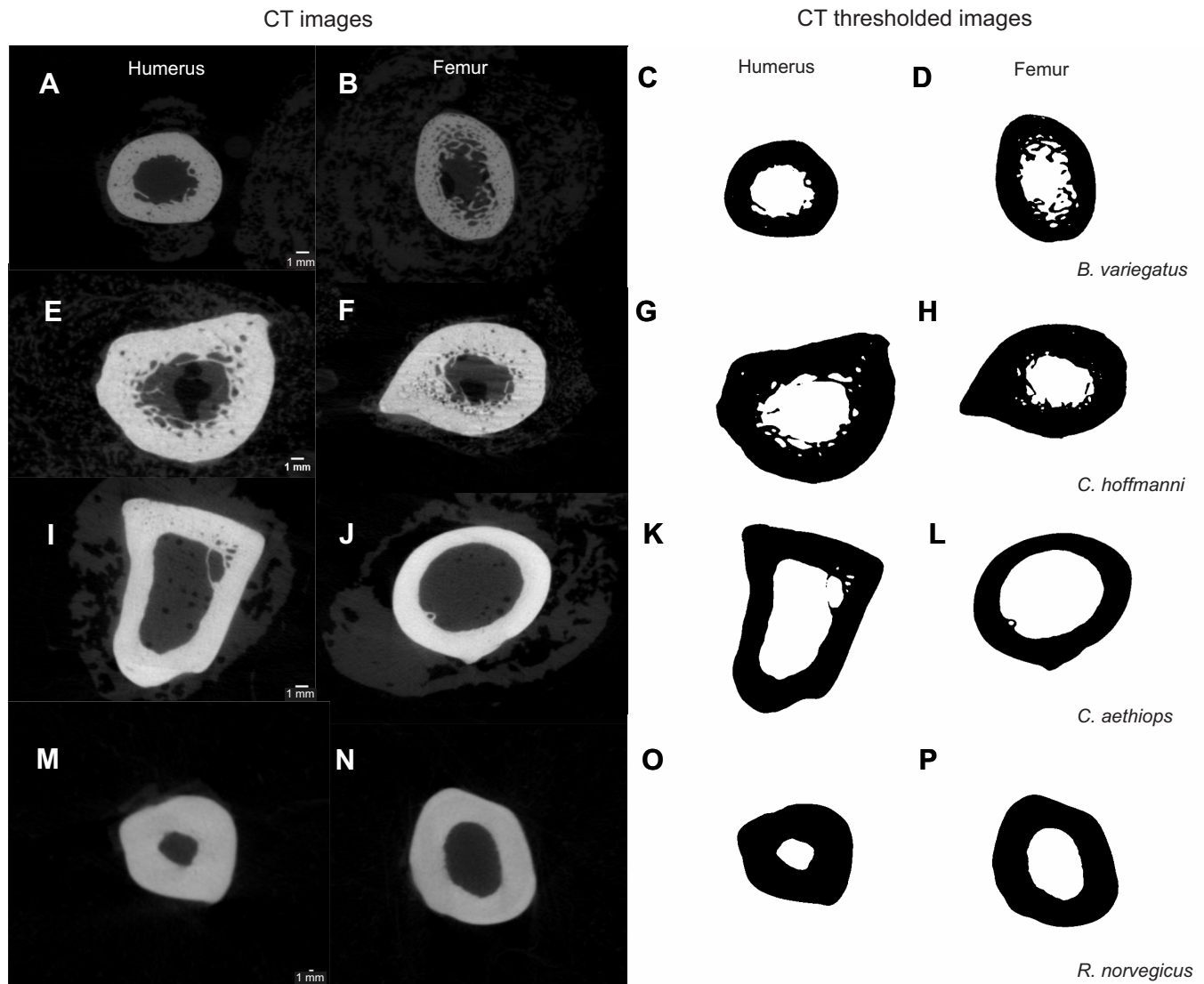


Fig. 1. Exemplar computed tomography (CT)-scans of bone cross-sectional area (CSA) for bone bending analysis. Shown are CT images (left) and thresholded images (right) of the mid-shaft of the humerus and femur, respectively, for *Bradypus variegatus* (A–D), *Choloepus hoffmanni* (E–H), primates (*Chlorocebus aethiops*: I–L) and rats (*Rattus norvegicus*; M–P). The thresholded images are included to show the cortical bone tissue resolved for calculations of CSA.

neutral axis of bending (c) (Turner and Burr, 1993). I and c were taken as the mean of maximum and minimum values (i.e. I_{\max} and I_{\min} and c_{\max} and c_{\min}) across a subsample of slices centered on the bone mid-shaft, with the total length of the sub-sampled region set to 10% of overall bone length (total number of slices, $n=170\text{--}370$; see Young et al., 2020, for further details). Cross-sectional images were thresholded prior to analysis to include only those pixels bright (i.e. dense) enough to qualify as bone tissue. Threshold values were specifically selected using the automated algorithm in BoneJ. There is potential for introducing confounding error using this procedure, in particular if threshold values are too low and include pixels of reduced ossification (e.g. with increased porosity as observed in sloth limb bones: Montanez-Rivera et al., 2018). However, sensitivity analyses were performed to establish that increasing threshold values up to 150% of the automated selection had minimal effect on the calculated material properties from the sloth bones sampled (Fig. S1). Output of I and c values from the CT-scan files was saved as .txt documents for analysis.

Testing

Compressive testing was performed with a uniaxial Instron Material Testing System (Tensiometer Model 5697, Instron, Norwood, MA, USA) equipped with a 10 kN load cell attached to the movable crosshead of the testing frame. Bone sections were placed between two flat plates on the testing rig (the top plate was capable of flexing with loading of the bone section to remain flush throughout the test) (Fig. 2A). Bone sections were loaded until failure at a strain rate of 2 mm min^{-1} (Currey, 1990; Erickson et al., 2002). Records of raw load and displacement were continuously recorded during testing along with output from the load cell to Instron data acquisition software (v. 2.25). All recorded data were exported as .csv files to MS Excel (Microsoft Corp., Redmond, WA, USA) for calculation of compressive strength, elastic (Young's) modulus, bone stiffness and extensibility (Fig. 3A). Final values of stress (in MPa) and strain (%) were calculated from records of load and displacement using digitally calculated values of bone CSA and initial (resting) length (in mm) of the bone section, respectively, and plotted as a normalized stress–strain curve. Strain was consistently determined

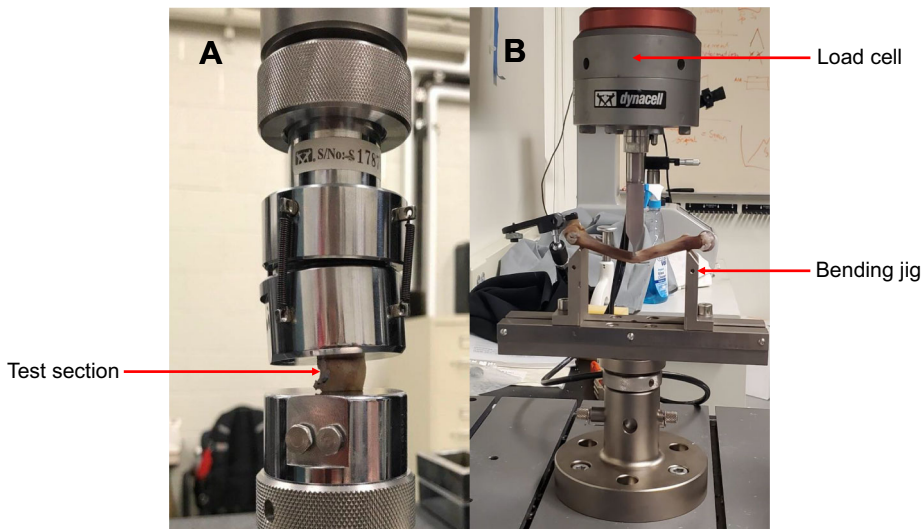


Fig. 2. Instron set-up for material property testing on sloth limb bones. (A) Cut section of a sloth femur being loaded in compression. (B) Sloth femur in the 3-point bending jig during a bending test.

as a change in length, but records of displacement were treated as absolute values rather than deriving negative (i.e. a decrease in length) strain measurements from the bone sections.

Bending tests were performed with a uniaxial Instron Material Testing System (E3000) mounted with a 250 N load cell. Briefly, whole bones were placed in a 3-point bending jig consisting of two rest arms attached to the testing frame base that could be adjusted in their distance apart. The bones were positioned to have the metaphyses at the point of contact on the rest arms of the jig with the natural curvature of the bone facing downwards (Fig. 2B). The crosshead anvil attached to the load cell was then positioned to contact the bone diaphysis at mid-shaft within the range sampled in the microCT scans. The distance between the anvil and rest arms of the jig was measured with digital calipers (Mitutoyo) prior to testing and taken as the bending moment arm (in mm). Humeri and femora were loaded in bending at a strain rate of 2 mm min^{-1} using a displacement control protocol consistent with previous studies (e.g. Currey, 1990; Erickson et al., 2002; Young et al., 2014).

None of the humeri/femora from sloths fractured during bone bending. Instead, those bones underwent substantial plastic deformation and returned close to their original shape after they were unloaded. Testing on these bones was stopped once the amount of load being resisted reached a consistent plateau (i.e. post-yield region), and showed no remaining fluctuations. The remainder of the limb bones sampled from primates and rats fractured as expected. Following the test, all bones (or fractured bone halves) were recovered, rewrapped in gauze soaked in PBS, and returned to storage at -20°C . Records of load (in N) and displacement (in mm) were sampled at 10 Hz and saved after the test to be output as .csv files. Raw data on applied load and displacement during bending tests were imported into MATLAB (Mathworks, Natick, MA, USA) for analysis using a custom-written program (available from figshare: <https://doi.org/10.6084/m9.figshare.16663960.v1>). Deformation was transformed into strain (ϵ) and load into stress (σ) using standard formulas for three-point bending of hollow cylinders (Turner and Burr, 1993):

$$\epsilon = \frac{12cd}{L^2}, \quad (1)$$

$$\sigma = \frac{FLc}{4I}, \quad (2)$$

where c represents the maximum distance from the periosteal surface of the bone to the neutral axis of bending (in m), d is displacement (in m), L is moment arm of the bending load (in m; equal to the distance between the supports of the flexure fixture), F is the bending load (in N) and I is the second moment of area relative the neutral axis of bending (in m^4). Calculated bending stress and strain data were then used to derive estimates of ultimate bending strength and elastic (Young's) modulus; the latter was taken as the slope of the linear region of the stress-strain curve (Fig. 3B). Last, species data were averaged across forelimbs and hindlimbs for strength in both bending and compression and calculated as a bending to compressive strength ratio.

Statistics

Mixed-effects two-way analyses of variance (ANOVA) were used to examine variation in bone material properties, including main effects for broad taxonomic group (e.g. sloth versus primate versus rat) and limb pair (e.g. humerus versus femur), as well as the interaction between the two factors. Individual was specified as the random factor in all mixed-effects models, permitting a repeated-measures type design for comparisons of forelimb and hindlimb bones within a single animal. In the presence of significant interactions, single degree of freedom tests (Quinn and Keough, 2002) were used to test for significant variation between species within limb pairs, or between limb pairs within species. *Post hoc* comparisons were performed using Tukey's honestly significant difference test (Sokal and Rohlf, 2011) for significant main effects without interactions, using the false discovery rate method (Benjamini and Hochberg, 1995) to properly control experiment-wise error rates during multiple comparisons. Degrees of freedom were adjusted using the Welch-Satterthwaite approximation to mitigate heteroscedasticity. In Results, we focus on the broad statistical outcomes; however, detailed information on each test, including effect sizes for all pairwise comparisons, are presented in Tables S1–S4. All analyses were conducted in R (<http://www.R-project.org/>), supplemented by the add-on libraries dplyr (<https://CRAN.R-project.org/package=dplyr>), ggplot2 (Wickham, 2016), lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017) and emmeans (<https://CRAN.R-project.org/package=emmeans>). All custom-written R scripts used for analysis, along with requisite data files, are freely available from figshare: <https://doi.org/10.6084/m9.figshare.16663960.v1>.

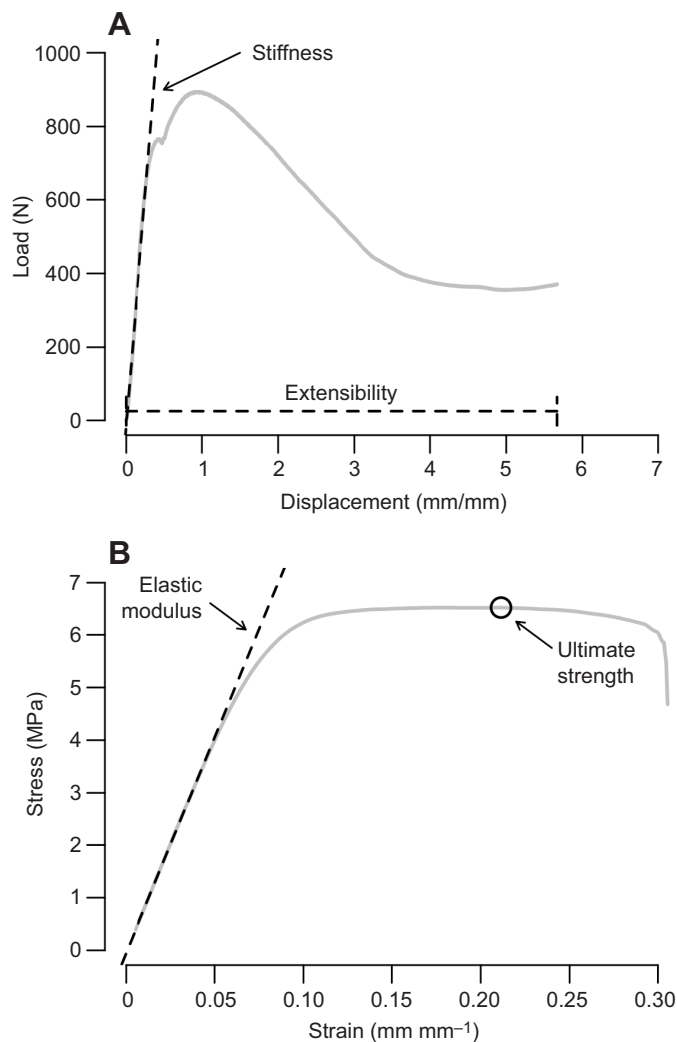


Fig. 3. Exemplar material property loading curves and measurement from sloth limb bones. (A) Stiffness and extensibility were quantified from raw load–displacement curves (demonstrated using compressive load data from a *C. hoffmanni* ulna). (B) Elastic (Young’s) modulus and ultimate strength were quantified by size-adjusted stress–strain curves following formulae discussed in Materials and Methods (demonstrated using bending load data from a *B. variegatus* humerus).

RESULTS

Compression

Variation in limb bone material properties in compression between *B. variegatus* and *C. hoffmanni* is shown in Fig. 4A–D and outcomes of all statistical tests are summarized in Table S1. Overall, compressive strength and elasticity were low for sloth limb bones (Table 2), whereas values of stiffness and extensibility were moderate. The forelimb bones were stronger ($P=0.004$) than the hindlimb bones in both species; however, no significant differences in the other material properties were observed between limb pairs, nor were there any differences found between the two species of sloths for any compressive material property.

Given the lack of significant differences for mean compressive material properties between the two species (Table 2), data for sloths were pooled for statistical comparisons with other taxa (Fig. 5A–D; Table S2). Sloths had stronger forelimb bones in compression than primates, whereas primates had significantly larger compressive strength ($P\leq 0.029$) in their hindlimb bones. Similarly, hindlimb

bone elastic (Young’s) modulus and stiffness were significantly greater in primates than in sloths ($P<0.001$), though forelimb bone elasticity and stiffness did not vary between the taxonomic groups. Sloth limb bones were also characterized by greater values of extensibility ($P=0.001$), regardless of forelimb or hindlimb pair.

Within each taxon, primates had significantly stronger and stiffer hindlimb bones than forelimb bones (both raw stiffness and elastic modulus; $P\leq 0.01$), whereas sloths had the opposite pattern of stronger forelimb bones than hindlimb bones ($P=0.032$). Stiffness and elastic (Young’s) modulus did not vary between the limb pairs in sloths. Last, extensibility in compression was similar across taxa and forelimb and hindlimb pairs in the test sample.

Bending

Variation in limb bone material properties in bending between *B. variegatus* and *C. hoffmanni* is shown in Fig. 4E,F and outcomes of all statistical tests are summarized in Tables S3. The forelimbs (humeri) were significantly stronger ($P\leq 0.003$) and characterized by a larger elastic modulus than the hindlimbs (femora) in both species. No significant interspecific differences between means were observed (Table 2). Given the lack of significant interspecific differences, data for sloths were again pooled for comparisons of bending properties across sloths, primates and rats (Fig. 5E,F; Table S4).

Among the taxa sampled, rats were characterized by the strongest humeri in bending, and primates by the weakest, with sloths having intermediate values that differed significantly from those of rats and primates ($P\leq 0.003$). In contrast, sloths had the weakest femora in bending ($P\leq 0.024$), with bending strength in rat and primate femora not differing from each other. Rat humeri additionally showed the largest values for elastic (Young’s) modulus in bending ($P\leq 0.015$), whereas those for primates and sloths were statistically similar. Conversely, the femora of primates were characterized by the greatest elastic modulus (all $P<0.001$), with that of rat and sloth femora not differing from each other. Finally, within each taxon, ultimate strength and elastic (Young’s) modulus in the humeri were greater than these properties in the femora of sloths and rats (all $P<0.01$), whereas both values for the femora were notably larger than those for the humeri in primates ($P\leq 0.002$).

DISCUSSION

This study is the first to determine material properties from limb bones within a tensile limb system of an obligate suspensorial mammal. Despite absolute differences in adult body mass (Grand, 1978), distal limb form (Mendel, 1981a,b; Marshall et al., 2021) and frequency of use of suspensory locomotion and posture (Sunquist and Montgomery, 1973; Urbani and Bosque, 2007), the collective material properties quantified are statistically similar for two- and three-toed sloths. Both the compressive and bone bending datasets show this pattern, pointing to the remarkable degree of evolutionary convergence for suspensory habits observed between *Bradypus* and *Choloepus* (Gaudin, 2004; Nyakatura, 2012). However, despite the limb pairs playing an equal role for body weight support (Granatosky et al., 2018b), some inter-limb variation in sloths was seen in both compression and bending, which was not predicted *a priori*.

Potential differences in bone strength and elasticity between limb pairs could be explained by previous locomotor data showing that sloths apply propulsive force with their forelimbs following touchdown (grasp-on) on the substrate, then apply braking force with their hindlimbs late in the contact phase prior to liftoff (grasp-off) (Granatosky and Schmitt, 2017). Thus, their forelimbs are creating the propulsive forces required to advance the center of mass

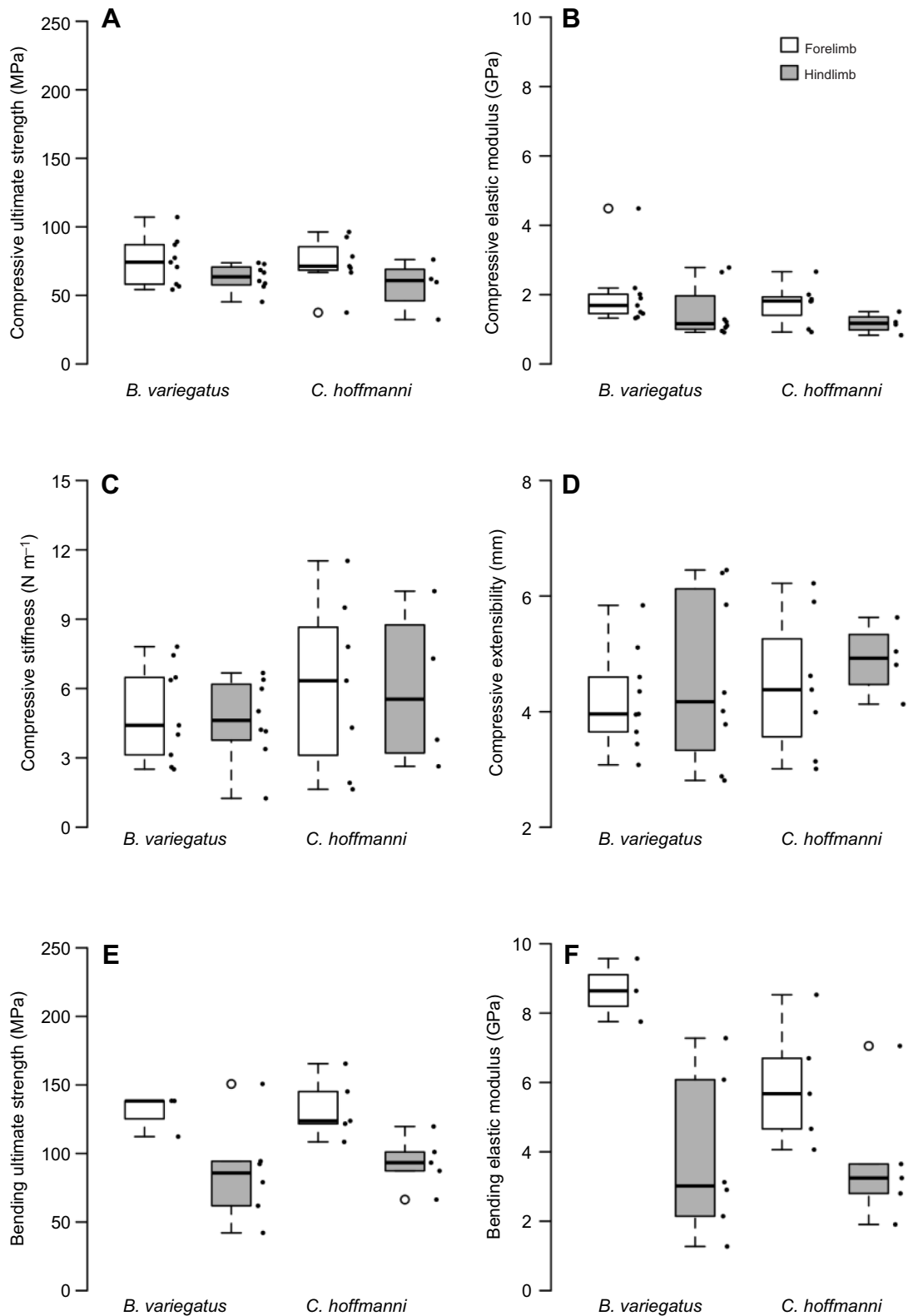


Fig. 4. Hybrid box and dot plots for material properties of limb bones from *B. variegatus* and *C. hoffmanni*. Data trends for both genera and limb pair (forelimb versus hindlimb) were observed across compressive (A, strength; B, elastic modulus; C, stiffness; D, extensibility) and bending (E, strength; F, elastic modulus) testing. Box plots of mean data show median, upper and lower quartiles, 1.5 \times interquartile range (whiskers) and outliers (open circles). Dot plot representations of all individual data points analyzed per genera and limb bone grouping are presented to the right of the corresponding box plot.

forward along the substrate, whereas the hindlimbs are primarily modulating forward velocity. Sloths may also use co-contraction of forelimb and hindlimb muscles to distribute their body weight evenly

between limb pairs (Granatosky et al., 2018b; Gorvet et al., 2020; Spainhower et al., 2021), preventing unwanted accelerations of the center of mass that might be caused by the application of a large

Table 2. Compressive and bending strength and elastic (Young's) modulus from the limb bones of all species tested

Species	Compressive strength (MPa)	Bending strength (MPa)	Compressive modulus (GPa)	Bending modulus (GPa)
<i>Bradypus variegatus</i>	74.96±17.51 ^F	129.67±15.06 ^F	2.00±9.84 ^F	8.66±9.09 ^F
	62.85±9.54 ^H	86.76±37.03 ^H	1.49±0.76 ^H	3.80±2.35 ^H
<i>Choloepus hoffmanni</i>	73.26±19.44 ^F	134.97±23.56 ^F	1.72±6.00 ^F	6.05±1.76 ^F
	57.52±18.30 ^H	92.53±17.62 ^H	1.17±0.28 ^H	3.58±1.82 ^H
<i>Chlorocebus aethiops</i>	48.21±14.42 ^F	59.05±69.61 ^F	1.90±0.57 ^F	4.30±2.25 ^F
	82.92±20.07 ^H	104.38 ^H	3.08±0.64 ^H	13.42 ^H
<i>Alouatta caraya</i>	78.8 ^H	170.29 ^H	3.34 ^H	27.2 ^H
<i>Rattus norvegicus</i>	–	196.67±33.08 ^F	–	11.88±4.62 ^F
	–	151.27±45.79 ^H	–	4.00±1.09 ^H

Forelimbs (F) and hindlimbs (H) have been separately denoted with superscripts. Values are means±s.d.

flexor moment at the shoulder joint early in the contact phase of suspensory walking (Nyakatura and Andrada, 2013). Sizable joint torque induced by flexor muscle contractions would cause the limb bones of sloths to experience bending in addition to resisting appreciable tensile loading during suspensory walking. Kinematic observations in two-toed sloths (*Choloepus didactylus*; Granatosky et al., 2018b) validate that suspensory locomotion involves predominantly the movement of the proximal limb segments while the distal limb segments show relatively little displacement, which limits step length and forward velocity (Nyakatura et al., 2010).

Sloths must also be able to climb, which likely causes their long limb bones to experience appreciable compressive/bending loads from gravitational torques. While the relative magnitudes of *in vivo* strains experienced by the humerus and femur are unknown during vertical climbing, muscle activation patterns in *B. variegatus* (Gorvet et al., 2020) indicate that sloths share (to some degree) functional roles in weight-bearing support and propulsion (up a vertical substrate) between limb pairs, as do several species of primates (Reynolds, 1985; Cartmill et al., 2002; Schmitt and Lemelin, 2002; Hanna et al., 2017; Granatosky et al., 2019). Specifically, in lorises (e.g. *Nycticebus*), for which sloths are ecologically and physiologically convergent, the forelimbs shift to a predominant role in propulsion by pulling up during climbing behavior (Hanna et al., 2017). It is possible that sloths have similar functional requirements for propulsion by their forelimbs in both suspensory walking and vertical climbing, and it is further probable that material properties (as observed herein) must be closely related for the humerus. Alternatively, the femora of sloths may have a greater role in weight bearing during suspension (Goffart, 1971), demanding greater mechanical resistance in tension, in addition to supplemental body weight support and propulsion for vertical climbing. Future studies are needed to measure substrate reaction forces and evaluate muscle activation patterns in the hindlimbs to verify these functional hypotheses.

Several species of primates display a range of arboreal maneuverability with their forelimbs, including suspensory postures that are similar to those of sloths. Interestingly, variation in material properties was observed between the forelimb bones of sloths and those of the primates tested here, whereas strength and elasticity were substantially greater in primate hindlimb bones. In fact, bending strength was lower in the humeri of the monkeys sampled compared with that from either *B. variegatus* or *C. hoffmanni*. Despite specialization for suspensory behaviors throughout the order Primates, most taxa are hindlimb biased and bear a larger percentage of body weight on their hindlimbs than on the forelimbs during pronograde quadrupedalism (Demes et al., 1994; Schmitt and Hanna, 2004; Young, 2012; Granatosky et al.,

2016a,b). This form of differential limb support is proposed to free weight-bearing constraints on the forelimbs for use in climbing, complex three-dimensional arboreal maneuvering and manual foraging. Nevertheless, primates routinely shift body weight to their forelimbs during quadrupedal suspension (Granatosky et al., 2018a) and these observations have led to predictions that their forelimb bones may have reduced compressive strength for terrestrial support, but they are enhanced in tensile strength/elasticity for suspensory support. Our findings that both ultimate compressive and bending strength are even less than those in sloth forelimb bones are the first to provide direct verification for these predictions.

In addition, potential limitations on compressive load resistance in primate forelimbs may be functionally analogous to loading conditions for the limb bones in sloths as well as colugos (Dermoptera; both sloths and colugos are obligate suspenders). Colugos have elongated, extremely gracile limb bones that cannot support their body weight for terrestrial locomotion without critical risk of failure (Vaughan et al., 2013). Sloths too have long, more gracile limb bones and a relative lack of extensor muscle mass (Goffart, 1971; Olson et al., 2018; Windle and Parsons, 1899; Butcher et al., 2022), and would appear to be incapable of supporting their body weight with an upright limb posture when moving on the ground. Instead, sloths opt for a 'crawling' mode of terrestrial locomotion where the hindlimbs assume a semi-erect limb posture, but the forelimbs support body weight on the antebachial segments, distributing load along the length of the ulna (Mendel, 1985; M.T.B., personal observations). It is not possible, however, to explain this behavior based on larger values of compressive/bending strength and elasticity in sloth forelimb bones compared with those properties for their hindlimb bones. Sloths could have adapted this terrestrial locomotor mode as a result of overall low safety factors in compression and bending, although it may just as likely be a behavioral preference to protect their foreclaws and avoid an increased metabolic cost of bearing load on extended limbs.

Tensile versus compressive limb system function

Our results provide novel evidence for improving understanding of bone properties expected for a tensile limb system in arboreal mammals (Swartz et al., 1989) and how the material properties differ from those of pronograde mammals. Moreover, differences were observed among the arboreal species in our sample. For example, the limb bones of sloths are more compliant (greater extensibility) and possibly tougher than those of primates, and these bone properties could be related to differing levels of arboreality versus terrestriality and other patterns of substrate use. It is therefore likely that greater diversity exists in bone strength and elasticity than previously accounted for in vertebrates (Erickson et al., 2002), thus

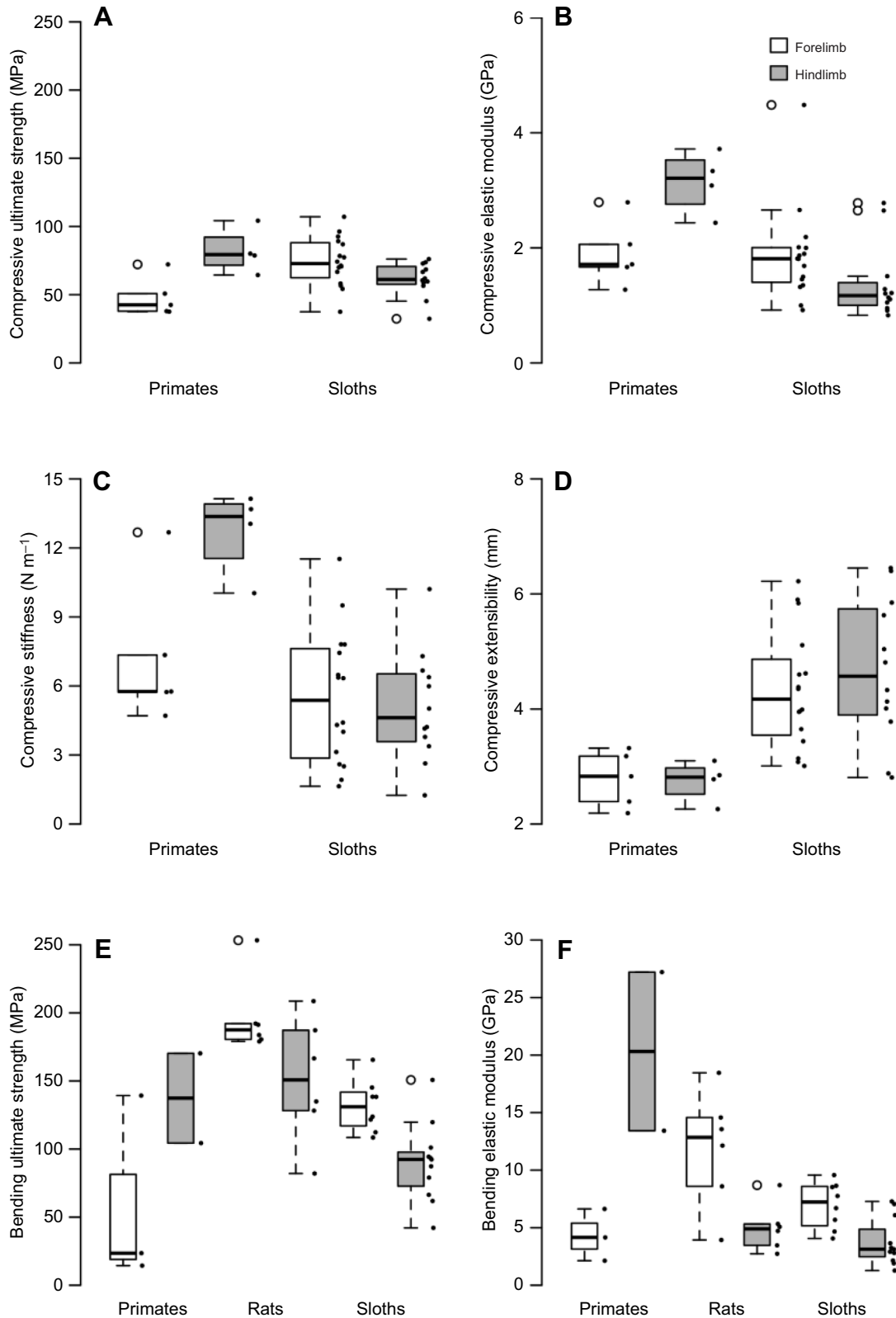


Fig. 5. Hybrid box and dot plots for material properties of limb bones from all sampled taxa. Data trends for taxonomic group and limb pair (forelimb versus hindlimb) were observed across compressive (A, strength; B, elastic modulus; C, stiffness; D, extensibility) and bending (E, strength; F, elastic modulus) testing. Material properties in compression were quantified for tree sloths (*B. variegatus* and *C. hoffmanni*) and primates (vervet and howler monkeys); material properties in bending were quantified for sloths, primates and lab rats. Box plots and dot plots as in Fig. 4.

challenging the existing paradigms by suggesting that arboreal mammals that routinely load their limbs in tension may also be characterized by unusual limb bone material properties.

Reduced stiffness and potentially greater toughness and tensile strength directly suggest modifications to the microstructure of bone tissue. The matrix microstructural array of collagen fibers and mineral composition of hydroxyapatite at the tissue level have ensured strength/stiffness in vertebrate bones to resist large compressive forces. Within a cross-section of compact bone, concentric layers of collagen fibers (within Haversian systems) are arranged at different angles in adjacent columns of bone tissue for increased compressive/bending strength (Rho et al., 1999). However, because sloth bones were more compliant and showed lower bending strength than that of the other bones tested, it is possible that sloth limb bones have collagen fibers that are aligned in a more parallel array for increased tensile strength and toughness beyond what is typical for upright mammals.

Decreased bone mass in tree sloths also could be correlated with lower observed stiffness in limb bones. Representative cross-sections of the humeri and femora of the species used here for bone bending analysis are shown in Fig. 1. Density was also calculated in ImageJ by calibrated gray values in CSA images (data not shown). Despite comparable estimates of bone density, the proximal limb bones of both species of sloth are more porous compared with the femur of primates and both the humerus and femur of rats. These observations agree with recent findings of high cortical porosity in the limb bones of arboreal tree sloths (Montanez-Rivera et al., 2018; Alfieri et al., 2020). Future analyses are needed to confirm our values of apparent bone density with the possibility of revealing additional modifications to bone microstructure (e.g. bone tissue level: collagen and mineral content) in sloths, although we hypothesize that it is unlikely that the observed levels of porosity are affecting the reported bone material properties alone. For example, a low rate of bone remodeling that results in elevated cortical compactness while minimizing bone tissue mass could be a consequence of the critical need for sloths to conserve metabolic energy.

One of the most striking observations from our study is that strength and elasticity in both compression and bending were low for the long limb bones of sloths (and moderate for primates) compared with those often reported for pronograde mammals (Figs 6 and 7). The comparative analysis presented here further suggests that the material properties of sloth limb bones have been modified for suspensory function. A continuum of strength/elasticity between arboreal and upright, terrestrial taxa demonstrates functional trade-offs in load resistance appropriate for variable substrate preference and use. This is perhaps best evidenced by the overlap in taxa grouped by locomotor habit with respect to bone bending loads as seen in Fig. 7. Common values for strength and elastic (Young's) modulus in compression and bending are 170–200 MPa and 18–20 GPa, respectively (Vogel, 2013; Currey, 1987, 1990; Biewener, 1989a,b, 1990). These data ranges are substantially greater than those determined for sloth limb bones and the primate humerus. We acknowledge that the data reported for bending strength and elasticity in our study may be underestimated as a consequence of the analytical methods used for calculating these values (which depend on modeling the loaded bones as hollow beams; Turner and Burr, 1993). Nonetheless, the repeatability of the measurements reported in our study, regardless of loading regime, provides confidence that the results of low strength and elasticity for sloths are precise even if the values are not accurate.

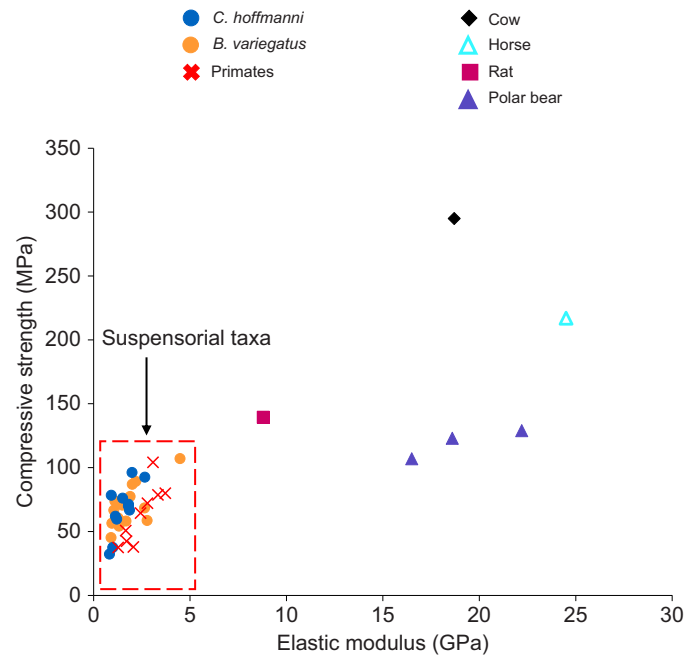


Fig. 6. Relationship between compressive strength and elastic (Young's) modulus across mammals. Experimental data were assembled from available sources in the literature: cow femur and horse femur from Currey (1987); rat femur from Cory et al. (2010) polar bear femur from Currey and Brear (1990). Sloth and primate data are from this study. Data outlined by the dashed red box were placed in the proposed 'suspensorial' functional grouping by emphasizing low compressive strength and/or elasticity of bone tissue from these taxa.

Mammalian limbs bones are typically 75% stronger in compression than in tension (Biewener and Bertram, 1993). This support function arises from the highly conserved microstructure of bone and pervasive remodeling mechanisms, which are effective at reducing large tensile loads (Currey, 1970; Biewener and Taylor, 1986; Biewener and Bertram, 1993; Hall, 2013). As bending results from both compression and tension (depending on the position of the neutral axis), and bending loads are superimposed on axial compression during pronograde support, any reduction in compressive strength without a concomitant decrease in bending strength would suggest tangential support for increased tensile strength, such that tensile strength would compensate for the loss of compressive strength to maintain bending performance. Compared with upright, cursorial mammals, tree sloths have larger ratios of bending to compressive strength (Fig. 8). Unlike most other mammals, sloths spend a large proportion of their active time in suspensory postures (Montgomery and Sunquist, 1978; Adam, 1999; Urbani and Bosque, 2007) where their limb bones likely undergo potentially high tensile loading for support. This may have been the main selective pressure influencing a potential shift to lower compressive strength and enhanced tensile strength. This relationship provides additional support for the hypothesis that tree sloths may have evolved modified organ-level limb bone material properties and compact bone matrix composition, permitting increased tensile strength to ensure adequate load resistance during suspension.

Study limitations

The selection of rats as the upright mammal baseline out-group may not have been the best fit based on their body size and bone microstructural properties, which are markedly different from those of other, larger mammals (Shipov et al., 2013). Perhaps the limb

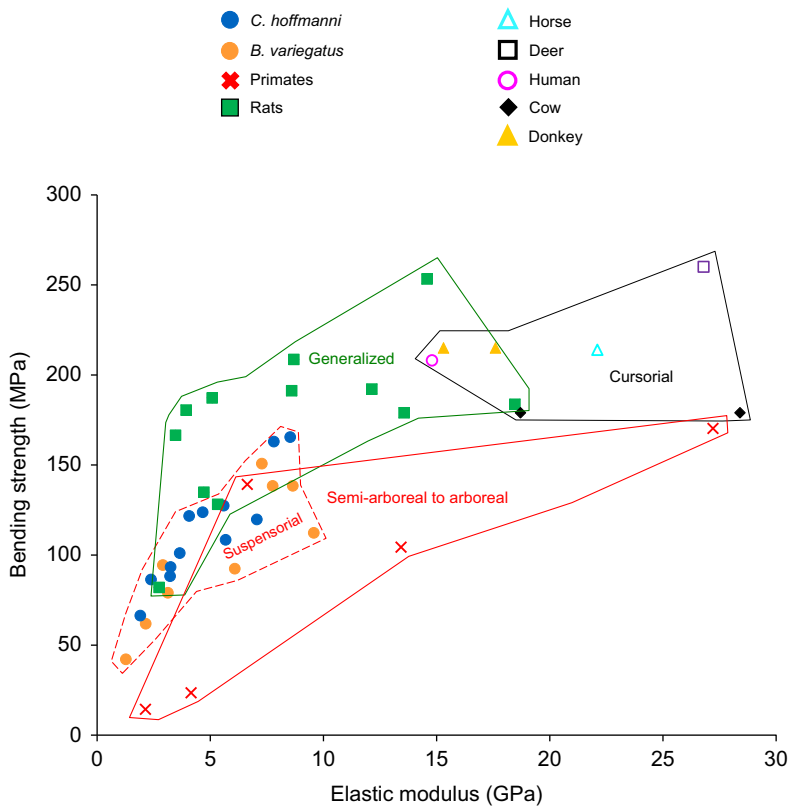


Fig. 7. Relationship between bending strength and elastic (Young's) modulus across mammals. Experimental data were assembled from numerous sources in the literature using similar methods: cow femur, donkey radius, deer tibia and horse femur from Currey (1987); human femur from Erickson et al. (2002). Sloth, primate and rat data are from this study. Colored groupings show functional categorizations of bone properties based on locomotor habits (i.e. substrate use). Data outlined by the red dashed line were placed in the proposed 'suspensorial' functional grouping by emphasizing low-to-moderate bending strength and/or elasticity of bone tissue from these taxa.

bones from upright cats or dogs would have been more appropriate to sample for this study; however, rats were chosen based on lab access/availability and their widespread use in previous studies of mammalian bone biology. Bones that were tested in compression and bending also had relatively small sample sizes for complete forelimb and hindlimb pairs in both sloths and primates because of specimen availability. Having a larger sample size would improve statistical power for resolving variation among species, which could potentially provide greater support for the hypothesis and predictions. Compressive test values may additionally have some

degree of error/inaccuracy, albeit systematic, as a result of bone cross-sectional geometry being left naturally irregular versus milled to a standardized shape (e.g. rectangular) as in some previous studies (Currey, 1984, 1987). Another factor to consider is that during bending tests it cannot be assumed that principal axes are the same as neutral planes of bending. Therefore, bending values calculated here may not account for the orientation and magnitude as they would *in vivo* (Lieberman et al., 2003). That said, however, we are confident in the selection of thresholded images used for calculation of bone CSA to normalize bending test data and further contend that changes in image threshold are unlikely to be biologically significant, particularly in relation to the magnitude of interspecific differences observed for values of bending strength and elastic (Young's) modulus in sloths. Last, a method for tensile testing of sloth long limb bones could not be effectively executed to provide data to directly support the prediction of enhanced tensile bone strength in suspensory taxa. It also would be helpful to have *in vivo* data from strain gauge recordings to compare magnitudes of tensile/compressive bone strain in sloths with those reported in primates.

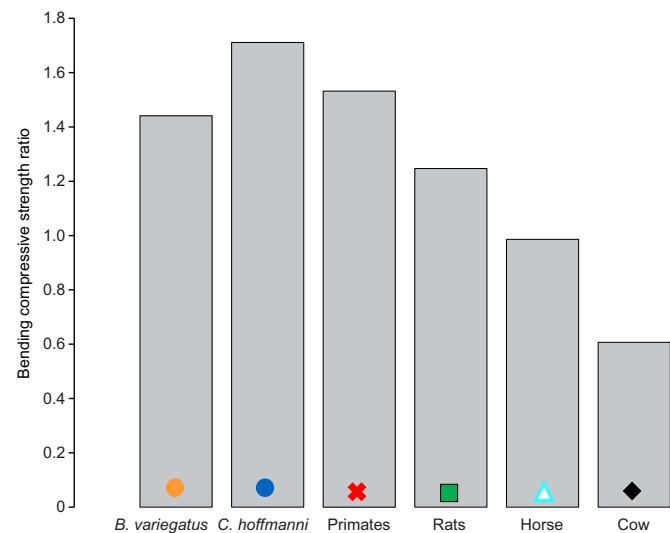


Fig. 8. Ratios of bending strength to compressive strength for selected mammals. Data for sloths, primates and rats are from this study. Data for cow and horse femora were compiled from Currey (1987).

Conclusions

Strength and elasticity for the limb bones of sloths are reduced in comparison with values of these material properties typical of pronograde quadrupedal mammals. Large ratios of bending to compressive strength suggest that sloths may have evolved modifications at both organ and matrix levels to ensure that their limb bones would not fail under the common tensile loads they experience during suspension. This conclusion, however, remains to be directly verified by axial tensile strength data and analyses of bone collagen and mineral content. Nonetheless, one trend that remains clear is that bone material properties are on a continuum across arboreal (suspensorial)-to-terrestrial (cursorial) taxa and are

more variable than previously considered. Bone properties are likely influenced by functional adaptation for specific niches (or lifestyles) that animals occupy in accordance with their substrate preference and predominant loading regime. Moreover, bone properties do not vary between two- and three-toed sloths, providing further evidence of a remarkable degree of morphological and physiological convergence between phylogenetically distant genera. Last, the findings of this study help to explain limb modifications necessary in a tensile limb system and clarify mechanical requirements appropriate for differing levels of arboreality. Future studies should aim to evaluate similar features in suspensory primates as well as make more detailed assessments of osteon remodeling, in sloths and other arboreal taxa.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.M.M., M.T.B.; Methodology: A.M.M., J.W.Y., M.T.B.; Software: J.W.Y.; Validation: A.M.M., M.T.B.; Formal analysis: A.M.M., J.W.Y.; Investigation: A.M.M.; Resources: M.T.B.; Data curation: A.M.M., J.W.Y.; Writing - original draft: A.M.M., M.T.B.; Writing - review & editing: A.M.M., J.W.Y., M.T.B.; Visualization: A.M.M., J.W.Y.; Supervision: M.T.B.

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Data availability

All custom-written R scripts used for analysis, along with requisite data files and the custom-written MATLAB program are available from figshare: <https://doi.org/10.6084/m9.figshare.16663960.v1>.

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