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Ontogeny and variability of trabecular bone in the chimpanzee humerus, femur and tibia

Running header: Chimpanzee trabecular ontogeny and variability

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Abstract

Objectives: Trabecular bone structure is known to be influenced by joint loading during life. However, many additional variables have the potential to contribute to trabecular bone structure of an adult individual, including age, sex, body size, genetics and overall activity level. There is little research into intraspecific variability in trabecular bone and ontogeny of trabecular bone structure, especially in non-human primates.

Materials and methods: This study investigates trabecular structure in adult and immature chimpanzees from a single population using high resolution micro-computed tomographic scans of the proximal humerus, proximal femur and distal tibia. Trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular spacing (Tb.Sp) and degree of anisotropy (DA) were quantified in specific regions of adult and immature chimpanzees, and colour maps were generated to visualise the distribution of BV/TV throughout the joint in the metaphysis of immature specimens.

Results: The results demonstrate that variability in adult trabecular structure cannot be explained by sex or body size. During ontogeny, there is a general increase in trabecular BV/TV and Tb.Th with age, and ratios of trabecular parameters between the fore- and hindlimb may be consistent with locomotor transitions during ontogeny.

Discussion: Variation in trabecular morphology among adult individuals is not related to sex or body size, and the factors contributing to intraspecific variability, such as overall activity levels and genetic differences, require further investigation. Trabecular ontogeny in chimpanzees differs from humans in some respects, most notably the absence of a high BV/TV at birth.

INTRODUCTION

Reconstructing locomotor behaviour in extinct species requires identification of plastic features that reflect actual behaviour during life (Ward, 2002). The internal structure of bone is able to remodel in response to loading, a process known as bone functional adaptation (Cowin, 2001; Kivell, 2016; Ruff, Holt, & Trinkaus, 2006), and thus features of both cortical and trabecular bone may hold a functional signal that could be used to interpret behaviour of extinct taxa. Experimental studies have demonstrated the ability of trabecular bone to remodel in response to changes in joint loading (Barak, Lieberman, & Hublin, 2011; Biewener, Fazzalari, Konieczynski, & Baudinette, 1996; Pontzer et al., 2006), and several studies have identified behavioural correlates in the trabecular structure of extant (Griffin et al., 2010; MacLatchy & Müller, 2002; Maga, Kappelman, Ryan, & Ketcham, 2006; Ryan & Ketcham, 2002b; Tsegai et al., 2013) and fossil (Barak, Lieberman, Raichlen, et al., 2013; Ryan & Ketcham, 2002a; Skinner et al., 2015; Su & Carlson, 2017; Su, Wallace, & Nakatsukasa, 2013; Zeininger, Patel, Zipfel, & Carlson, 2016; Ryan et al., 2018) primate taxa. However, we currently lack an understanding of how non-biomechanical factors may influence trabecular structure, particularly within non-human primates. Here, we help to fill this gap through an analysis of how trabecular bone structure changes throughout ontogeny within the chimpanzee hind- and forelimb to provide insight into the underlying patterning of trabecular bone, and how the structure may change in response to locomotor changes throughout development.

Cortical bone morphology of the adult humerus and femur reflects variation in human activity levels (Macintosh, Pinhasi, & Stock, 2014; Macintosh, Pinhasi, & Stock, 2017; Shaw & Stock, 2013; Stock & Macintosh, 2016), as well as locomotor differences in extant apes, enabling the reconstruction of limb loading in fossil taxa (Ruff, 2008; Ruff, 2002; Ruff, Burgess, Ketcham, & Kappelman, 2016). Humans, gorillas and chimpanzees undergo changes in locomotion during ontogeny (Doran, 1992, 1997; Sarringhaus, MacLatchy, & Mitani, 2014; Sutherland, 1997; Sutherland, Olshen, Cooper, & Woo, 1980), which are reflected in limb bone cross-sectional geometry at various life stages (Cowgill, Warrener, Pontzer, & Ocobock, 2010; Gosman, Hubbell, Shaw, & Ryan, 2013; Ruff, 2003a; Ruff, Burgess, Bromage, Mudakikwa, & McFarlin, 2013; Sarringhaus, MacLatchy, & Mitani, 2016). In humans, analysis of long bone cross-sectional geometry, from neonates to adulthood, reveals ontogenetic changes in relative femoral to humeral strength (i.e. polar section modulus), and femoral and tibial diaphyseal strength and shape (i.e. subperiosteal area, and the ratio of the maximum and minimum second moments of intertia, respectively), which reflect the acquisition of bipedal locomotion (Gosman et al., 2013; Ruff, 2003a). The increased stability that is acquired as bipedal locomotion becomes more refined is also reflected in changes in femoral cross-sectional shape (Cowgill et al., 2010). In chimpanzees, there is an increase in strength of the femur compared to the humerus during ontogeny, with a significant change between infant and juvenile periods (Sarringhaus et al., 2016). This transition coincides with a reduction in forelimb loading and an increase in hindlimb loading, as the hindlimb becomes more dominant in locomotion (Doran, 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016). Similarly, young mountain gorillas, which engage in more arboreal and suspensory behaviour than adults (Doran, 1997), have forelimb to hindlimb strength ratios similar to more arboreal adult Western lowland gorillas, but have relatively stronger forelimbs than hindlimbs when compared to their less arboreal adult counterparts (Ruff et al., 2013). The ratio of forelimb to hindlimb strength changes to the adult condition at around two years of age, which corresponds to the reduction of arboreal locomotion, and adoption of a more terrestrial, adult-like locomotor repertoire (Doran, 1997; Ruff et al., 2013). Evidence from cortical bone diaphyseal morphology demonstrates that both adult and non-adult cortical structure reflects locomotor behaviour at different life stages.

In contrast to cortical bone, comparisons of trabecular architecture between the adult humeral and femoral head does not clearly distinguish bipedal humans from other primates (Shaw & Ryan, 2012; Tsegai, Skinner, Pahr, Hublin, & Kivell, 2018). In general, across primate taxa, regardless of their locomotor behaviour, the femoral head has a more robust trabecular structure (i.e. higher bone volume/total volume, or BV/TV) than the humeral head (Chirchir et al., 2015; Ryan & Walker, 2010; Shaw & Ryan, 2012; Tsegai et al., 2018). However, trabecular bone does show signals of locomotor changes during ontogeny. In humans, changes in trabecular structure in the lower limb occur around the time of acquisition of bipedal locomotion; the human proximal femur (Ryan & Krovitz, 2006) and proximal tibia (Gosman & Ketcham, 2009) have a shared pattern of trabecular ontogeny that is characterised by an initially high volume of numerous, thin trabeculae, with both BV/TV and uniformity of orientation (i.e. degree of anisotropy, or DA) reducing until 1-2 years of age. At this time, perhaps driven by commencement of bipedal locomotion, there is an increase in BV/TV and DA, reaching adult levels at around 4-7 years. The human calcaneus shares this pattern of high BV/TV, followed by a rapid reduction after birth, subsequently increasing around three years of age (Saers, 2017). The DA follows the inverse pattern, unlike the femur and tibia, there is an increase in DA from birth until three years of age, followed by a reduction until DA stabilises at around five years of age (Saers, 2017). Furthermore, throughout human ontogeny, there is a reduction in the number of trabeculae and an increase in trabecular thickness (Tb.Th) in the lower limb and vertebrae, reaching adult-like values during adolescence (proximal tibia: Gosman & Ketcham, 2009; lumbar vertebra: Kneissel et al., 1997; proximal femur: Ryan & Krovitz, 2006; calcaneus: Saers, 2017).

Although these ontogenetic changes in trabeculae fit well with the onset and development of human bipedal locomotion, both the proximal humerus and proximal femur show a similar pattern of initial trabecular reduction, followed by an increase in BV/TV around 1-2 years of age, indicating the potential for genetic, rather than mechanical, influence on the timing of trabecular structural change (Ryan, Raichlen, & Gosman, 2017). In other words, these commonalities between the upper limb and lower limb bones may suggest, a “general model for trabecular development” (Ryan et al., 2017, p294), which may also be shared with the vertebral column (Acquaah, Robson Brown, Ahmed, Jeffery, & Abel, 2015). During this period, bone is adapting from the initial grid-like structure laid down during endochondral ossification (Carter & Beaupré, 2000; Martin, Burr, Sharkey, & Fyhrie, 2015; Scheuer & Black, 2000), and undergoing changes in both physical properties and bone mineral content (Mølgaard, Thomsen, & Michaelsen, 1998; Nafei, Danielsen, Linde, & Hvid, 2000), while adjusting to a rapidly increasing body mass (Tanck, Homminga, van Lenthe, & Huiskes, 2001). In the ilium, characteristic adult-like trabecular organisation is observed in neonates, further indicating the potential roles of genetics, or other factors such as joint morphology, in determining trabecular structure (Abel & Macho, 2011; Cunningham & Black, 2009a, 2009b). Other non-primate, domesticated mammalian taxa also demonstrate an increase in BV/TV and DA during ontogeny (sheep tibia: Nafei, Kabel, Odgaard, Linde, & Hvid, 2000; pig vertebra and tibia: Tanck et al., 2001; dog ulna: Wolschrijn & Weijs, 2004).

Nonetheless, in humans divergence of trabecular bone characteristics of the humerus and femur, with a higher rate of increase in BV/TV, Tb.Th and Young’s Modulus in the femur, begins at around one year of age, indicating that although the general pattern of ontogeny is similar between these elements, differences in the developmental trajectory between elements may reflect biomechanical changes in limb function (Ryan et al., 2017). At later stages of ontogeny, the refinement of bipedal locomotion, which involves increased stability and reduced variability (Cowgill et al., 2010; Raichlen et al., 2015), is indeed reflected in increased homogeneity of trabecular organisation across the distal tibia (Raichlen et al., 2015). There is perhaps evidence of changes in trabecular morphology of the human foot related to the acquisition of the characteristic human heel-strike and toe-off stance phases (Saers, 2017; Zeininger, 2013).

Among non-human primates, ontogenetic changes in trabecular structure have, to our knowledge, only been studied in the African ape foot (Zeininger, 2013). Similar to humans, African apes show an increase in BV/TV and Tb.Th between infant and adult age groups across taxa (Zeininger, 2013). However, whether the timing of trabecular structural changes in African apes is similar to that of humans is unknown. With very little comparative context, it remains unclear to what extent ontogenetic changes in human trabecular architecture are driven by biomechanical changes associated with the onset of bipedal locomotion, or by pre-determined genetic transitions in bone microarchitecture.

Bone is a complex structure, with adult form being determined by a combination of factors that can change throughout life, including body mass, sex, age, hormonal and genetic differences. For example, non-behavioural factors, such as genetic and hormonal differences, can influence trabecular structure and/or the responsiveness of trabecular bone to mechanical load between species or populations, and even between anatomical sites (Lovejoy, McCollum, Reno, & Rosenman, 2003; Wallace, Judex, & Demes, 2015; Wallace, Kwaczala, Judex, Demes, & Carlson, 2013; Wallace, Pagnotti, et al., 2015; Wallace, Tommasini, Judex, Garland, & Demes, 2012). Our understanding of these factors in non-human primates, and their contribution to bone structure at different life-stages, is limited. For example, chimpanzees and bonobos have been shown to vary in the ontogenetic timing of changes in thyroid hormone, regulating among other things somatic growth and metabolism (Behringer, Deschner, Murtagh, Stevens, & Hohmann, 2014). Sex differences have the potential to affect bone structure at all life stages. During growth, the body mass of male and female chimpanzees diverges at around seven years of age, with males and females showing different growth rates and ages of growth cessation (Leigh &

Shea, 1995, 1996), potentially contributing to sex differences in adult bone morphology. There are locomotor differences between male and female chimpanzees from the Taï National Park (Doran, 1993), with females engaging in a greater proportion of arboreal quadrupedalism and, to a lesser extent, suspension than males, who use more quadrupedal climbing and bipedal locomotion compared to females. The percentage of locomotor time spent on different substrates, comprising the ground and five types of arboreal substrate, are similar between sexes (Doran, 1993).

In this study, we use a sample of chimpanzee (*Pan troglodytes verus*) specimens from a single population – the Taï National Forest, Cote D'Ivoire – to investigate trabecular ontogeny and within population variability in the proximal humerus, proximal femur and distal tibia. We hypothesise that:

1. Across taxa Tb.Th, trabecular separation (Tb.Sp) and BV/TV scale positively with size (Doube, Klosowski, Wiktorowicz-Conroy, Hutchinson, & Shefelbine, 2011; Fajardo et al., 2013; Ryan & Shaw, 2013), trabecular number (Tb.N) scales negatively with size (Fajardo et al., 2013; Ryan & Shaw, 2013), and there is a weak relationship between the relative uniformity of trabecular orientation (i.e. DA) and body size (Doube et al., 2011). Among humans, it has been found that Tb.Th, BV/TV and bone mineral density of the calcaneus are correlated with body size (Best, Holt, Troy, & Hamill, 2017), however, this is only the case in individuals who do not engage in regular running. Here, we test whether there is a relationship between body size and trabecular variables in adult chimpanzees within a single subspecies of chimpanzee, with a limited range of size variability.

2. Trabecular bone will change during ontogeny in a manner similar to that found in humans, following the model for trabecular bone development outlined by Ryan et al. (2017) and identified in other studies (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Saers, 2017; Zeininger, 2013). Trabecular BV/TV and DA will be initially high followed by a reduction and then subsequent increase. We also predict Tb.Th will increase and Tb.N will decrease, from birth to adulthood.

3. Locomotor ontogeny in chimpanzees is characterised by a reduction in arboreal locomotion and an increase in the frequency of quadrupedal knuckle-walking with increasing age (Doran,

1992, 1997; Sarringhaus et al., 2014). It is difficult to directly characterise loading experienced by the skeleton in chimpanzees, as their complex locomotor repertoire includes a diverse range of positional and locomotor behaviours (Hunt, 1991), and there are both practical and ethical limitations to research on chimpanzee locomotion. To simplify interpretation of skeletal signals of locomotion, a dichotomy of reduced forelimb and increased hindlimb loading during ontogeny has been used, however, this may not fully capture changing loads on the skeleton. This concept is generally based on reduced torso-orthograde suspension and increased frequencies of knuckle-walking, which incurs higher vertical reaction forces on the hindlimb than the forelimb (Demes et al., 1994). Together this implies a reduction in forelimb loading and an increase in hindlimb loading. However, infants and juveniles use some hindlimb loaded modes more frequently than adults, specifically, they have higher frequencies of bipedalism, leaping and vertical climbing (for limb loading during vertical climbing: Hanna, Granatosky, Rana, & Schmitt, 2017). Despite this complexity of locomotor changes, there is morphological evidence for increased loading of the hindlimb relative to the forelimb across ontogenetic stages, as the femur increases in torsional and bending strength (i.e. polar second moment of area and polar section modulus) compared to the humerus during ontogeny (Sarringhaus et al., 2016). As such, we predict that, as with diaphyseal cross sectional properties, trabecular bone will reflect increased hindlimb (femoral head and distal tibia) loading compared to the forelimb (proximal humerus), with this change occurring after five years of age (Doran, 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016).

4. During ontogeny, the distribution of trabecular BV/TV within each metaphysis will be initially homogeneous, with increasing heterogeneity over time (Ryan & Krovitz, 2006). Previous studies have not assessed the stage in humans at which heterogeneity stabilises, due to absence of adult or adolescent specimens in the study samples. Therefore, we make no prediction as to which age adult-like distributions of trabecular BV/TV will be reached.

MATERIALS AND METHODS

Study sample

The study sample was taken from a single population of *Pan troglodytes verus,* wild collected in the Taï National Park, Republic of Côte d'Ivoire. This is a long-term study site for which age, sex, behavioural data and cause of death is available for some individuals (Boesch & Boesch-Achermann, 2000). Trabecular bone was analysed in the proximal humerus, proximal femur and distal tibia of 21 adult and 21 immature individuals (Table 1), although all elements were not present for every individual in the sample. The proximal humerus, proximal femur and distal tibia were selected based on preservation and availability, with femora and tibiae taken from the same side where possible.

Age categories

The locomotor repertoire of chimpanzees comprises a range of positional and locomotor modes, and changes in frequencies of these behaviours occur at different life stages (Doran, 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016). As such, the study sample was divided into five age categories based on locomotor transitions reported by Sarringhaus et al. (2014; 2016) and, for this particular population/subspecies, by Doran (1992, 1997). Age of each individual was based on the collection records, as the identification, sex and date of birth for most individuals was known (see Supporting Information).

Age categories were defined as follows. Baby: from birth to 5 months, the period prior to onset of independent locomotion where chimpanzees are carried by the mother, although locomotor play occurs after 3 months (Doran, 1992). Infant: from 5 months until 4 years, after the commencement of locomotion but before complete independence from the mother. During this stage torso-orthograde suspension is the most frequent locomotor mode (42.8%: Sarringhaus et al., 2014), but individuals continue to engage in higher frequencies of arboreal locomotion than adults (Doran, 1992; Sarringhaus et al., 2014). Juvenile: 5-9 years, after independence from the mother but before the adolescent period; quadrupedal walking is the most frequent locomotor mode for this age category (42.3%: Sarringhaus et al., 2014), however, a higher frequency of suspensory behaviour is used during arboreal locomotion than in adults (Doran, 1992). Adolescent: from 10 years until epiphyseal fusion, locomotor modes are approaching adult-like frequencies of each locomotor mode. Adult: defined by complete epiphyseal fusion. The oldest individual included in the sample of known age was 38 years old. Among the individuals of unknown age, only one male individual had pronounced tooth wear, however, the trabecular structure of this individual showed no evidence of age related reduction in BV/TV, compared to other males in the sample. For four immature individuals in the study sample, age was estimated based on dental eruption data (B. H. Smith & Boesch, 2011; B. H. Smith, Crummett, & Brandt, 1994; see Supporting Information). One individual was considered juvenile (M2 erupted) and three were considered to be infants (two individuals with incomplete deciduous dentition and one individual with M1 erupted). One indiviual (MPI 15003) was very small (see Supporting Information for femoral length), and could potentially be younger than five months, however, to be conservative, this individual was included in the Infant category.

Scan acquisition

Micro-computed tomographic scans (micro-CT) were collected using three scanners (BIR ACTIS 225/300, diondo d3, and SkyScan 1173) housed at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). Specimens were scanned at an isotropic voxel size of 13.7-42.8 microns. All scans were reconstructed as 16-bit TIFF image stacks. Specimens were downsampled in Avizo 6.3 (FEI Visualization Sciences Group) to reduce file size for subsequent processing stages. The downsampled resolutions, along with relative resolution, are shown in Table 1 for each age category. Adequate resolution for analysis of trabecular structure was retained, as demonstrated by the range of relative resolutions, which indicates the number of pixels representing trabeculae with mean thickness (Kivell, Skinner, Lazenby, & Hublin, 2011; Sode, Burghardt, Nissenson, & Majumdar, 2008).

Segmentation

All specimens were reoriented into standardised positions in Avizo 6.3, and segmented using the Ray Casting Algorithm (Scherf & Tilgner, 2009). Scans were subsequently segmented into three regions (cortex, trabeculae, and internal region of the bone) using an in-house script for medtool 4.0 (www.dr-pahr.at), following Gross et al. (2014). The cortex was removed to create the inner mask, where the internal region of the bone and the trabecular bone had different grey values (Fig 1a).

3D morphometric mapping

Morphometric maps of BV/TV were generated for immature individuals following Gross et al. (2014) using medtool v4.0 (www.dr-pahr.at) as shown in Figure 1c. A 3D tetrahedral mesh was created of the internal region of the bone using CGAL (http://www.cgal.org). For adolescent individuals a mesh size of 1 mm was used, and a mesh size of 0.5mm was used for younger individuals. A 2.5mm background grid was applied in three dimensions to the inner mask, and BV/TV was quantified at each node of the background grid using a 5mm sampling sphere. The values from each sampling sphere were interpolated and applied to elements of the 3D tetrahedral mesh in order to generate morphometric maps of BV/TV. For some specimens in the immature sample (four tibia, four femora and six humeri) BV/TV maps were not generated, either due to the presence of matrix or, due to high porosity, the cortex could not be segmented (see Supporting Information).

Definition of regions and volumes of interest

Definition of biomechanically-homologous subregions in trabecular analyses in adult specimens is often complex (Kivell et al., 2011; Lazenby, Skinner, Kivell, & Hublin, 2011; Maga et al., 2006), and this complexity only increases when investigating immature specimens. Therefore, different methods of defining subregions were applied here for adult and immature specimens. To investigate variability in adult chimpanzees, trabecular bone was quantified in large regions of interest (ROIs; following Tsegai et al., 2018), which were defined according to the following protocol (Fig 1b and Fig 2). The proximal humerus was defined from the point at which curvature of the shaft expands towards the humeral head (Fig 2a). The femoral head was defined by the most inferior and lateral points of the femoral head (Fig 2b). The ROI in the distal tibia was defined by increasing curvature of the shaft in medial and anterior views (Fig 2c).

In immature individuals, it is difficult to define homologous regions, especially in very young individuals, due to continued longitudinal growth and changing morphology over time (Ryan & Krovitz, 2006). As such, definition of a large ROI, similar to that used for adult individuals, across an ontogenetic series is challenging. Instead, volumes of interest (VOIs) were extracted from the inner mask using medtool v4.0, and trabecular bone parameters were quantified in these subregions (Fig 2d-f). A spherical VOI, with a 5mm diameter, was placed 5mm deep to the epiphyseal surface in each individual, centred in the mediolateral and anteroposterior planes of the proximal humerus and distal tibia following approximately the VOI size and placement protocols of Ryan and Krovitz (2006) and Ryan et al. (2017). In the proximal femur, centering the VOI in the mediolateral and anteroposterior planes, resulted in VOIs located in clearly different locations between different age categories. Thus, in the youngest individuals (N = 3) VOIs were placed centred in the mediolateral and anteroposterior plane, whereas in older individuals the VOI was centered in the anteroposterior plane, the mediolateral location was determined by the most proximal extent of the metaphysis and the proximodistal location by the most medial extent of the metaphysis. This resulted in similar VOI placement between individuals. Specimens with partially fused epiphyses were included in the study, if the epiphyseal line remained clearly visible.

An advantage of this methodology is that it enables comparison of the results of the present study to previous studies of trabecular ontogeny in humans. There are potential advantages to using scaled VOIs due to the problem of oversampling (Lazenby, Skinner, Kivell, et al., 2011), and scaled VOIs have been applied to investigate trabecular ontogeny of the proximal and distal tibia (Gosman & Ketcham, 2009; Raichlen et al., 2015). However, for many volume based variables, including most variables quantified in this study, VOI size has less of an impact than location (Kivell et al., 2011; Lazenby, Skinner, Kivell, et al., 2011).

Quantification of regional trabecular variables

Trabecular bone architectural variables were quantified in each defined ROI or VOI (Fig 1b and Fig 2). These were quantified from the inner mask using a script available in medtool v4.0. Bone volume fraction (BV/TV) was calculated as the total number of bone voxels divided by the total number of voxels. Trabecular thickness (Tb.Th) and trabecular spacing (Tb.Sp) were calculated following the sphere fitting method of Hildebrand and Rüegsegger (1997), and trabecular number (Tb.N) was calculated as Tb.N = 1/(Tb.Th+Tb.Sp). The mean intercept length method was used to quantify the second order fabric tensor and the degree of anisotropy (DA) was calculated as DA = 1 – [smallest eigenvalue/largest eigenvalue], with a DA of 0 reflecting complete isotropy and a DA of 1 representing “complete” anisotropy.

Statistical analysis

Non-parametric tests were used for the statistical analysis, as the Shapiro-Wilk test for normal distribution found that trabecular variables were not normally distributed in the adult and immature samples. A p-value of <0.05 was considered significant. All statistical tests were conducted using R v3.3 (R Core Team 2016) and ggplot2 (Wickham, 2009) and cowplot (Wilke, 2017) were used for plot generation.

Adult analysis. In the adult sample, Mann-Whitney U tests were applied to test for significant differences between sexes. As no significant differences were found between sexes in any trabecular variable, sexes were pooled for subsequent analyses. To test for significant differences in trabecular parameters between elements in the adult sample, Kruskal-Wallace tests were used, followed by post-hoc Nemenyi tests for pairwise comparisons. The relationship between trabecular variables both within and between elements was tested using Spearman's rank correlation tests with a post-hoc Bonferroni correction. To investigate the relationship between body size and trabecular structure among adult chimpanzees, two measures of size were used. Firstly, the superoinferior femoral head height (FHH) was used as a proxy for body size. Although, primate taxa differ slightly in the relationship between femoral head height and body mass, only one taxon is included in the study sample and there is a strong correlation between FHH and body mass in chimpanzees (Burgess, McFarlin, Mudakikwa, Cranfield, & Ruff, 2018; Jungers, 1991; Ruff, 2003b). Moreover, this measurement has been used in previous analyses of allometric relationships in trabecular bone structure (e.g. Doube et al., 2011; Ryan & Shaw, 2013). The second measure of size was the total joint volume (TJV) included within each ROI, which was used to investigate the relationship between joint size and trabecular bone structure. TJV was found to correlate significantly with FHH in each element, using Spearman's rank correlation test (humerus: $r = 0.86$, $p < 0.01$; femur: $r = 0.75$, $p < 0.01$; tibia: $r = 0.77$, $p < 0.01$). The relationship between trabecular variables and both size proxies, FHH and TJV, were tested using Spearman's rank correlation tests with a post-hoc Bonferroni correction.

Ontogenetic analysis. The four individuals for whom exact age was not known were included in all statistical analyses conducted by age category, but excluded from those analyses that were based on exact age. To test for significant differences in trabecular variables between age groups, a Kruskal-Wallace test was applied, followed by post-hoc pairwise Nemenyi tests. To test for changes in trabecular structure over time, OLS regressions with a post hoc Bonferroni correction of age against each trabecular variable in each element were conducted. To compare changes in trabecular structure between elements over time, the ratio of trabecular variables between elements was calculated. Kruskal-Wallace tests were applied to test for differences in these ratios between elements.

RESULTS

Adults

Mean and standard deviations of each trabecular parameter from ROIs in adult individuals are shown in Table 2, and box-and-whisker plots of trabecular variables are shown for each element in Figure 3. Mann-Whitney U tests find no significant differences between males and females in any trabecular parameter in the femur, humerus and tibia, with box-and-whisker plots of trabecular variables by sex shown in Figure 4. Results of Kruskal-Wallace tests indicate significant differences between elements in all trabecular variables (Table 2) and post-hoc pairwise Nemenyi tests (Table 3) reveal that this is due to differences between the proximal femur and the other two elements in the majority of trabecular variables. The only variable that differs significantly between the humerus and tibia is DA.

Results of Spearman's correlation test to identify correlations between trabecular variables in each element are shown in Table 4. There is a significant correlation between the humerus and femur in BV/TV, Tb.Th, Tb.Sp and Tb.N, and between the humerus and tibia in Tb.N. These relationships between trabecular variables are shown in Figure 5 as the values for the femur and tibia compared to the humerus. Spearman's correlation test to identify the relationship between trabecular variables within each element (Table 5), shows that in both the femur and tibia, BV/TV and Tb.Th are significantly correlated, and in the femur DA is significantly correlated with both BV/TV and Tb.Th. In all elements, there is a significant correlation between Tb.Sp and Tb.N. Within the humerus there are no additional significant correlations. Spearman's correlation tests between trabecular structure and both FHH and TJV of each element find no significant relationship between either size variable and trabecular bone structure, with no R^2 value above 0.08 (Table 6). Figure 6 shows the relationship between TJV and trabecular structure.

VOIs during ontogeny

For the immature individuals, means and standard deviations of trabecular variables measured in VOIs are shown in Table 7. Scatterplots of trabecular variables by age are shown in Figure 7, with four individuals for whom exact age is unknown plotted in the shaded area on the right side of each plot. These plots show a general trend of increasing BV/TV and Tb.Th with age, a relatively constant Tb.N and Tb.Sp over time, and DA that is higher at a younger age in the humerus and tibia, but in the femur remains relatively similar across age groups. There appears to be an increase in DA in all three elements in the oldest individuals. Significant differences between age categories are only present in the femur for BV/TV, Tb.Th and Tb.N (Table 7). Post-hoc Nemenyi tests (Table 8) find significant differences between the Baby and Adolescent groups in both Tb.Th and Tb.N and significant differences between the Infant and Adolescent groups in Tb.Th. Results of OLS regressions (Table 9) show a significant relationship between age and Tb.Th and BV/TV in both the femur and tibia, but not in the humerus.

The ratio of each trabecular variable between the femur and humerus, femur and tibia, and tibia and humerus, is shown by age group in box-and-whisker plots in Figure 8. No significant differences in ratios between age groups are found in the Kruskal-Wallace tests (Table 10). However, the pattern of change over time does demonstrate potential differences between age categories, and a different pattern between the humerus and each hind limb element.

Comparisons between age groups in the ratio of femoral to humeral trabecular variables demonstrate that both BV/TV and Tb.Th in the femur increases relative to the humerus between Juvenile and Adolescent age groups. Between the Baby and Infant age groups, there is a slight reduction in the ratio of BV/TV, but not in Tb.Th. Tb.N and Tb.Sp show an inverse pattern, having more numerous, closely spaced trabeculae in the femur than humerus in the Baby group compared to older age groups. The ratio of Tb.N and Tb.Sp is highly variable among the other three age groups. The ratio of femoral to humeral DA follows a similar pattern to that of BV/TV, with a decrease between Baby and Infant groups, and then increasing with age.

The ontogenetic pattern of tibial to humeral trabecular ratios differs from the femoral to humeral pattern. There is an increase in the BV/TV ratio between the Infant and Juvenile age groups, but this ratio is similar between Baby and Infant, and between Juvenile and Adolescent groups. For Tb.Th ratios, although the median shows an increase with age, there is a great deal of variability and overlap between all age groups. There is overlap between all age groups in the ratios for

Tb.Sp and there is no clear pattern between age groups. The ratio of DA appears to reduce between Baby and Infant stages, and to subsequently increase with increasing age.

Comparisons between the two hindlimb elements, the tibia and femur, demonstrate that the ratio of BV/TV, Tb.N, Tb.Sp, and DA remains fairly constant over time in these elements. There is a slight trend toward a lower ratio of Tb.N and a higher ratio of Tb.Sp with increasing age. However, there is a greater increase in Tb.Th in the femur compared to the tibia between the Infant and Juvenile, and between the Juvenile and Adolescent age groups.

Morphometric maps during ontogeny

Figures 9-11 show cross-sections of segmented micro-CT data and colour maps of the distribution of BV/TV in a subset of the study sample (see Supporting Information for images of the other individuals in the sample). In the proximal humerus, distal tibia, and proximal femur, the youngest individual (MPI_11787) has a relatively homogenous trabecular bone structure, with BV/TV values within a narrow range. When scaled to the range of BV/TV values in this individual, it is apparent that despite the fairly homogenous distribution, the medial region of the proximal humerus and femur, and the posterior region of the distal tibia, have the highest BV/TV. With increasing age, BV/TV increases and more regional differences become apparent, notably regions of high BV/TV on both the medial and lateral edges of the proximal humerus, femoral neck, and anterior and posterior edges of the distal tibia.

DISCUSSION

Trabecular bone structure is thought to reflect behavioural differences and joint loading (Barak et al., 2011; Ehrlich & Lanyon, 2002; Lanyon, 1974; Pontzer et al., 2006), however, the precise relationship between trabecular structure and behaviour in primates requires further consideration (Kivell, 2016). To accurately reconstruct behaviour in fossil species, it is essential to better understand all factors that can influence trabecular structure in extant taxa. To address this, the present study explores how trabecular structure changes throughout ontogeny, and intraspecific variability, within a single sub-species of chimpanzee from the Taï National Park, Republic of Côte d’Ivoire, using a multi-element approach. Amongst adult chimpanzees, there is no clear link between trabecular bone structure and sex or size, emphasising the importance of further investigation of factors that contribute to adult bone structure. These results establish that adult-like trabecular structure and distribution in chimpanzees is not present from birth but, rather, is acquired over time during ontogeny. Changes in chimpanzee trabecular structure during growth reveal both similarities and differences compared to published studies of humans.

Variability of trabecular structure in adult chimpanzees

Numerous factors have the potential to influence intraspecific variation in trabecular structure, and consequently the degree to which trabecular architecture reflects behavioural differences. These include differences between sexes that may be due to sexual dimorphism in bone lengths (Behringer et al., 2016; Zihlman, Stahl, & Boesch, 2008) and body mass (Leigh & Shea, 1995, 1996; R. J. Smith & Jungers, 1997), or due to hormonal differences (Behringer, Deschner, Deimel, Stevens, & Hohmann, 2014; Behringer, Deschner, Murtagh, et al., 2014). Trabecular structure correlates with body size both across primates (Barak, Lieberman, & Hublin, 2013; Cotter, Simpson, Latimer, & Hernandez, 2009; Fajardo et al., 2013; Ryan & Shaw, 2013), and a broad sample of both mammalian and non-mammalian taxa (Doube et al., 2011). Trabecular structure may differ across anatomical sites for multiple reasons including, for example, as an adaptation to reduce mass distally and maximise locomotor efficiency (Chirchir, 2015; Saers, Cazorla-Bak, Shaw, Stock, & Ryan, 2016), due to differences in susceptibility to remodelling (Wallace, Pagnotti, et al., 2015), or individual trabecular parameters varying in their contribution to Young's modulus (i.e. bone stiffness; Ulrich, van Rietbergen, Laib, & Ruegsegger, 1999). In addition, genetic differences (Paternoster et al., 2013; Wallace, Demes, & Judex, 2017), diet and the intestinal microbiome (Cao, Gregoire, & Gao, 2009; Cashman, 2007; Charles, Ermann, & Aliprantis, 2015; McCabe, Britton, & Parameswaran, 2015; Prentice, 1997; Shea et al., 2002), and activity levels (Best et al., 2017; Chirchir et al., 2015; Chirchir, Ruff, Junno, & Potts, 2017; Ryan & Shaw, 2015; Saers, 2017; Saers et al., 2016) influence bone morphology.

Contrary to our prediction, we find no relationship between size proxies FHH or TJV and trabecular structure in either the proximal humerus, proximal femur or distal tibia of chimpanzee adults. This indicates that within a species with limited size variation, body size may not be critical in determining trabecular structure. Trabecular structure does not differ significantly between sexes in the study sample in any element. Male individuals are underrepresented in the sample, with only eight males included, perhaps due to a female bias in the sex ratio of the Taï population (Boesch & Boesch-Achermann, 2000). Sex specific median values are similar with overlapping ranges of variation within all three elements and, as such, the smaller sample of male individuals is unlikely to explain the statistical results. Trabecular structure of other anatomical regions does not differ significantly between male and female adult chimpanzees in the vertebra (Cotter et al., 2009) or metacarpals (Lazenby, Skinner, Hublin, & Boesch, 2011). Sexual dimorphism in body size is relatively low in the Taï chimpanzees, with males only having significantly longer clavicles and broader scapulae across the postcranial skeleton (Zihlman et al., 2008). Correlations between elements and trabecular variables within elements were identified, indicating the complex interrelationships between trabecular variables across the skeleton. We found DA of the proximal femur to be correlated with BV/TV, which differs from the findings of Ryan et al. (2018). This is likely due to the different methodological approach, as DA results differ between analyses which focus on the entire joint and those which quantify trabecular structure in a smaller sub-region (Tsegai et al., 2018).

The absence of a relationship between trabecular structure and body size or sex in this population of adult chimpanzees suggests alternative factors, such as ontogeny, locomotor behaviour, activity level and/or genetic differences, may influence adult trabecular structure. With regards to activity level, at a broad scale, across a range of mammalian taxa, longer daily travel distances are associated with higher trabecular mass (Chirchir, Ruff, Helgen, & Potts, 2016). Within living humans, comparison between runners and non-runners reveals that only in non-runners is body mass correlated with trabecular structure of the calcaneus, specifically trabecular density, Tb.Th and BV/TV (Best et al., 2017). In contrast, in runners, weekly running distance, number of years running and age of onset of running is correlated with Tb.Th, together explaining 68.2% of variation (Best et al., 2017).This demonstrates that behavioural differences, such as weekly running distance, have the potential to mask allometric requirements of bone structure at the intraspecific level. Across an archaeological sample, trabecular robusticity of the calcaneus, talus and first metatarsal is related to subsistence strategies based on predictions of overall activity level (Saers, 2017). The degree of sexual dimorphism in trabecular structure of the foot differs between populations, but does not clearly relate to predictions based on cross-sectional geometry of the long bones of male and female individuals, indicating a complex relationship between sex, activity level/type and bone morphology (Saers, 2017). Across human populations, subsistence strategies requiring higher activity levels are associated with a more robust trabecular (Chirchir et al., 2015; Chirchir et al., 2017; Ryan & Shaw, 2015; Saers, 2017; Saers et al., 2016; Scherf, Wahl, Hublin, & Harvati, 2016) and cortical (Macintosh et al., 2014; Macintosh et al., 2017; Shaw & Stock, 2013; Stock & Macintosh, 2016) structure. Although this degree of intraspecific variability is likely unique to modern humans, due to the greater degree of behavioural and technological variability (both geographical and temporal), activity levels could be an important factor determining intraspecific variability in chimpanzees.

The contribution of travel distance and locomotor repertoire to both cortical and trabecular bone structure between populations of chimpanzee is as yet uncertain. Daily travel distance varies between chimpanzee populations from 2.1km to 4.8km at different study sites (Gruber, Zuberbühler, & Neumann, 2016), and locomotor repertoires differ between groups, in part due to variation in local ecology (Carlson et al., 2006; Carlson et al., 2008; Hunt, 1991). Cortical bone cross-sectional geometry differs between populations and subspecies of chimpanzee, specifically at the study sites of Mahale, Gombe, Kibale and Taï, and ecological/locomotor variables such as the degree of arboreal behaviour, the amount of forest cover, elevation and incline of the terrain, as well as genetic differences, may explain bone structural differences across subspecies (Carlson et al., 2006; Carlson et al., 2008; Carlson et al., 2011). Comparison of trabecular structure between populations of chimpanzee has only been conducted in the 1st, 2nd and 5th metacarpals (Lazenby, Skinner, Hublin, et al., 2011). Taï chimpanzees have a less robust trabecular structure in the hand compared to *Pan troglodytes troglodytes* from Cameroon, which cannot be explained by either differences in body mass or daily travel distance between the subspecies. Potentially, the contribution of terrestrial knuckle-walking to the locomotor repertoire of each subspecies, or more frequent manipulation during tool use in the Taï population, contributes to this structural difference (Lazenby, Skinner, Hublin, et al., 2011). With regard to the Taï population, daily travel distance differs between the three study groups living in the Taï National Park, and varies seasonally within each study group (Herbinger, Boesch, & Rothe, 2001). Within two groups for whom sufficient data is available, there are no sex differences in daily travel distance (Herbinger et al., 2001), despite the distances travelled by male chimpanzees during territorial patrols (Amsler, 2010; Boesch & Boesch-Achermann, 2000; Mitani, 2009; Watts & Mitani, 2001). As the study sample was collected over a long period, a potential factor contributing to bone structure is temporal changes in home range size, which have occurred, for example due to changes in group composition, i.e. the number of adult males in the group, but not with fruit availability (Lehmann & Boesch, 2003). Thus, activity levels may differ across individuals in the sample.

Ontogenetic changes in trabecular structure

Understanding how chimpanzee trabecular structure changes throughout ontogeny and how this compares with humans may provide further insight into the functional inferences that can be drawn from adult trabecular bone. We find that chimpanzee trabecular structure in the Baby category is characterised by thin, numerous, closely spaced trabecular struts, with a low BV/TV compared to older age groups. In this youngest age group, trabecular structure is more uniformly oriented in the proximal humerus and distal tibia, than in the proximal femur. With increasing age, both BV/TV and Tb.Th increase in all elements, however, this is only significant for the femur and tibia. Trabeculae become less numerous and more widely spaced with age, although this is relatively subtle, the only significant difference being higher Tb.N in the femur of the Adolescent group compared to the Baby group. After birth, trabeculae in the humerus and tibia become less uniformly organised, subsequently increasing in uniformity after five years of age. In the femur, the uniformity of orientation shows no clear pattern of change during ontogeny. These changes to trabecular structure during ontogeny may reflect locomotor transitions. Increasing BV/TV and Tb.Th of the hindlimb elements during ontogeny may reflect increased loading of these elements as the frequency of knuckle-walking increases in the locomotor repertoire. Moreover, the ratio of femoral to humeral BV/TV increases with age, suggesting increased loading of the femur in comparison to that of the humerus during ontogeny. Although comparisons of trabecular structure between elements is complex, due to differences in external joint morphology and VOI placement protocols, the higher DA of the distal tibia may reflect the more restricted movement of this joint, compared to the ball-and-socket joints included in this study (proximal humerus and femur).

In humans, there is a shared pattern of trabecular ontogeny across the proximal femur, proximal tibia and proximal humerus, with structural changes coinciding with the adoption of bipedal locomotion after one year of age (Gosman & Ketcham, 2009; Milovanovic et al., 2017; Ryan & Krovitz, 2006; Ryan et al., 2017). An increase in BV/TV, Tb.N, and DA occurs at around 1-2 years in the human proximal femur and proximal tibia, at the time of acquisition of bipedal locomotion (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006), and BV/TV, Tb.Th and DA continue to increase into early adulthood in the proximal tibia (Gosman & Ketcham, 2009). The sample used for the proximal femur does not extend beyond nine years (Ryan & Krovitz, 2006). The humerus follows a similar pattern of changing trabecular structure to these hindlimb elements, but differs from the femur in having a slower rate of change, reflecting increased loading of the femur with the acquisition of bipedal locomotion (Ryan et al., 2017). At a later stage of ontogeny, reduced regional variation in DA, i.e. more homogeneous structure, across the distal tibia reflects increasing stability in bipedalism (Raichlen et al., 2015). These findings in humans emphasise the complex interplay between regulation of growth and biomechanical influences on the skeleton. The pattern of trabecular ontogeny identified here in chimpanzee long bones has both similarities and, contrary to our prediction, differences to the findings of previous analyses of humans. This emphasises the potential for both different regulatory mechanisms underlying trabecular ontogeny in these taxa, and the influence of locomotion, i.e. mechanical loading.

The most often discussed trabecular structural measurement is BV/TV, which explains 88 % of variation in trabecular stiffness (Stauber, Rapillard, van Lenthe, Zysset, & Müller, 2006), and as such is of great biomechanical importance. Trabecular ontogeny in humans is characterised by an initially high BV/TV at birth, which drops until around 1-2 years of age, followed by an increase in BV/TV in both the proximal femur, proximal tibia, and proximal humerus. This initial reduction in BV/TV is due to a rapid reduction in Tb.N, alongside a gradually increasing Tb.Th (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017). This trabecular structure characteristic of neonatal humans appears to be acquired during gestational bone development through increasing BV/TV and Tb.Th in the human vertebral column (Acquaah et al., 2015) and proximal femur (Glorieux, Salle, Travers, & Audra, 1991; Salle, Rauch, Travers, Bouvier, & Glorieux, 2002). However Reissis and Abel (2012) found an increase in Tb.Th, but not BV/TV, during gestation in the proximal humerus and proximal femur. Unlike the pattern identified in humans, we do not find an initially high BV/TV in any of the three elements in chimpanzees at the youngest ages included in this sample, rather, BV/TV increases in all three elements during ontogeny, with a significant increase in BV/TV with age in the femur and tibia. This gradual increase in BV/TV is associated with increasing Tb.Th, also significant in the femur and tibia, similar to humans (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017), but with no rapid reduction in Tb.N. Tb.Sp remains relatively constant across ontogeny in all elements.

This contrast in ontogenetic changes between humans and chimpanzees is unlikely to be explained entirely by our small sample size below one year of age. Three individuals in the study sample are known to be younger than one year of age, and the suspected cause of death in these individuals is starvation (MPI_11787: 0.04 years) and respiratory disease (MPI_14993: 0.74 years; MPI_15015: 0.18 years). There are two individuals in the study sample whose age is not known, but appear to be around 1 year of age (MPI_15000 and MPI_15003), based on dental eruption and femoral length (see Supporting Information). Neither of these individuals has high BV/TV, and their values are similar to those known to be younger than one year of age. Younger chimpanzees in this skeletal collection have delayed dental eruption when compared to captive chimpanzees, and have a higher incidence of dental anomalies, indicating the potential influence of external factors, such as illness or nutritional stress, on their development (B. H. Smith and Boesch, 2011). As human specimens included in previous publications may also have died of disease and/or starvation, it seems unlikely, although remains a possibility, that health related factors contributed to this absence of high BV/TV in our sample prior to one year of age. The VOI protocol adopted in this study is similar to that of previous studies in humans (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017), and studies using a very different VOI protocol have found similar high BV/TV at birth in the human proximal femur (Milovanovic et al., 2017). In general, in chimpanzees there is an increase in trabecular bone robusticity (i.e. higher BV/TV and Tb.Th) with age in all three elements. This pattern is likely related to increasing body size with age, but perhaps also to increasing activity levels, such as greater locomotor independence from the mother (Doran 1992) and longer day ranges as individuals get older (Pontzer & Wrangham, 2006).

In non-primate, domesticated taxa, trabecular BV/TV follows a similar pattern to humans, being high early in ontogeny (dog ulna: Wolschrijn & Weijs, 2004), however, not all studies could determine the presence or absence of this pattern, as they did not include individuals from birth (e.g. pig mandible: Mulder, Koolstra, Weijs, & van Eijden, 2005; sheep tibia: Nafei, Kabel, et al., 2000; pig vertebra and tibia: Tanck et al., 2001). In contrast, there is no postnatal reduction in BV/TV in the distal tibia and talus of Dutch warm-blooded horses, rather, BV/TV and Tb.Th increase after birth (Gorissen et al., 2018). Acquaah et al. (2015) have suggested several potential explanations for the presence of a high BV/TV at birth in the human skeleton. Firstly, prenatally bone structure is likely to be, primarily, under genetic regulation, whereas postnatally biomechanical loading may be more influential for bone structure. Secondly, development of a dense trabecular structure where bone is subsequently removed and its structure is refined, may enable greater flexibility for adaptation to mechanical load (see Tanck et al., 2001). Finally, a high BV/TV at birth may function as a calcium reserve, as human breast milk may contain lower levels of calcium than required, and this bone loss may be regulated by elevated parathyroid hormone levels at this stage of development in humans. The pattern of trabecular ontogeny in horses, which differs from that of humans and dogs, has been explained by Gorissen et al (2018; 2016) as an adaptation for a precocial species whereby, in anticipation of the onset of locomotion only hours after birth, genetic factors prepare the skeleton for the mechanical loading it will experience. Potentially, all taxa could undergo a similar stage of reduction in BV/TV, but at different developmental stages, i.e. at birth in humans but in-utero in horses and chimpanzees. Future studies of trabecular bone structure in other mammalian taxa are required to fully understand this variability. Further, analysis of a larger sample of non-human apes at the youngest developmental stages are required to determine whether a high BV/TV and Tb.N at birth is a feature absent from their trabecular ontogeny, as the results of the present study suggest.

The uniformity of trabecular bone orientation (i.e. DA) and primary trabecular orientation is often found to reflect locomotor differences across primate taxa (e.g. Barak, Lieberman, Raichlen, et al., 2013; Barak, Sherratt, & Lieberman, 2017; Griffin et al., 2010; MacLatchy & Müller, 2002; Ryan & Ketcham, 2002b; Su et al., 2013; Tsegai et al., 2017) and to reflect both experimental or natural changes in loading during ontogeny (Barak et al., 2011; Pontzer et al., 2006; Raichlen et al., 2015). DA is thought to reflect whether a joint is more stereotypically loaded in a particular direction (i.e. a high DA) or loaded from a wide range of joint positions (i.e. a low DA). In the chimpanzee humerus and tibia, DA reduces from birth until around five years, when it begins to increase. In contrast, DA of the femur remains relatively constant across ontogeny. The pattern in the humerus and tibia is consistent with that of the human proximal femur (Ryan & Krovitz, 2006), although the age at which DA begins to increase is earlier in humans. In humans, locomotor transitions occur at a young age, with bipedalism acquired at 1-2 years and a stable gait at 3.5-4 years (Raichlen et al., 2015; Sutherland, 1997; Sutherland et al., 1980). In contrast, in chimpanzees locomotor changes are more gradual and occur throughout ontogeny (Doran, 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016), with a transition to increased terrestrial knuckle-walking at five years (Sarringhaus et al., 2014; Sarringhaus et al., 2016). Thus, in both humans and chimpanzees, changes in DA occur at a similar time to locomotor transitions, indicating that this parameter may be indicative of locomotor transitions during ontogeny. The only exception to this is the chimpanzee femur in which DA values suggest a consistency in the variability of load orientation throughout ontogeny.

Through analysis of ontogenetic changes in trabecular bone structure it is possible to characterise the process by which adult-like bone structure is obtained. A characteristic feature of the trabecular bone structure of adult primates is higher BV/TV in the femur compared to humerus (Chirchir et al., 2015; Ryan & Walker, 2010; Tsegai et al., 2018). Comparison of trabecular structure across the skeleton of chimpanzees and humans demonstrates that this similar pattern is driven by high BV/TV in the chimpanzee femur and low BV/TV in the human humerus compared to other anatomical sites (Tsegai et al., 2018). The pattern of ontogeny identified here in the chimpanzee skeleton demonstrates that adult-like trabecular ratios between the femur and humerus are not present at birth, but rather are acquired during life, with femoral and humeral BV/TV similar at birth but very different by adolescence. The femur of adult chimpanzees may have a higher BV/TV than the humerus for several reasons. Quadrupedal knuckle-walking is the largest component of the locomotor repertoire of chimpanzees (Doran, 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016), and although both the forelimb and hindlimb are loaded, the hindlimb experiences higher vertical ground reaction forces than the forelimb at various speeds, including both walking and galloping (Demes et al., 1994; Kimura, Okada, & Ishida, 1979). Furthermore, during vertical climbing, which has been considered to load both the forelimb and hindlimb equally (Sarringhaus et al., 2014; Sarringhaus et al., 2016), the hindlimb produces greater propulsive forces than the forelimb in most primates (Hanna et al., 2017; Hirasaki, Kumakura, & Matano, 1993). The relationship between vertical ground reaction forces and trabecular bone structure has not been tested. However, the domestic dog has higher vertical reaction forces on the forelimb (Kimura et al., 1979; Lee, Bertram, & Todhunter, 1999; Lee, Stakebake, Walter, & Carrier, 2004), while trabecular ash (correlated with trabecular density)

(Gong, Arnold, & Cohn, 1964; Kang, An, & Friedman, 1998) and Young's modulus (Kang et al., 1998) are higher in the femoral compared to the humeral head. Thus, dogs may have a similar pattern of femoral compared to humeral trabecular structure to primates, despite the difference in vertical reaction forces. Apart from biomechanical loading of the femur, potential differences in response to loading between the forelimb and hindlimb, as demonstrated experimentally in mice (Wallace, Pagnotti, et al., 2015), is an additional factor that could contribute to this pattern.

Ontogenetic changes in bone distribution

Ontogenetic studies are an ideal way to identify plastic features of bony morphology to recognise phylogenetic and functional signals in the fossil record (Congdon, 2012; Kivell & Schmitt, 2009; Richmond, 1998; Tardieu, 1999; Tardieu & Trinkaus, 1994; Ward, 2002). In adult individuals, the distribution of trabecular BV/TV is consistent with habitually-loaded joint positions in the adult hand (Skinner et al., 2015; Stephens et al., 2016; Tsegai et al., 2013), and in the talus and distal tibia (Tsegai et al., 2017). During ontogeny, regional variability of several trabecular parameters increases in the human proximal femur, reflecting the development of complex regional trabecular patterns characteristic of the adult proximal femur (Ryan & Krovitz, 2006). In contrast, DA becomes increasingly homogeneous with age throughout the human distal tibia as a more stable bipedal gait is acquired (Raichlen et al., 2015). This demonstrates that the way in which trabecular structure changes during ontogeny differs between anatomical sites, reflecting the loading environment of each joint during ontogeny. Thus, it is important to explore these changes in different skeletal sites. For example, in contrast to the limb bones discussed here, an adult-like trabecular pattern is present from birth in the human ilium (Abel & Macho, 2011; Cunningham & Black, 2009a, 2009b).

The distribution patterns of BV/TV during ontogeny in the chimpanzee proximal humerus, distal tibia and proximal femur demonstrate that the sub-epiphyseal distribution of BV/TV differs at different ontogenetic stages. In all elements, the trabecular structure is initially homogenous, with increasing regional differences in BV/TV with age, reaching more adult-like distributions in juvenile or adolescent stages. The changes in BV/TV distribution that occur during ontogeny may be due to biomechanical changes caused either by locomotion and/or by the general process of growth, and the changing morphology of the external joint. During growth, trabecular bone develops beneath the epiphyseal plate via the process of endochondral ossification (Carter &

Beaupré, 2000; Martin et al., 2015; Scheuer & Black, 2000), where columnar rows of chondrocytes form cartilage which is subsequently ossified to form primary trabeculae. These are then remodelled to form secondary trabeculae. This process of endochondral ossification, by which trabecular bone is formed, could explain the initially homogeneous trabecular bone distribution in all three elements (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Ryan et al., 2017). However, BV/TV is not entirely homogeneous and all elements have regions of higher BV/TV, even before the onset of locomotion. These early appearing regions of differentiated trabecular structure could be attributed to joint morphology and non-locomotor loading both pre- and postnatally, or to genetically predetermined patterns of deposition (Abel & Macho, 2011; Cunningham & Black, 2009a, 2009b).

During subsequent growth, trabecular bone is deposited at the metaphyseal surface, likely partly in response to the local mechanical environment. As experimental studies are often performed on immature individuals (e.g. Barak et al., 2011), it is unclear whether trabecular bone orientation is remodelled after its initial deposition, or whether adult-like morphology is entirely a result of strain during deposition (Bertram & Swartz, 1991). Although we are unable to address this question using a quantitative approach, due to the difficulty in selecting homologous regions in an ontogenetic series, qualitative comparison of both mid-slices and BV/TV maps appear to show changes in BV/TV in regions deeper to the articular surface across the ontogenetic series. As growth is not only characterised by increased bone length, via deposition at the epiphyseal plate, but also by appositional growth leading to increasing anteroposterior and mediolateral width (Carter & Beaupré, 2000; Martin et al., 2015; Scheuer & Black, 2000), it would be expected that BV/TV in regions deep to the epiphyseal plate would be characterised by reduction in BV/TV, i.e. due to increases in TV with an unchanged BV. This is not the case, and BV/TV does increase deeper to the articular surface, most notably in the proximal femur, and does not appear to reduce over time with increasing size. Further research is required to compare trabecular bone distributions in ontogenetic series of both metaphyseal and epiphyseal regions in extant apes, in order to identify potential signals of locomotor changes during ontogeny. These results suggest such analyses would be useful for understanding adult trabecular structure, and potentially the trabecular morphology of immature fossil hominoids.

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Figure Captions

Figure 1. Processing stages for analysis of trabecular bone structure, shown here in the proximal humerus. a) Mid-plane images of the segmented bone (left) and the masked image (right), where trabecular bone, cortical bone, and internal region of the bone are assigned different grey values. b) Trabecular variables are quantified in a large ROI for adult individuals and a small spherical VOI for immature individuals. c) Trabecular bone is quantified using a spherical VOI at each node of a 3D grid (left), using a mesh of the internal region of the bone (middle), the values are interpolated to produce a 3D morphometric map of BV/TV (right).

Figure 2. Definition of cubic ROIs in a) the proximal humerus, b) the proximal femur and c) the distal tibia of adult chimpanzees. Definition of 5mm spherical VOIs in d) the proximal humerus, e) the proximal femur and f) the distal tibia of immature chimpanzees.

Figure 3. Trabecular bone quantified from ROIs in the humerus, femur and tibia of adult chimpanzees.

Figure 4. Sex specific trabecular bone structure quantified from ROIs in the humerus, femur and tibia of adult chimpanzees. Females are shown in red, males in blue, and individuals of unknown sex in green.

Figure 5. Trabecular bone structure in the humerus compared to the femur and tibia of adult chimpanzees.

Figure 6. The relationship between total joint volume and trabecular bone structure in the humerus, femur and tibia of adult chimpanzees.

Figure 7. Trabecular bone ontogeny in the proximal humerus, proximal femur and distal tibia in immature chimpanzees. Individuals of unknown age are shown in the shaded area to the right of each plot.

Figure 8. Ratios of femoral to humeral (left), tibial to humeral (centre) and femoral to tibial (right) trabecular structure in each age category. B: Baby; I: Infant; J: Juvenile; A: Adolescent.

Figure 9. Ontogenetic changes in BV/TV of the chimpanzee proximal humerus. Shown in the mid-coronal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0-60% BV/TV (center) and scaled to the range of each specimen (right), with a 5mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison.

Figure 10. Ontogenetic changes in BV/TV of the chimpanzee proximal femur. Shown in the mid-coronal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0-60% BV/TV (center) and scaled to the range of each specimen (right), with a 5mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison.

Figure 11. Ontogenetic changes in BV/TV of the chimpanzee distal tibia. Shown in the mid-sagittal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0-60% BV/TV (center) and scaled to the range of each specimen (right), with a 5mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison.

Table 1. Study sample

Age Group	Age	Number of individuals (M/F/U)	Number per Element			Resolution	Relative resolution [*]
			Humerus	Femur	Tibia		
Adult	Fused epiphyses	8/12/1	19	21	20	0.035-0.045	4.25-8.10
Adolescent	10 -	2/2/0	4	4	3	0.025-0.035	5.03-11.85
Juvenile	5-10yrs	6/2/1	9	7	6	0.015-0.030	4.41-12.54
Infant	5m-4yrs	2/1/3	6	6	5	0.014-0.020	7.99-13.07
Baby	0-5m	1/0/1	2	2	2	0.014-0.015	6.48-9.82

[*] Relative resolution is calculated as mean trabecular thickness divided by the resolution. This demonstrates how many pixels represent trabeculae of mean thickness.

Table 2. Trabecular variables from ROIs in adult individuals. Mean and standard deviation, in parentheses, for each trabecular variable in the proximal humerus, proximal femur and distal tibia. Results of Kruskal-Wallace test for significant differences between elements, with significant differences in bold.

	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (mm^{-1})	DA
Humerus	24.82 (2.91)	0.22 (0.02)	0.69 (0.11)	1.12 (0.12)	0.16 (0.02)
Femur	37.56 (5.13)	0.28 (0.03)	0.56 (0.07)	1.20 (0.12)	0.09 (0.06)
Tibia	25.26 (3.22)	0.23 (0.02)	0.73 (0.14)	1.07 (0.15)	0.35 (0.05)
p-value	**<0.01**	<**0.01**	<**0.01**	**0.01**	<**0.01**

Table 3. Results of post-hoc Nemenyi test to identify significant differences between elements in adult chimpanzees, with significant differences shown in bold.

	BV/TV	Tb.Th	Tb.Sp	Tb.N	DA
Humerus-Femur	**<0.01**	**<0.01**	**<0.01**	0.12	**0.03**
Humerus-Tibia	0.94	0.45	0.75	0.61	**<0.01**
Femur-Tibia	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Table 4. Spearman’s correlation test between elements in adult chimpanzees, with a post-hoc Bonferroni correction and significant correlations in bold.

	BV/TV		Tb.Th		Tb.Sp		Tb.N		DA	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p
Humerus-Femur	0.64	**0.047**	0.67	**0.023**	0.81	**<0.001**	0.75	**0.003**	-0.43	1.000
Humerus-Tibia	0.49	0.569	0.26	1.000	0.64	0.060	0.66	**0.044**	-0.04	1.000
Femur-Tibia	0.41	1.000	0.45	0.726	0.59	0.091	0.52	0.274	0.33	1.000

Table 5. Spearman's correlation between trabecular variables within elements in adult chimpanzees, with a post-hoc Bonferroni correction and significant correlations in bold.

		BV/TV		Tb.Th		Tb.Sp		Tb.N	
Humerus		*r*	p	*r*	p	*r*	p	*r*	p
	Tb.Th	0.38	1.000						
	Tb.Sp	-0.64	0.104	0.12	1.000				
	Tb.N	0.54	0.547	-0.31	1.000	-0.97	**<0.001**		
	DA	0.29	1.000	0.25	1.000	0.29	1.000	-0.29	1.000
Femur	Tb.Th	0.75	**0.002**						
	Tb.Sp	-0.29	1.000	0.17	1.000				
	Tb.N	-0.03	1.000	-0.52	0.502	-0.91	**<0.001**		
	DA	-0.69	**0.015**	-0.91	**<0.001**	-0.20	1.000	0.49	0.728
Tibia	Tb.Th	0.68	**0.029**						
	Tb.Sp	-0.52	0.598	0.02	1.000				
	Tb.N	0.42	1.000	-0.18	1.000	-0.97	**<0.001**		
	DA	0.04	1.000	-0.04	1.000	-0.31	1.000	0.34	1.000

Table 6. Spearman's correlation between size proxies and trabecular structure in adult chimpanzees, with a post-hoc Bonferroni correction.

Element	Size proxy	BV/TV		Tb.Th		Tb.Sp		Tb.N		DA	
		r	p	*r*	p	*r*	p	*r*	p	*r*	p
Humerus	FHH	-0.22	1.00	0.24	1.00	0.33	1.00	-0.35	1.00	-0.15	1.00
	TJV	-0.30	1.00	0.24	1.00	0.35	1.00	-0.36	1.00	-0.15	1.00
Femur	FHH	-0.20	1.00	-0.06	1.00	0.40	1.00	-0.23	1.00	0.05	1.00
	TJV	-0.26	1.00	0.01	1.00	0.42	1.00	-0.27	1.00	-0.11	1.00
Tibia	FHH	-0.41	1.00	-0.49	0.81	0.46	1.00	-0.34	1.00	-0.48	0.97
	TJV	0.00	1.00	-0.12	1.00	0.21	1.00	-0.09	1.00	-0.29	1.00

FHH: Femoral head height

TJV: Total joint volume

Table 7. Trabecular variables from VOIs in immature chimpanzees. Age group mean and standard deviation, in parentheses, for each trabecular variable in the proximal humerus, proximal femur and distal tibia, with results of Kruskal-Wallace tests for differences between age groups.

		BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (mm^{-1})	DA
Humerus						
	Baby	18.87 (3.50)	0.13 (0.02)	0.39 (0.02)	1.94 (0.15)	0.48 (0.02)
	Infant	23.53 (4.52)	0.15 (0.02)	0.45 (0.08)	1.70 (0.26)	0.46 (0.06)
	Juvenile	25.84 (4.62)	0.16 (0.01)	0.47 (0.07)	1.60 (0.18)	0.40 (0.06)
	Adolescent	26.89 (9.74)	0.18 (0.04)	0.47 (0.08)	1.55 (0.15)	0.39 (0.05)
	Significance	0.361	0.087	0.608	0.225	0.112
Femur						
	Baby	20.98 (2.16)	0.14 (0.00)	0.39 (0.05)	1.89 (0.17)	0.43 (0.10)
	Infant	24.73 (7.66)	0.17 (0.03)	0.53 (0.05)	1.41 (0.06)	0.35 (0.04)
	Juvenile	28.22 (7.82)	0.19 (0.03)	0.53 (0.09)	1.41 (0.15)	0.33 (0.05)
	Adolescent	41.43 (4.98)	0.27 (0.03)	0.51 (0.03)	1.29 (0.07)	0.37 (0.04)
	Significance	**0.041**	**0.005**	0.151	**0.031**	0.141
Tibia						
	Baby	9.33 (0.57)	0.10 (0.00)	0.53 (0.08)	1.61 (0.23)	0.55 (0.08)
	Infant	12.99 (5.40)	0.14 (0.02)	0.75 (0.25)	1.18 (0.25)	0.38 (0.08)
	Juvenile	15.93 (6.14)	0.14 (0.01)	0.72 (0.43)	1.30 (0.36)	0.41 (0.07)
	Adolescent	20.53 (5.82)	0.16 (0.02)	0.56 (0.06)	1.38 (0.09)	0.49 (0.03)
	Significance	0.177	0.070	0.227	0.109	0.078

Table 8. Results of post-hoc Nemenyi test between age groups in immature chimpanzees with significant differences in bold.

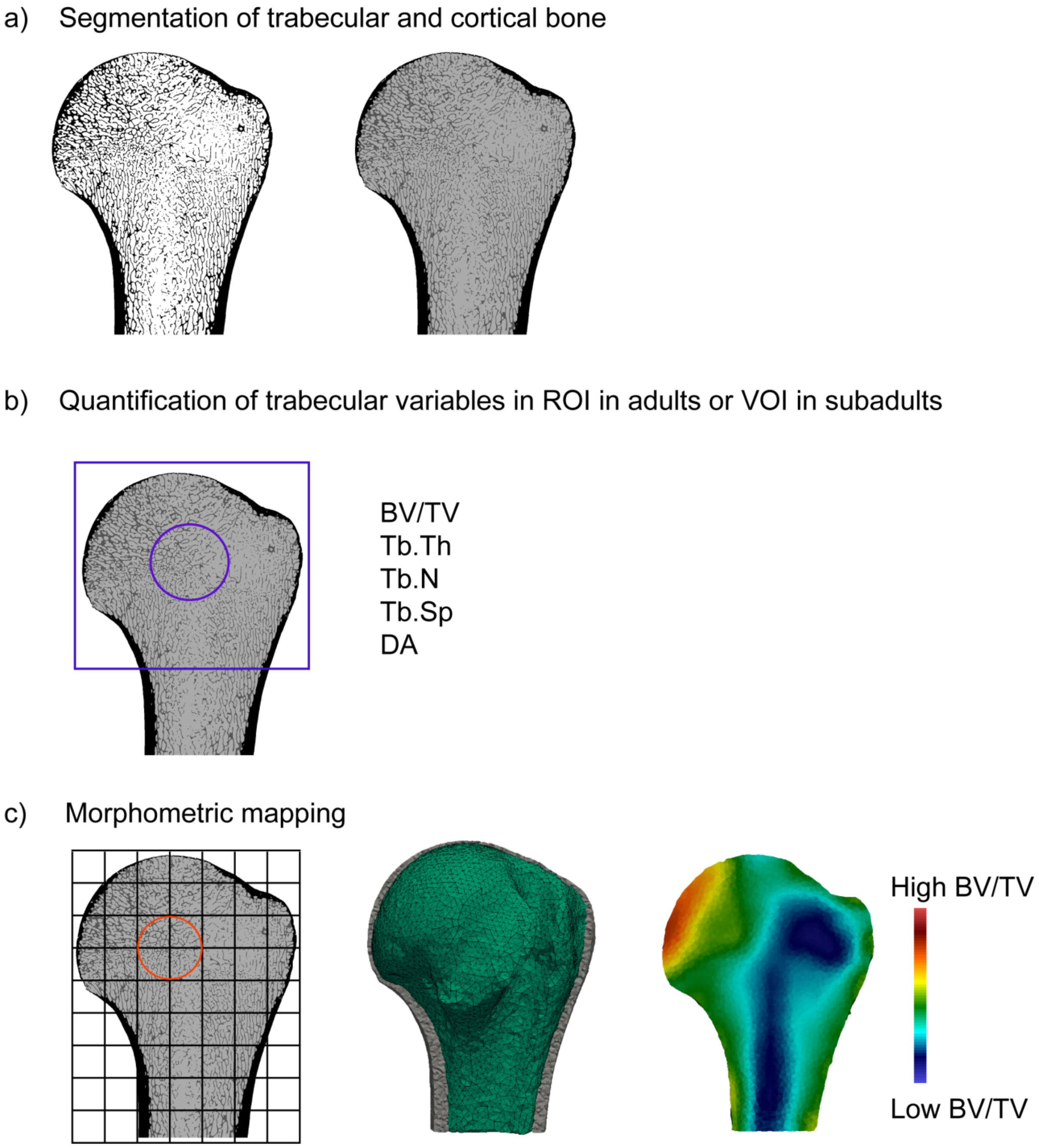
Element	Variable		Baby	Infant	Juvenile
Femur	BV/TV	Infant	0.972		
		Juvenile	0.804	0.911	
		Adolescent	0.096	0.053	0.175
	Tb.Th	Infant	0.559		
		Juvenile	0.208	0.810	
		Adolescent	**0.006**	**0.030**	0.171
	Tb.N	Infant	0.321		
		Juvenile	0.264	0.999	
		Adolescent	**0.018**	0.290	0.318

Table 9. Results of OLS regression of age against trabecular variables with Bonferroni post-hoc correction in immature chimpanzees with signficant p-values in bold.

	Variable	Intercept	Slope	R^2	p-value
Humerus	BV/TV	23.24	0.31	-0.01	1.000
	Tb.Th	0.15	0.00	0.17	0.884
	Tb.Sp	0.43	0.00	-0.04	1.000
	Tb.N	1.77	-0.02	0.06	1.000
	DA	0.45	-0.01	0.11	1.000
Femur	BV/TV	19.50	1.65	0.67	**0.002**
	Tb.Th	0.14	0.01	0.85	**<0.001**
	Tb.Sp	0.50	0.00	-0.05	1.000
	Tb.N	1.61	-0.03	0.34	0.203
	DA	0.35	0.00	-0.08	1.000
Tibia	BV/TV	9.26	0.99	0.63	**0.011**
	Tb.Th	0.11	0.00	0.58	**0.023**
	Tb.Sp	0.78	-0.02	0.04	1.000
	Tb.N	1.28	0.01	-0.05	1.000
	DA	0.44	0.00	-0.09	1.000

Table 10. Results of Kruskal-Wallace tests to compare ratios of trabecular variables between age groups in immature chimpanzees.

Ratio	Variable	p-value
Femur/Humerus	BV/TV	0.245
	Tb.Th	0.085
	Tb.Sp	0.501
	Tb.N	0.210
	DA	0.139
Femur/Tibia	BV/TV	0.745
	Tb.Th	0.156
	Tb.Sp	0.555
	Tb.N	0.183
	DA	0.459
Tibia/Humerus	BV/TV	0.433
	Tb.Th	0.817
	Tb.Sp	0.865
	Tb.N	0.830
	DA	0.210


a) Segmentation of trabecular and cortical bone

b) Quantification of trabecular variables in ROI in adults or VOI in subadults

c) Morphometric mapping

Figure 1. Processing stages for analysis of trabecular bone structure, shown here in the proximal humerus. a) Mid-plane images of the segmented bone (left) and the masked image (right), where trabecular bone, cortical bone, and internal region of the bone are assigned different grey values. b) Trabecular variables are quantified in a large ROI for adult individuals and a small spherical VOI for immature individuals. c) Trabecular bone is quantified using a spherical VOI at each node of a 3D grid (left), using a mesh of the internal region of the bone (middle), the values are interpolated to produce a 3D morphometric map of BV/TV (right).

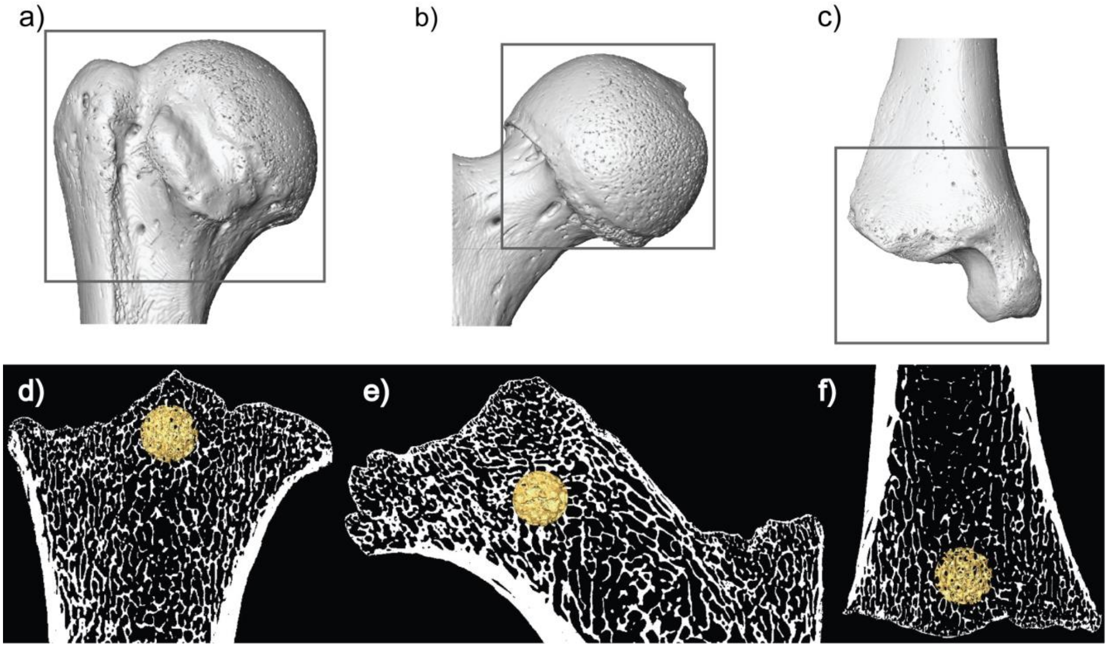


Figure 2. Definition of cubic ROIs in a) the proximal humerus, b) the proximal femur and c) the distal tibia of adult chimpanzees. Definition of 5mm spherical VOIs in d) the proximal humerus, e) the proximal femur and f) the distal tibia of immature chimpanzees.

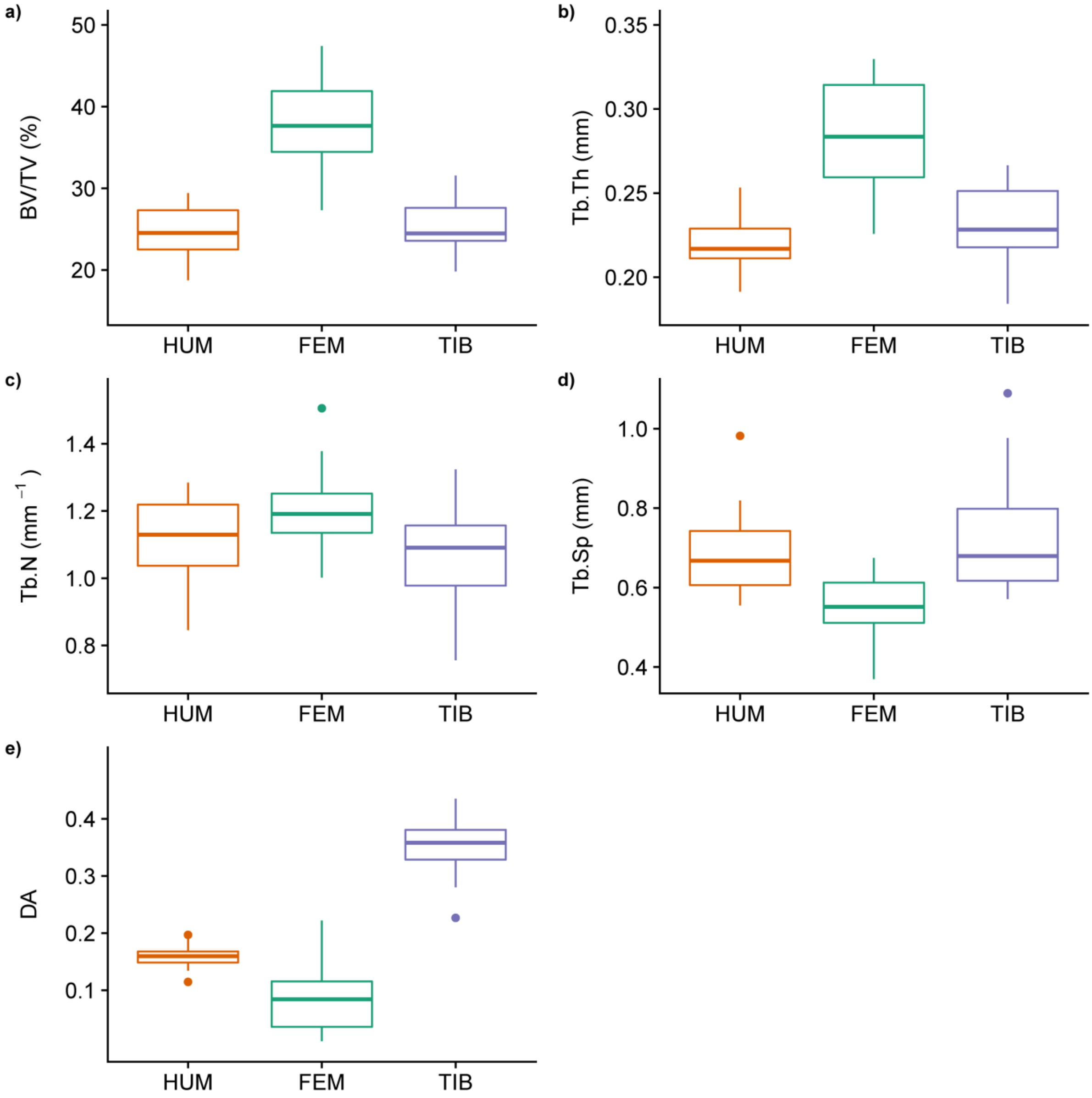


Figure 3. Trabecular bone quantified from ROIs in the humerus, femur and tibia of adult chimpanzees.

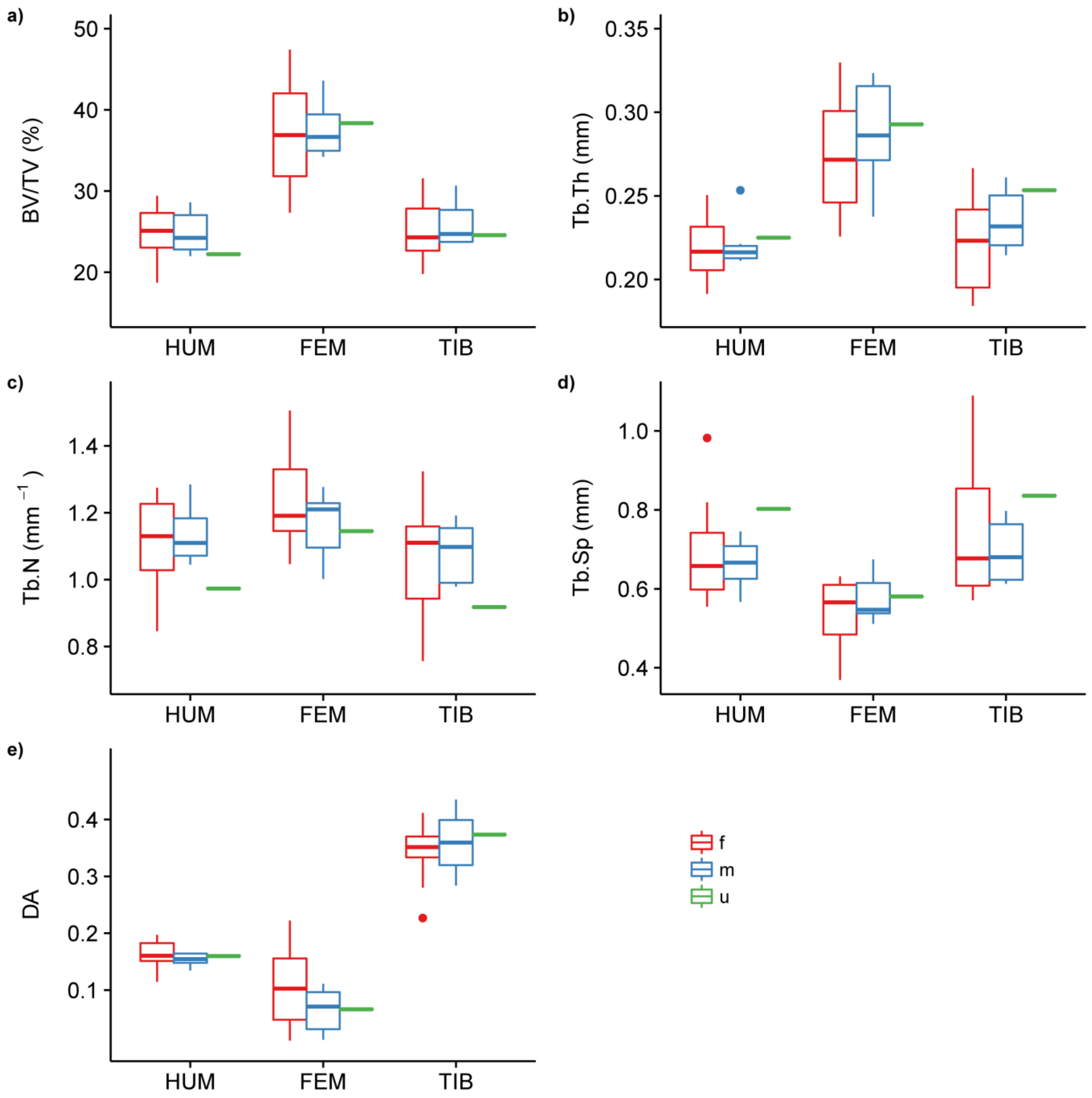


Figure 4. Sex specific trabecular bone structure quantified from ROIs in the humerus, femur and tibia of adult chimpanzees. Females are shown in red, males in blue, and individuals of unknown sex in green.

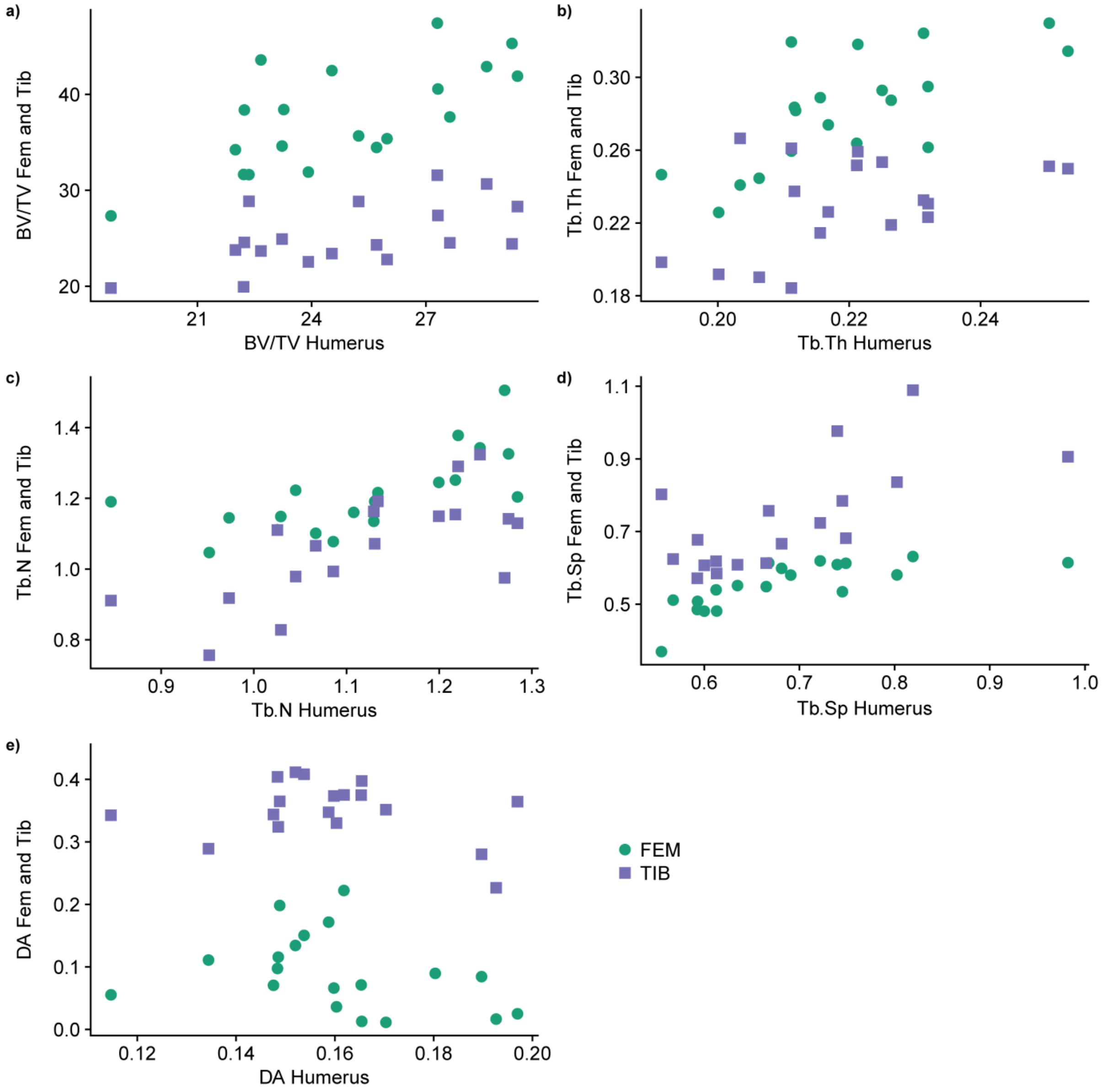


Figure 5. Trabecular bone structure in the humerus compared to the femur and tibia of adult chimpanzees.

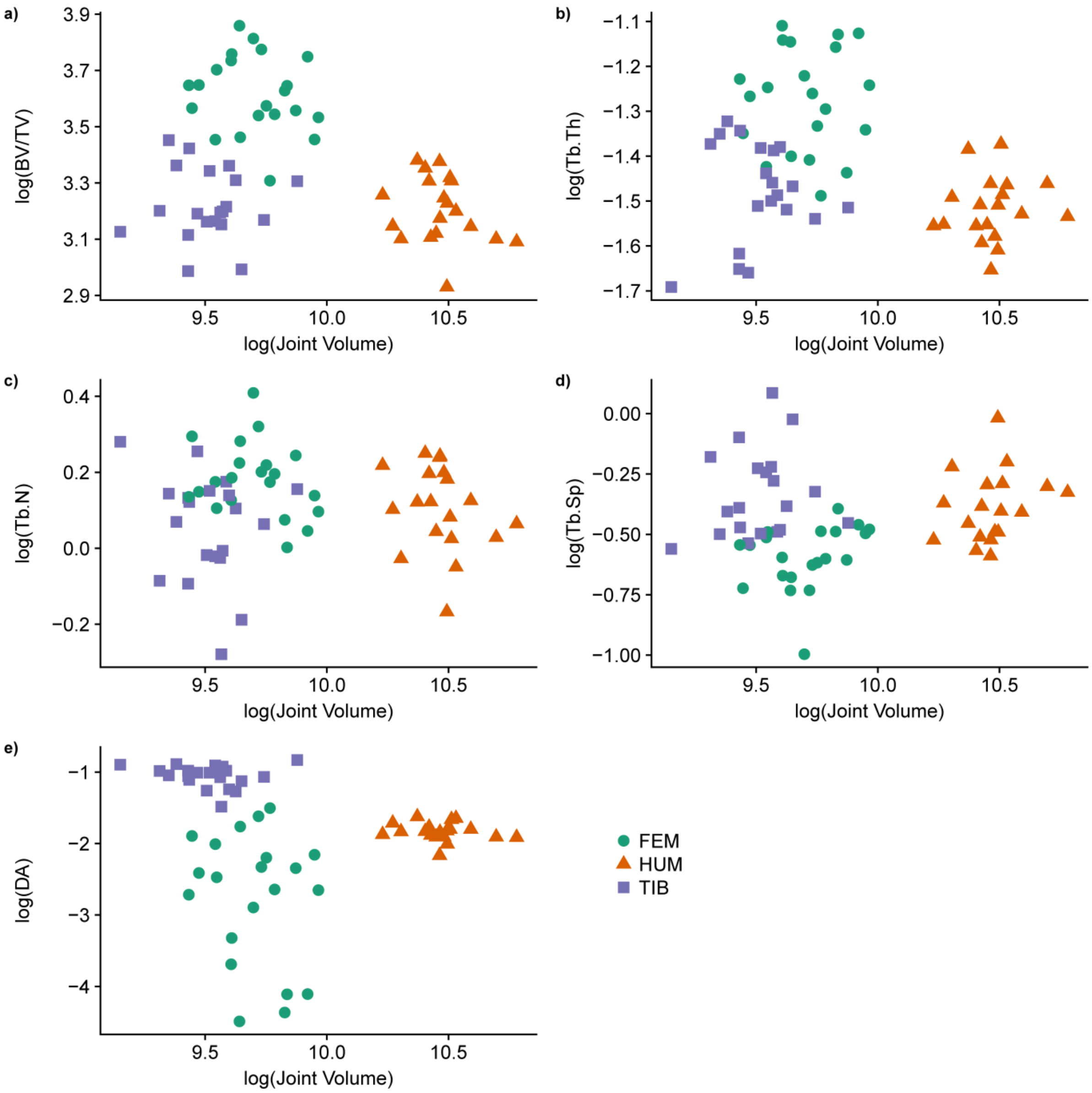


Figure 6. The relationship between total joint volume and trabecular bone structure in the humerus, femur and tibia of adult chimpanzees.

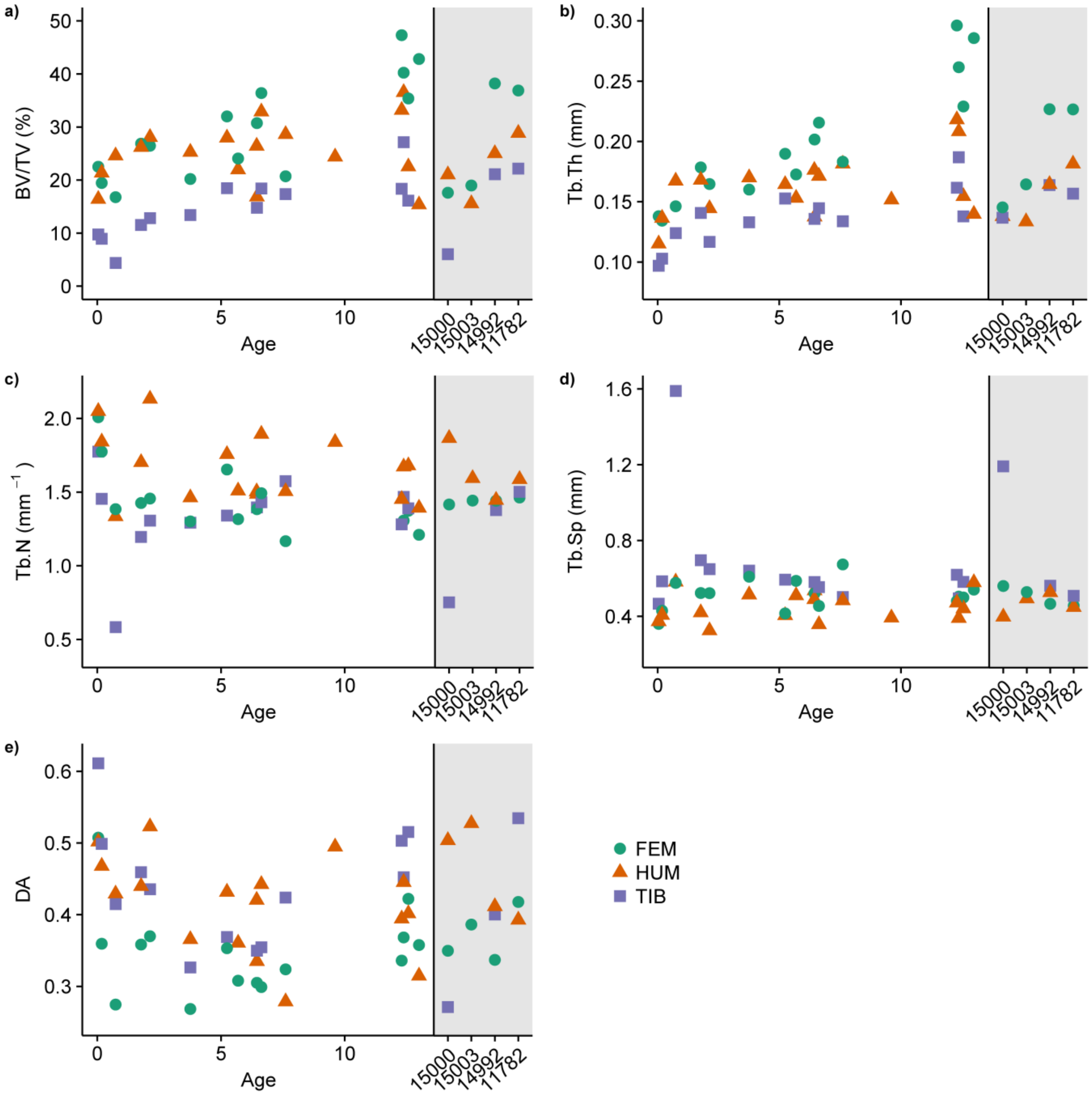


Figure 7. Trabecular bone ontogeny in the proximal humerus, proximal femur and distal tibia in immature chimpanzees. Individuals of unknown age are shown in the shaded area to the right of each plot.

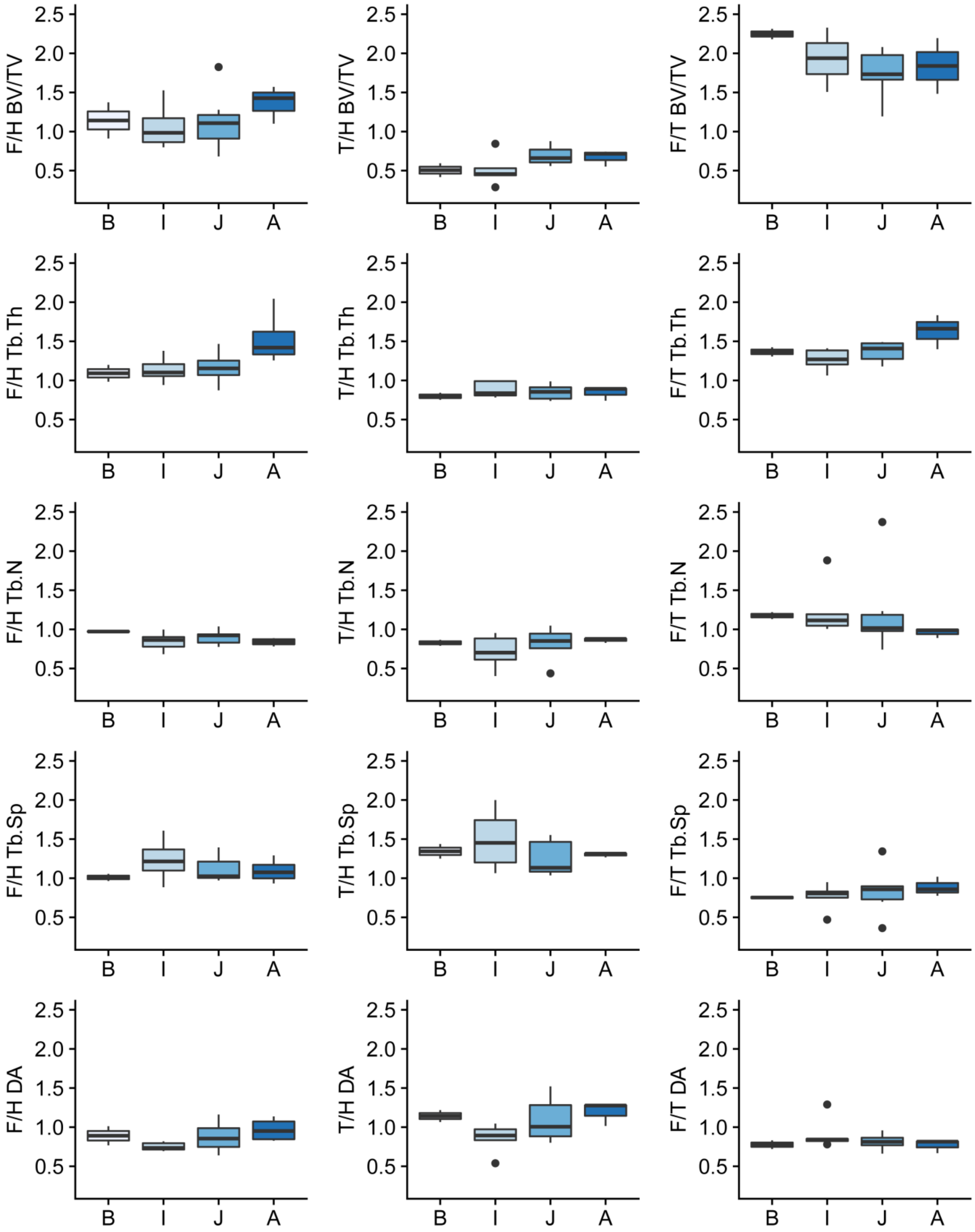


Figure 8. Ratios of femoral to humeral (left), tibial to humeral (centre) and femoral to tibial (right) trabecular structure in each age category. B: Baby; I: Infant; J: Juvenile; A: Adolescent.

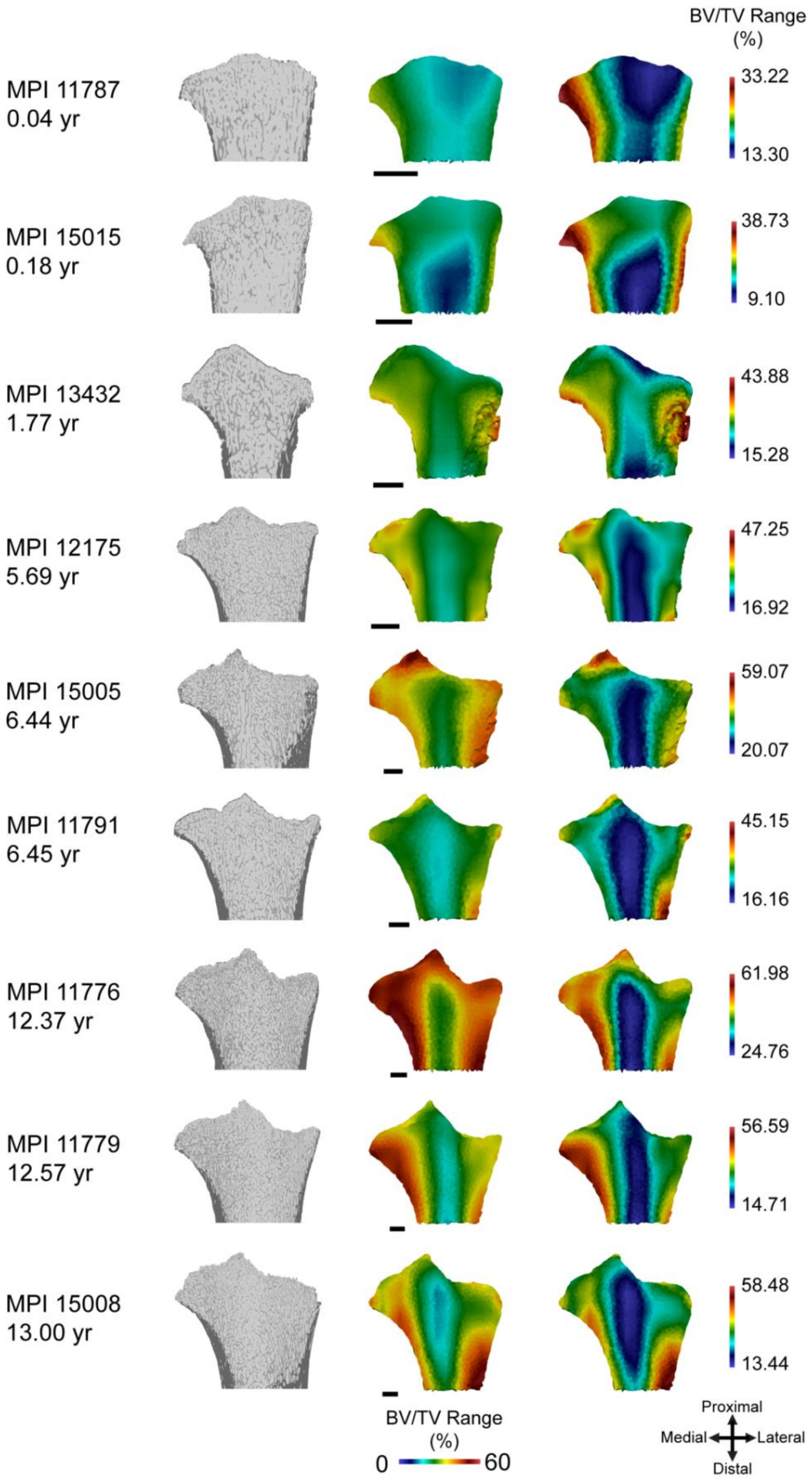


Figure 9. Ontogenetic changes in BV/TV of the chimpanzee proximal humerus. Shown in the mid-coronal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0-60% BV/TV (center) and scaled to the range of each specimen (right), with a 5mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison.

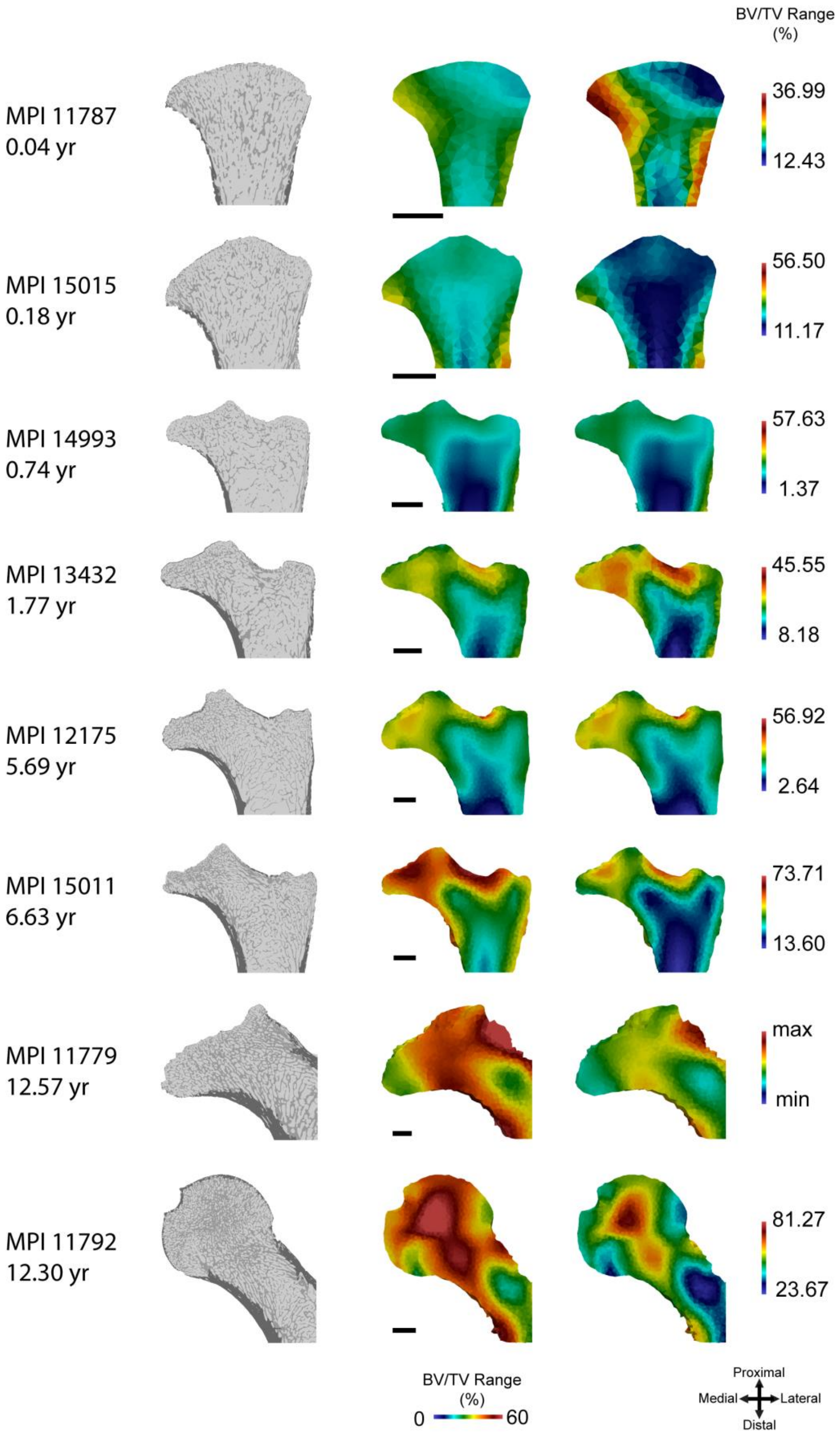


Figure 10. Ontogenetic changes in BV/TV of the chimpanzee proximal femur. Shown in the mid-coronal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0-60% BV/TV (center) and scaled to the range of each specimen (right), with a 5mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison.

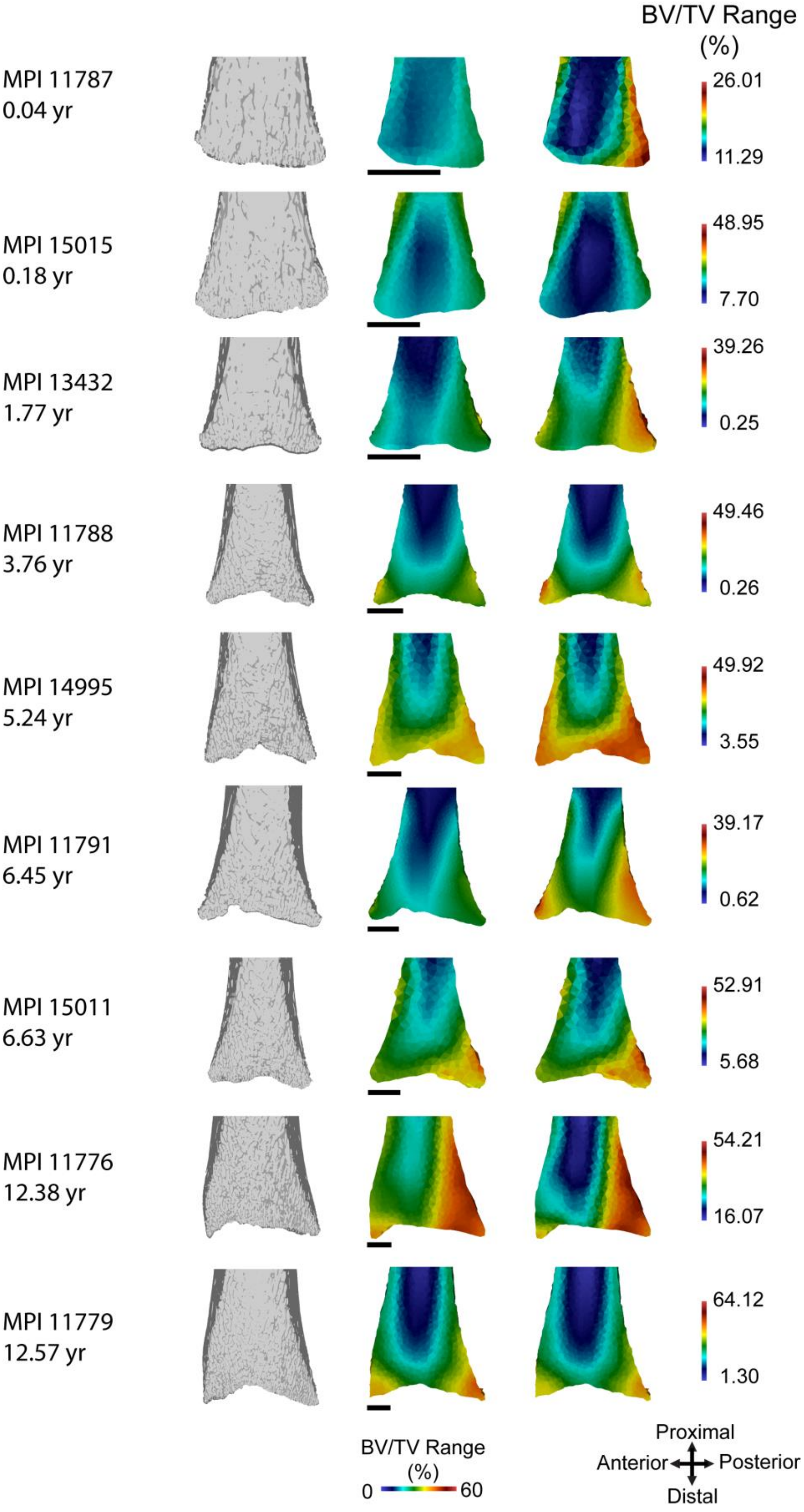


Figure 11. Ontogenetic changes in BV/TV of the chimpanzee distal tibia. Shown in the mid-sagittal plane, segmented trabecular and cortical bone (left), trabecular structure scaled from 0-60% BV/TV (center) and scaled to the range of each specimen (right), with a 5mm scale bar. The age of each individual is shown in years and specimens from the right side are flipped for comparison.