



# An ontogenetic framework linking locomotion and trabecular bone architecture with applications for reconstructing hominin life history

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## ABSTRACT

The ontogeny of bipedal walking is considered uniquely challenging, due in part to the balance requirements of single limb support. Thus, locomotor development in humans and our bipedal ancestors may track developmental milestones including the maturation of the neuromuscular control system. Here, we examined the ontogeny of locomotor mechanics in children aged 1–8, and bone growth and development in an age-matched skeletal sample to identify bony markers of locomotor development. We show that step-to-step variation in mediolateral tibia angle relative to the vertical decreases with age, an indication that older children increase stability. Analyses of trabecular bone architecture in the distal tibia of an age-matched skeletal sample (the Norris Farms #36 archaeological skeletal collection) show a bony signal of this shift in locomotor stability. Using a grid of eleven cubic volumes of interest (VOI) in the distal metaphysis of each tibia, we show that the degree of anisotropy (DA) of trabecular struts changes with age. Intra-individual variation in DA across these VOIs is generally high at young ages, likely reflecting variation in loading due to kinematic instability. With increasing age, mean DA converges on higher values and becomes less variable across the distal tibia. We believe the ontogeny of distal tibia trabecular architecture reflects the development of locomotor stability in bipeds. We suggest this novel bony marker of development may be used to assess the relationship between locomotor development and other life history milestones in fossil hominins.

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## Introduction

Bipedalism is a hallmark trait for the human lineage and its evolution generated changes in the hominin skeleton from the skull to the feet (Robinson, 1972; Stern and Susman, 1983). While studies of comparative biomechanics and functional anatomy have yielded key insights into how and why bipedalism may have evolved (Stern and Susman, 1983; Wheeler, 1984, 1991; Chaplin et al., 1994; Hunt, 1994; Sockol et al., 2007; Thorpe et al., 2007), most work has focused exclusively on adult subjects and fossil specimens. However, the study of locomotor development can provide a unique

window into the evolution of morphology and behavior across species that is obscured when considering the adult phenotype alone (Inouye, 1994; Raichlen, 2005a, 2005b, 2006; Shapiro and Raichlen, 2005; Ryan and Krovitz, 2006; Shapiro and Raichlen, 2006; Gosman and Ketcham, 2009; Zollikofer and Ponce de León, 2010; Gosman et al., 2013; Harmon, 2013; Shapiro et al., 2014).

For example, previous studies used evidence of load-induced ontogenetic changes in bone morphology from living taxa to confirm the presence of locomotor behaviors such as climbing or terrestrial bipedalism in the fossil record (e.g., phalangeal curvature and bicondylar angle; Duncan et al., 1994; Tardieu and Trinkaus, 1994; Tardieu and Damsin, 1997; Tardieu, 1999, 2010; Shefelbine et al., 2002; Richmond, 2007). Due to the rising number of fossil elements attributed to juvenile hominins (e.g., Duarte et al., 1999; Alemseged et al., 2006; Cowgill et al., 2007), researchers may

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now have the unique ability to explore the *pattern* of locomotor development in early hominins, providing a novel perspective on the evolution of bipedal walking and hominin life history patterns. This concept of exploring locomotion itself as a life history characteristic was first introduced by Zihlman (1992), and we suggest this intriguing idea can be expanded by carefully examining bony development in the fossil record. To compare patterns of development across taxa, however, we must first determine how skeletal material reflects the ontogeny of human bipedalism. The goal of this study is to find skeletal markers of bipedal locomotor development that we can apply to fossil hominins in future studies.

One salient aspect of locomotion in bipeds that may have a major impact on the characteristics of skeletal loading during development (e.g., the magnitude and orientation of ground reaction forces), and consequently on bone growth, is the inherent instability of walking on two limbs (Sutherland et al., 1980; Beck et al., 1981; Bril and Brenière, 1992; Adolph, 2003; Adolph et al., 2003; Khammari and Poyil, 2013). Early in locomotor development, when this instability is most pronounced, walking is irregular with each step differing from the last as individuals work to maintain balance with an immature muscular control system (Sutherland et al., 1980; Adolph, 2003; Khammari and Poyil, 2013). As neuromuscular control improves, variation from step to step is reduced, and, therefore, forces become more predictable (Sutherland et al., 1980; Forssberg, 1985; White et al., 1999; Adolph et al., 2003). Since both cortical bone and trabecular bone respond to changes in loading patterns through remodeling processes (Wolff, 1982; Ruff and Hayes, 1982; Pontzer et al., 2006; Ruff et al., 2006; Carlson and Judex, 2007; Barak et al., 2011), the response of bone to irregular loading patterns early on, and more predictable loading patterns during late childhood, may provide a unique morphological indicator of the development of mature and stable gaits.

Here we examine trabecular bone in the distal tibia as a potential marker of bipedal maturation in humans. Trabecular bone architecture is highly responsive to changes in loading orientations (Ryan and Ketcham, 2002b, 2005; Pontzer et al., 2006; Barak et al., 2011; Wallace et al., 2013) and the distal tibia represents the fulcrum for the body over the fixed foot during single limb support. Additionally, recent work shows that trabecular architecture in the tibia differentiates loading patterns in bipedalism and quadrupedalism (humans vs. chimpanzees), and these gait-related differences are detectable in the hominin fossil record (Barak et al., 2013). Thus, we predict changes in stability and balance during human growth are reflected in distal tibia trabecular bone architecture. If supported, then researchers would have a powerful method to capture patterns of locomotor development and maturation using fossil tibiae in hominin taxa.

#### *Linking locomotor and morphological maturation*

As described earlier, bone growth may capture the shift from unstable to stable locomotion, driven by a combination of neuromuscular maturation and changes in muscle strength (McGraw, 1935, 1943; Thelen, 1984; Brenière and Bril, 1987; Assaiante et al., 1993). In order to predict how bone will respond to locomotor changes, however, we must first define the features of gait in new walkers that reflect instability and immaturity. One key marker of instability is step-to-step variation in locomotor parameters. For example, intra-individual coefficients of variation (CV) for spatio-temporal parameters (calculated over multiple steps within an individual) are generally higher in younger compared to older walkers (Lasko-McCarthy et al., 1990). Standard deviations (SD) of joint flexion/extension angles (hip, knee, and ankle) also decrease from new walkers to older children (Lasko-McCarthy et al., 1990).

Mediolateral trunk oscillations are significantly higher in new walkers as well, reflecting variation in joint angles from the ankle through the pelvis (Bril and Brenière, 1992; Assaiante et al., 1993; Yaguramaki and Kimura, 2002; Ivanenko et al., 2005). Supporting new walkers (holding their hands or otherwise providing postural support) reduces CVs for spatio-temporal parameters and SDs of joint angles, suggesting step-to-step variation in these parameters is due to insufficient balance control (Lasko-McCarthy et al., 1990; Ivanenko et al., 2005). In addition to variation during a single set of walking trials, measurements of joint angles and spatio-temporal variables show lower day-to-day repeatability in younger children compared to older children (Gorton et al., 1997; Loooper et al., 2006). Although more challenging to collect, ground reaction force (GRF) data from children also show greater variation than adults (Cowgill et al., 2010). Cowgill et al. (2010) show that GRFs in all three directions (vertical, fore-aft, mediolateral) are more variable at young ages and that peak magnitudes of mediolateral forces are highest at the youngest ages, possibly reflecting mediolateral instability in early walkers.

Shifts from unstable to stable locomotion (i.e., variable to less variable segment angles) may leave markers on bone during growth and development. For example, epiphyseal morphology, cortical bone morphology, and bone strength are all influenced by ontogenetic changes in loading patterns (Carter, 1987; Carter et al., 1989; Shefelbine et al., 2002; Ruff, 2003a, 2003b), and the shift from highly variable to highly predictable joint angles should lead to a significant change in load orientations throughout growth and development. While the response of cortical bone to loads during growth is well documented, less is known about ontogenetic changes in trabecular bone morphology in the hindlimb of growing humans (see Ryan and Krovitz, 2006; Gosman and Ketcham, 2009). We suggest that trabecular morphology may provide a sensitive marker of changes in locomotor stability, since the architecture of trabecular struts may hold more detailed information regarding both the magnitude and orientation of loading patterns during development (Pontzer et al., 2006; Barak et al., 2011).

One of the primary functions of trabecular bone is to transmit loads generated during activity through struts oriented to best resist these loads. Researchers have suggested a relationship between limb usage, inferred loading patterns (orientations), and trabecular architecture in extant adult primates (Ward and Sussman, 1979; Pauwels et al., 1980; Oxnard and Yang, 1981; Oxnard, 1993; Rafferty and Ruff, 1994; Oxnard, 1997; Rafferty, 1998; Fajardo and Müller, 2001; MacLatchy and Müller, 2002; Ryan and Ketcham, 2002b, 2005; Richmond et al., 2004; Fajardo et al., 2007), fossil primates (Galichon and Thackeray, 1997; Macchiarelli et al., 1999; Rook et al., 1999; Ryan and Ketcham, 2002a; Scherf et al., 2013; Su et al., 2013), archaeological populations (Mielke et al., 1972; Vogel et al., 1990; Brickley and Howell, 1999; Agarwal et al., 2004), a diversity of extant mammal species (Kummer, 1959), extant and extinct equids (Thomason, 1985a, 1985b), and experimentally in guinea fowl (Pontzer et al., 2006), mice (Wallace et al., 2013), and sheep (Barak et al., 2011).

We also note that there is some evidence that loading patterns are not always reflected in trabecular architecture (e.g., Carlson et al., 2008; Scherf, 2008; Ryan and Walker, 2010; DeSilva and Devlin, 2012). Some of these researchers suggest that the trabecular response to loading patterns depends on the bone (i.e., location in the body). For example, Carlson et al. (2008) suggested joints that are more constrained in their range of motion may show a lower trabecular response in DA to loading patterns than joints that have higher degrees of freedom (see also Wallace et al., 2013). The lack of consensus among studies suggests we must use caution when interpreting trabecular bone, but also argues for more direct studies linking locomotor mechanics and bony architecture. Thus, despite

studies suggesting a lack of straightforward correspondence between loading patterns and morphology, the majority of studies described above support our hypothesis that trabecular architecture may provide a unique marker of the changing loading patterns associated with the development of mature gait in humans.

Recent work highlights the ability of trabecular bone to track ontogenetic signals. Two previous studies have analyzed human trabecular bone development in the proximal humerus and proximal femur (Ryan and Krovit, 2006; Ryan et al., 2007) and proximal tibia (Gosman, 2007; Gosman and Ketcham, 2009) for growth series from two prehistoric populations. In the proximal femur, changes in trabecular number, thickness, and degree of anisotropy suggest a gradual change from varying loading patterns to the more stereotyped morphology associated with bipedal walking (Ryan and Krovit, 2006). The proximal tibia also sees a change in trabecular number and thickness, along with more highly oriented struts, with age that seems to track more predictable loading patterns associated with locomotor maturation (Gosman and Ketcham, 2009). These ontogenetic patterns from trabecular bone in postcranial long bones contrast with results from the fetal human ilium which suggest that trabeculae display patterns typically associated with locomotor loading pre-natally (Abel and Macho, 2011). Studies of trabecular bone growth and development in other mammals, however, have also demonstrated gradual increases in bone volume fraction, anisotropy, trabecular number, and trabecular thickness with age (Nafei et al., 2000a, 2000b; Tanck et al., 2001; Nuzzo et al., 2003; Mulder et al., 2005, 2007). Of particular relevance to the current study, an analysis of trabecular bone development in the ulnar coronoid process of dogs suggests that the timing of locomotor development (both onset and early maturation) may play a role in the inter-specific changes in trabecular bone morphology (Wolschrijn and Weij, 2004).

These studies suggest that trabecular architecture is particularly responsive to changes in loading patterns during growth and development. As yet, researchers have not explicitly linked locomotor and trabecular architecture data sets to clarify how bone growth tracks changes in gait mechanics. To better characterize this relationship, and to provide a framework for application to the fossil record, we compared locomotor kinematics with trabecular bone measurements in the distal tibia in an age-matched sample of children and a skeletal sample from the Norris Farms #36 site in Illinois (see Methods). We hypothesize that changes in trabecular bone architecture will reflect increasingly consistent locomotor parameters as individuals grow, develop, and mature.

## Methods

### Kinematics

A sample of children ( $n = 35$ ; 20 males and 15 females aged 1.3–8.5 years old; see Table 1) was recruited from the Tucson community. All experimental procedures were approved by the University of Arizona Institutional Review Board and all parents of subjects gave their informed consent for participation. Subjects had no history of gait abnormality or lower limb injury.

Kinematic data were collected at 200 Hz using a Vicon high-speed, six-camera, motion capture system. Reflective markers were affixed to the skin of subjects at joint centers, limb segments, and foot landmarks (markers were placed on the heads of the 1st and 5th metatarsals, the calcaneus, the lateral malleolus, midway between the knee and ankle on the shank, the knee, the greater trochanter, ASIS, and PSIS on both the left and right sides; see Fig. 1). Subjects were encouraged to walk unshod across a 4-m trackway at their preferred walking speed, with each pass down the trackway considered a trial. All trials for each individual were

**Table 1**  
Subject characteristics.

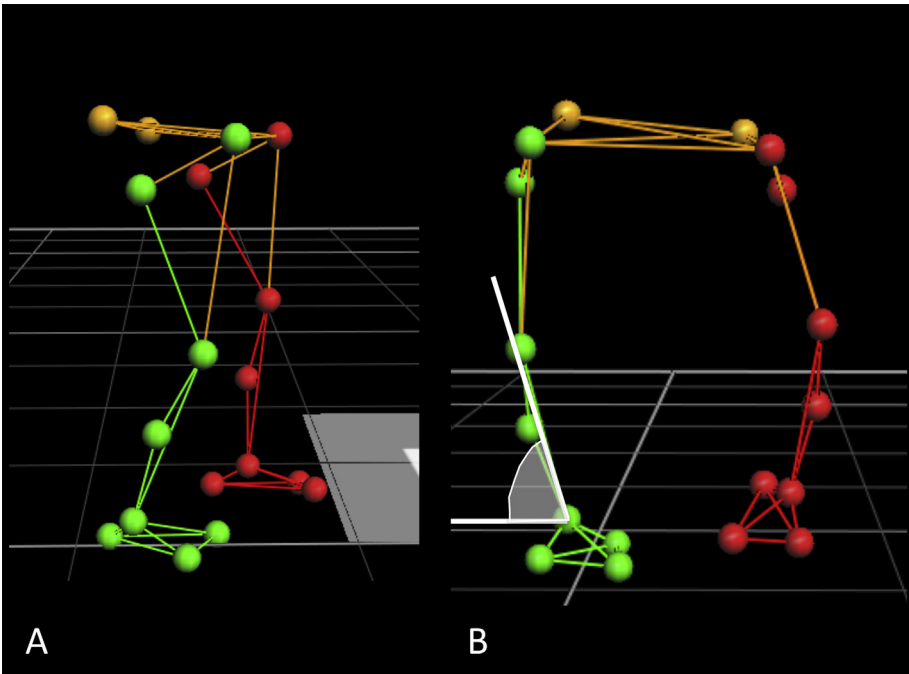
Sub	Age (years)	Sex	Body mass (kg)	Froude number <sup>a</sup>
24	1.3	f	8.7	0.08 (0.01)
31	1.3	m	13.1	0.12 (0.02)
36	1.3	f	11.0	0.07 (0.01)
18	1.5	m	10.4	0.30 (0.09)
3	1.6	m	11.2	0.09 (0.02)
29	1.6	f	10.1	0.09 (0.03)
30	1.6	f	11.5	0.07 (0.01)
34	1.6	f	10.9	0.12 (0.04)
11	2.0	m	13.75	0.16 (0.09)
28	2.25	m	17.3	0.19 (0.10)
33	2.5	m	13.1	0.06 (0.01)
23	2.75	f	11.6	0.35 (0.10)
6	2.8	f	12.7	0.30 (0.10)
15	2.8	f	13.3	0.17 (0.06)
8	3.0	f	14.7	0.19 (0.13)
22	3.2	f	15.5	0.22 (0.04)
41	3.25	f	13.1	0.17 (0.10)
1	3.5	m	15.2	0.17 (0.12)
12	3.7	m	16.0	0.15 (0.05)
38	4.15	f	15.3	0.07 (0.01)
39	4.25	m	18.8	0.11 (0.03)
40	4.25	m	16.9	0.11 (0.03)
43	4.4	m	19.0	0.17 (0.07)
32	4.8	f	15.3	0.12 (0.03)
17	5.0	f	16.6	0.27 (0.18)
35	5.3	m	22.1	0.29 (0.14)
16	5.5	m	19.0	0.13 (0.04)
7	5.7	m	16.5	0.17 (0.11)
27	5.7	m	24.2	0.12 (0.03)
19	5.8	m	17.9	0.11 (0.03)
9	6.0	m	38.0	0.17 (0.08)
10	6.0	m	25.3	0.12 (0.01)
26	7.3	f	27.5	0.08 (0.02)
5	8.5	m	29.1	0.25 (0.11)
20	8.5	m	24.7	0.22 (0.06)

<sup>a</sup> Froude number is a mean for all trials for each subject with standard deviation in parentheses.

collected on the same day. Steps were defined as the time between touchdown of one foot to lift-off of the same foot, and we analyzed all steps for all individuals. For comparisons across subjects, we converted raw speeds into the dimensionless Froude number ( $Fr = \text{velocity}^2 / (\text{hip height} \times \text{gravity})$ ); (Alexander and Jayes, 1983). Here, we analyze walking trials only.

We calculated the mediolateral (ML) angle of the tibia relative to the ground (90° refers to a tibia segment perpendicular to the trackway; segment defined by the ankle and knee markers) to measure balance and stability (see Fig. 1). Degree of variation in the ML angle from step-to-step is offered as a measure of the consistency in postural support system since children will have trouble balancing if they sway in the coronal plane. We extracted tibia angle at touchdown (TD) and mid-stance (MS) from each step for further comparisons. To compare variation in step characteristics across age, we calculated a mean and standard deviation (SD) for each subject's tibial angles at TD and MS (analyzed separately) across all available trials and all strides within trials (see Table 1 for sample sizes for each subject and the Supplementary Online Material [SOM] for angles). Mean angles provide a measure of whether the direction of load orientation changes during locomotor ontogeny. SD of the tibial angle provides a measure of step to step variability in loading patterns, as high SDs are associated with highly variable tibial angles across all steps within an individual.

Neither mean Froude number nor CV of Froude number were significantly correlated with age ( $p = 0.36$  and  $0.33$ , respectively). SDs of ML angles were also not significantly correlated with Froude number ( $p = 0.28$  and  $0.41$  for ML angles at TD and MS, respectively). Thus, we did not incorporate Froude number into



**Figure 1.** Marker placement and tibia angle. **A:** Lateral view of young subject during locomotor trial. Markers are placed on the heads of the 1st and 5th metatarsals, the calcaneus, the lateral malleolus, midway between the knee and ankle on the shank, the knee, the greater trochanter, ASIS, and PSIS on both the left and right sides. **B:** The angle of the tibia in the mediolateral plane relative to the horizontal.

subsequent analyses, but rather compared tibial angles directly with trabecular bone properties.

#### Quantification of trabecular bone fabric structure

The skeletal sample used in this analysis consisted of 25 juvenile human individuals from the Norris Farms #36 archaeological skeletal collection. The Norris Farms #36 site is a late Prehistoric cemetery site from the central Illinois River valley dating to ca. AD 1300 with graves containing between one and several individuals associated with the Oneota cultural tradition of village agriculturalists. For the current study, only individuals ranging in age from 1 to 9 years were used (Table 2). Age-at-death was determined previously based on tooth crown and root formation and eruption (Milner and Smith, 1990).

One distal tibia from each individual was scanned on the OMNI-X HD600 High-Resolution X-ray CT scanner (HRCT) at the Pennsylvania State University Center for Quantitative Imaging. Bones from both right and left sides were used in the sample, depending on the quality of preservation. Each bone was mounted upright in florist foam and transverse scans were collected for the entire distal metaphyseal region, encompassing approximately the distal third of the bone. The HRCT scans were collected using source energy settings of 180 kV and 0.11 mA. Images were reconstructed as 16-bit grayscale TIFF images with a 1024 × 1024 pixel matrix. Pixel sizes for the datasets ranged from 0.026 to 0.047 mm and slice thicknesses ranged from 0.028 to 0.050 mm depending on specimen

size. The best possible spatial resolution was used for each bone based on the size of the bone. Due to the specific configuration of the OMNI-X HRCT scanner at Penn State, datasets are not reconstructed with isotropic voxels. To facilitate quantification of trabecular bone architecture for this and related studies, the image data were resampled to produce isotropic voxels. The x,y pixel sizes were resampled to match the value for the slice thickness for each dataset using Avizo 7.1.

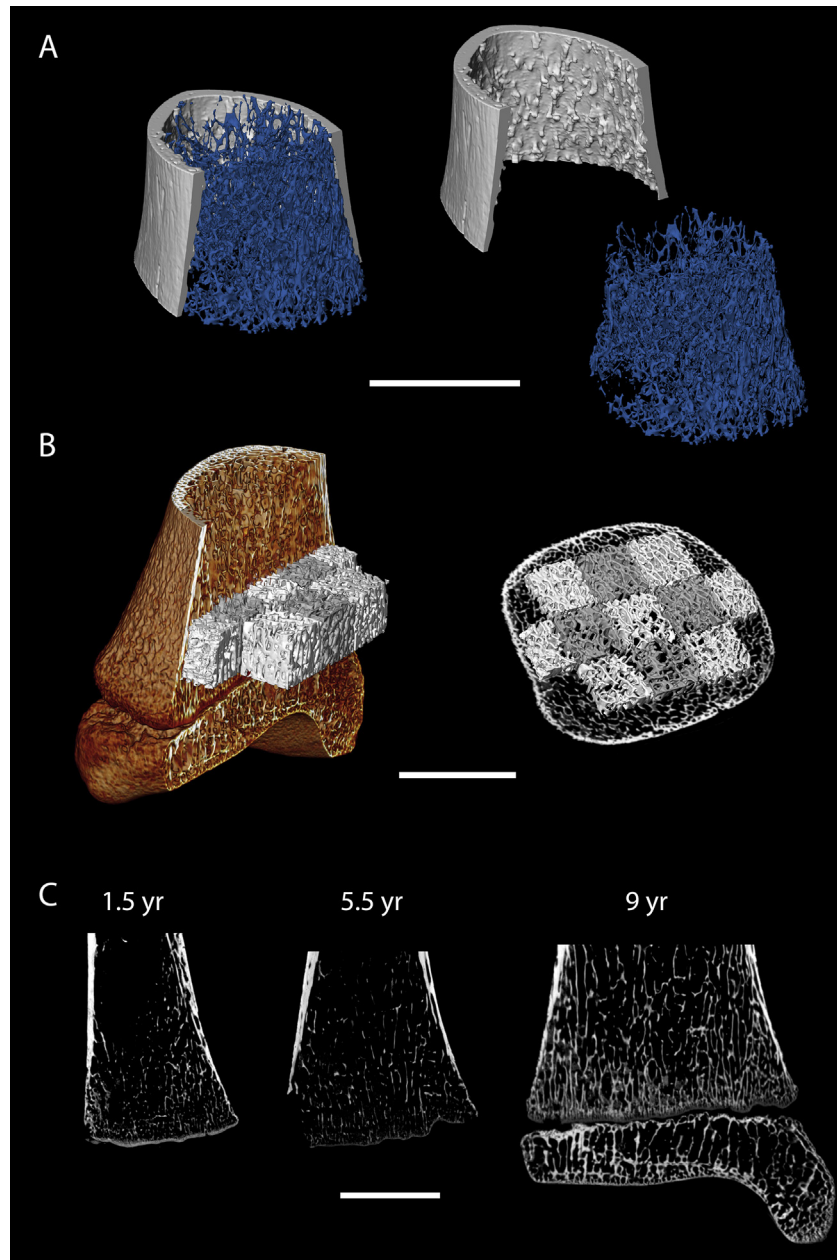
To facilitate analyses of trabecular bone fabric structure, the dual threshold segmentation technique of Buie et al. (2007) was used to remove the cortical shell from each specimen. This method uses two input threshold values and a series of basic image processing steps to define the periosteal and endosteal surfaces of an output mask. This segmentation routine effectively identifies the cortical and trabecular bone components of the bone dataset (Fig. 2A) and was used here to isolate the trabecular bone compartment. The two input threshold values used in this processing step were generally particular to each dataset and were determined manually through visual inspection of the resulting masks as suggested by Buie et al. (2007). This step ensured that no cortical bone was included in any volume of interest (VOI) used to analyze trabecular bone architecture.

A grid of eleven cubic VOIs was defined in the distal metaphysis of each tibia (Fig. 2B). The VOI grid was located centrally within the metaphysis to ensure homologous placement of the VOIs across individuals of different sizes and to quantify as much of the metaphyseal trabecular bone structure as possible. The VOIs were scaled based on the size of the individual using the anteroposterior breadth of the proximal femoral metaphysis as the size standard. The size of each VOI was calculated as 25% of the anteroposterior breadth of the proximal femoral metaphysis, resulting in cubic VOIs with edge lengths ranging from 2.1 to 5.2 mm, reflecting size increases associated with growth of the tibia across ages represented in this sample (Fig. 2C). The only exception to this protocol of using cubic volumes were the two VOIs positioned on the lateral and

**Table 2**  
Sample sizes for each age group for skeletal and kinematic datasets.

Age range (years)	Skeletal sample	Kinematic sample
<2.7	11	11
2.7–4.9	5	13
4.9–7.1	5	8
>7.1	4	3





**Figure 2.** Quantification of three-dimensional trabecular bone architecture in the distal tibia. **A:** Separation of cortical and trabecular bone in the distal metaphysis. **B:** Grid of 11 volumes of interest used for quantification of anisotropy. **C:** Coronal sections through the distal tibial metaphysis of 1.5, 5.5, and 9 year old individuals from the Norris Farms #36 collection. Scale bars for each image 5 mm.

medial sides of the metaphysis. The edges of these VOIs were truncated by the endosteal margin of the cortical shell, resulting in VOIs that were not fully cubic in shape (Fig. 2B).

Each VOI was segmented using an iterative histogram-based algorithm (Ridler and Calvard, 1978; Trussell, 1979). In all cases, the data were visually inspected to ensure that a reasonable threshold value was used due to the presence of a significant amount of loess filling intertrabecular spaces in some individuals. The loess is generally substantially lower density than bone. To quantify the fabric structure of each VOI, the degree of anisotropy (DA) was calculated using the star volume distribution (SVD) method. Star volume distribution characterizes the distribution of trabecular bone in 3D using a voxel-traversing directed secant method (Ketcham and Ryan, 2004). In the current study, intercept lengths were measured for 2,049 uniformly distributed

orientations at each of 2,000 points lying in the bone phase of each VOI. The SVD was calculated on a centered sphere within each cubic VOI to avoid edge and corner effects. A second rank tensor that describes the distribution of material in 3D space was derived using the orientation and intercept data (Odgaard et al., 1997; Ryan and Ketcham, 2002a, 2002b; Ketcham and Ryan, 2004). The magnitude and orientation of the principal material axes are represented by the eigenvectors and eigenvalues of this tensor. To facilitate intra- and inter-individual comparisons DA was normalized as 1-(tertiary eigenvalue/primary eigenvalue). This normalization of DA resulted in values ranging from 0 for an isotropic structure to 1 for a highly anisotropic structure. For each individual, the primary eigenvectors from the 11 VOIs were used to calculate the mean resultant primary vector,  $R$ , and the  $\alpha_{95}$  confidence limit of the mean vector, following the methods outlined in Ryan and Ketcham

(2005). Image segmentation and quantification of trabecular bone anisotropy were performed using QUANT3D (Ryan and Ketcham, 2002a, 2002b; Ketcham and Ryan, 2004).

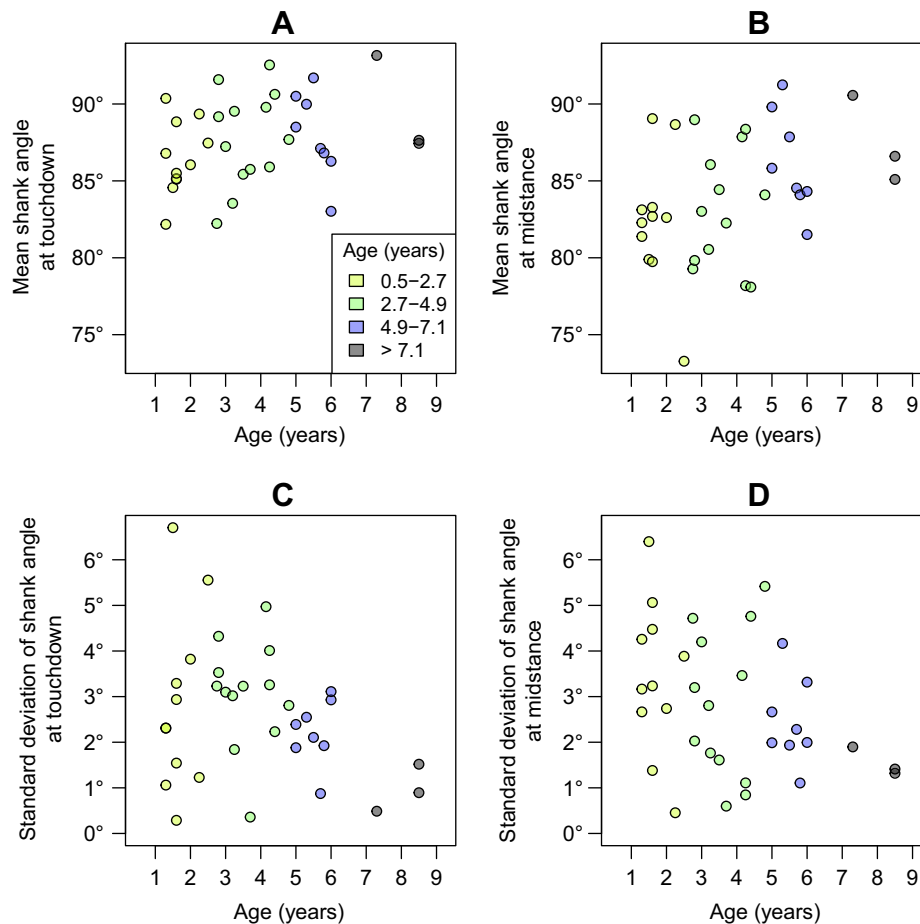
## Results

Mediolateral tibial angle is significantly correlated with age at TD and MS ( $r_{TD} = 0.28$ ,  $p = 0.048$ ;  $r_{MS} = 0.37$ ,  $p = 0.01$ ), converging on a more vertically oriented tibia at older ages (Fig. 3A,B). SDs of tibial angles are also significantly correlated with age (Fig. 3C,D;  $r_{TD} = -0.31$ ,  $p = 0.04$ ;  $r_{MS} = -0.37$ ,  $p = 0.01$ ), however variation in SDs is dependent on age (Park test of heteroscedasticity:  $p_{TD} = 0.03$ ,  $p_{MS} = 0.02$ ). Results do not change if we remove subjects with small samples ( $n = 2$ ) (correlation statistics:  $r_{TD} = -0.33$ ,  $p = 0.04$ ;  $r_{MS} = -0.38$ ,  $p = 0.02$ ; Park test of heteroscedasticity:  $p_{TD} = 0.02$ ,  $p_{MS} = 0.03$ ). High levels of inter-individual variation in intra-individual tibial angles in young children shift to less intra-individual variation as age increases. The change in distributions of SD with age suggest that at young ages, some individuals have more consistent tibial angles from step to step and resemble patterns observed in older children, while others have highly variable tibial angles from step to step.

Trabecular bone structure in the distal tibia displays distinctive patterns of change with age (Fig. 2C). Changes in mean DA across

the 11 VOIs in the distal tibia mirror the kinematic results. Inter-individual variation in mean DA is high at young ages, with some individuals having low mean DA, or relatively isotropic trabeculae, across the distal tibia (Fig. 4A). Mean DA converges on higher values in older children, leading to a significant correlation in mean DA with age ( $r = 0.36$ ,  $p = 0.04$ ). However, this relationship is significantly heteroscedastic ( $p_{\text{park}} = 0.02$ ) since there is a reduction in variation in mean DA at older ages. The heteroscedastic shift in DA variability with age is also apparent in an analysis of SDs of DA across the distal tibia within individuals. There is a pattern of changes in SD of DA with age, with younger aged subjects showing a higher degree of variation in SDs and older individuals showing lower overall SDs in DA across the distal tibia (Fig. 4B). While this correlation does not reach statistical significance ( $r = -0.23$ ,  $p = 0.14$ ), and is not significantly heteroscedastic ( $p = 0.35$ ), the overall shift from a wide spread of variation to lower levels of variation is evident and is consistent with kinematic analyses.

The 95% confidence limits for mean orientation angles across distal tibia volumes of interest decrease with age in a heteroscedastic pattern ( $r = -0.37$ ,  $p = 0.035$ ,  $p_{\text{park}} = 0.001$ ) and are significantly negatively correlated with mean DA ( $r = -0.84$ ,  $p < 0.0001$ ,  $p_{\text{park}} = 0.009$ ; Fig. 4C,D): as age increases, DA tends to increase in all VOIs within an individual (Fig. 4A), and the orientations of the primary vectors across those VOIs become less



**Figure 3.** Age and shank angles within and across individuals. In all plots, each point corresponds to all angles measured from the right leg of a single individual over multiple steps, measured at either touchdown or midstance. Colors correspond to age categories given in the lower right of Figure 2A. **A:** Mean of shank angle relative to the ground at touchdown within all trials for each individual. **B:** Mean of shank angle relative to the ground at midstance within all trials for each individual. In both **A** and **B**, shank angles tend to become more vertically oriented (i.e., closer to 90°) as individuals age. **C:** Standard deviation of shank angle relative to the ground at touchdown within all trials for each individual. **D:** Standard deviation of shank angle relative to the ground at midstance within all trials for each individual. In both **C** and **D**, as individuals age, shank angles become both less variable within individuals (decreasing standard deviation of mean angles) and less variable between individuals (narrowing spread of data points with respect to the y-axis).

variable within individuals (Fig. 4C). Orientations of the resultant primary eigenvectors across the distal tibia also change with age such that they become less variable between individuals as well (Fig. 4E). At young ages, there is a high degree of inter-individual variation in the resultant orientations, as well as a high degree of intra-individual variation, denoted by the size of the circles in Figure 4E. As subject ages increase, resultant primary eigenvectors become more consistently vertically oriented and there is a reduction in inter-individual variation. This more consistent vertical orientation mirrors the change in mean tibial angle with age, from highly variable to more vertically oriented.

To compare the living and archaeological datasets directly, we divided both kinematic and trabecular bone data into four age categories (categories were chosen to maximize sample sizes within each bin while also maintaining equal age ranges across bins; Fig. 5). When data are combined, there is a strong positive relationship between SD of tibial angle and SD of DA at TD ( $r = 0.995$ ,  $p = 0.004$ ; Fig. 5A). This pattern is also present at MS, although the correlation fails to reach significance because values of variation in kinematic data are more similar at young ages ( $r = 0.786$ ,  $p = 0.21$ ; Fig. 5B). Variation in the orientation of trabecular struts in a given age category generally matches variation in tibial angles.

## Discussion

Our results show a relationship between the patterns of locomotor and morphological change in the distal tibia of humans during the development of bipedal walking. We find the change in tibial angle variation with age tracks the shift from unstable to more stable locomotion as individuals age and gain walking experience. Variation in the ML tibial angle likely reflects immaturity in the postural support system of children and is an indicator of difficulty with balance and stability. Shifts in mean tibial angles to more vertical orientations, with reduced step-to-step variation, likely reflect improved balance at older ages. Interestingly, young children seem to show a high degree of inter-individual variation in tibia angle means and SDs, with some young children capable of highly stereotypical loading patterns. As steps become less variable, distal tibia trabecular struts become more uniformly oriented, and the orientations of struts in VOIs across the distal tibia converge on the same, more vertical direction. Thus, the overall pattern is that, at young ages, variation in both kinematic patterns and trabecular strut orientations can be either highly variable or highly stereotypical, but at older ages, both datasets converge on more stereotypical patterns.

Kinematics results are consistent with data from adults suggesting that instability and challenges to balance produce similar variation in step-to-step kinematics (Voloshina et al., 2013). For example, when walking on a treadmill that introduces variation in substrate height, step-to-step variability in step width increased significantly compared with walking on a smooth-surface treadmill (Voloshina et al., 2013). Our results for children are also similar to data from elderly subjects who show both increased step width and increased step-to-step variability in kinematic patterns when balance becomes more challenging with increasing age (Murray et al., 1969; Schrager et al., 2008). Across the age spectrum, reduced balance and stability are associated with similar kinematic effects during locomotion.

Our results suggest that early in development, trabecular bone seems well structured to manage the disorganized loading patterns created by variation in lower limb segment angles from step to step. It is only after maturation occurs, and individuals become more stable and consistent walkers, that trabecular struts become consistently more highly oriented and that trabecular orientation converges on a single more vertical direction across the distal joint

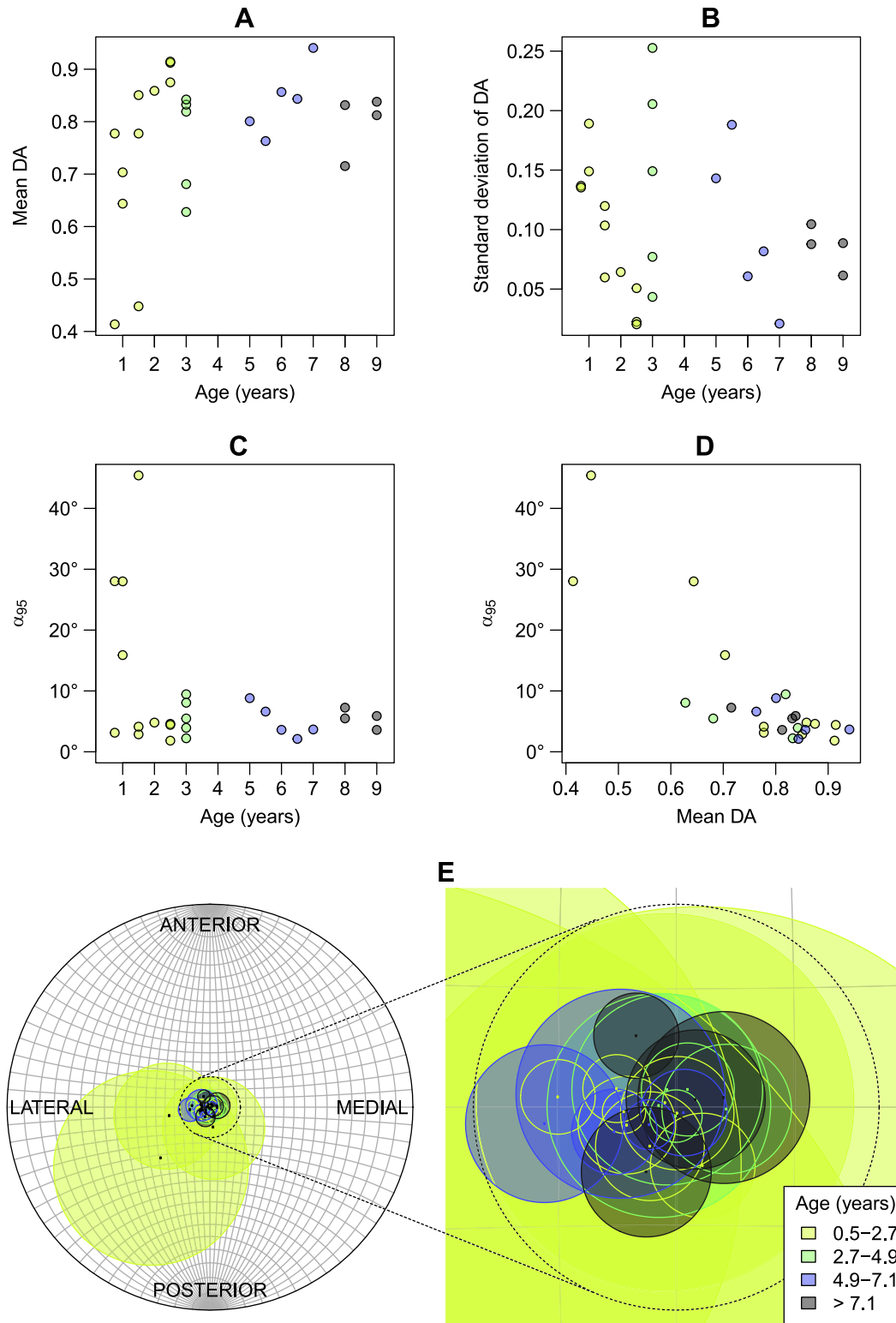
surface. The combination of a shift in both (1) direction of trabecular orientation across the joint and (2) degree of orientation in volumes of interest may provide a skeletal marker of locomotor maturation in bipeds.

Despite the signal, we note some constraints on our study design that could affect both the robusticity of the results and their interpretations. First, our kinematic data set had small sample sizes at older ages. While it is possible that patterns of change in kinematic variation with age are driven by these smaller samples at old ages, our data are consistent with many other studies showing reduction in intra- and inter-individual kinematic parameters in these same age ranges (Lasko-McCarthy et al., 1990; Bril and Brenière, 1992; Assaiante et al., 1993; Yaguramaki and Kimura, 2002; Ivanenko et al., 2005). Second, we do not know whether there are differences in the time of onset of independent bipedal walking between the Norris Farms archaeological sample and the living sample, so ages in our samples may not fully reflect the same amount of time spent walking. Third, we do not know how differences between these two samples in pre-bipedal behavior impacted both locomotor development and skeletal growth (e.g., proportion of time spent crawling, riding in a stroller, carried in sling, etc). Fourth, we do not know how the use of rigid-soled shoes from an early age alters the development of walking mechanics in humans living today. Finally, this analysis does not include data from ground reaction forces, limiting our ability to draw strong conclusions about loading patterns during locomotor development. Recent work suggests that mediolateral forces change in magnitude with age. For example, Cowgill et al. (2010) showed that peak mediolateral forces decrease with age relative to anteroposterior and vertical forces, and that inter-individual variation in ML forces also decrease with age. This shift towards lower magnitudes and less variability is likely associated with the more vertically oriented tibia at TD and MS and the less variable tibial angle at older ages that we detailed in the study. Thus, future work must explore the relationship between kinematics and the orientation of the ground reaction force vector in more detail to confirm our results in terms of tibial loading patterns. Despite these caveats for our study, we believe the predicted match between locomotor and morphological variables supports our overall hypothesis. Based on these results, we can explore the possible developmental milestones reflected in changes in trabecular bone architecture.

### *Bone as a marker of neurological maturation?*

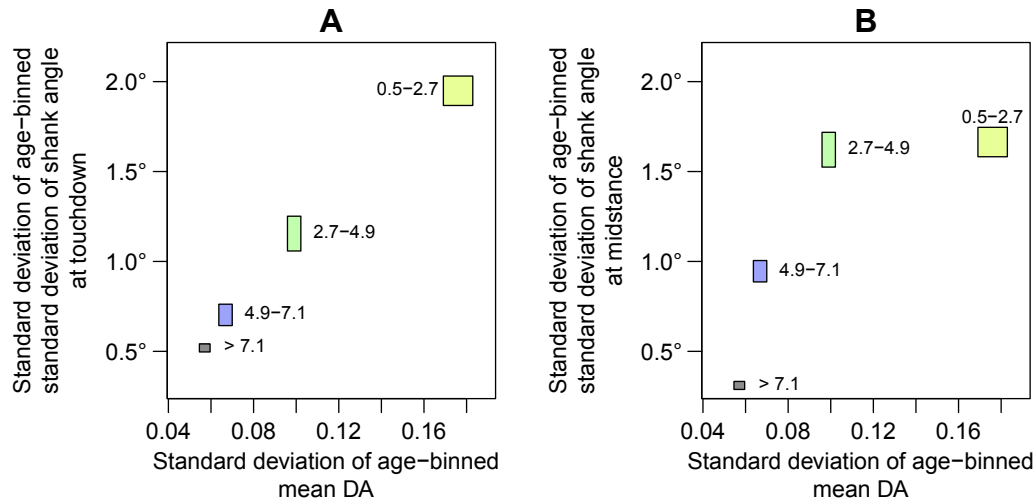
Across mammals, age of walking onset is strongly explained by the timing of brain development, with larger adult brain sizes associated with later onset of independent locomotion (Garwicz et al., 2009). Increasing stability and balance during locomotor ontogeny is likely related to changes in neuromuscular control due to brain growth and development during childhood (Adolph, 2003). Since trabecular bone in the distal tibia seems to track changes in balance, we believe the analysis presented here provides a framework for using bone to reflect broader changes in maturation.

Changes in brain structure and function affect muscle control, and ultimately, the control of gait characteristics that contribute to loading patterns supported by trabecular bone. For example, as the brain grows, neural fibers are myelinated, and glial cells multiply, with major changes in brain activity coinciding with the onset of walking (Bell and Fox, 1996; Adolph, 2003). Myelination of descending tracts is necessary for maturation of the central neuronal pathways required to control muscles during movement (Paus et al., 1999). The integration of supraspinal, intraspinal, and sensory controls all contribute to the maturation of gait, and this integration occurs slowly over time (Forssberg, 1999; Lacquaniti



**Figure 4.** Orientation properties of trabecular bone within and across individuals. In all plots, each circle represents the average of the 11 volumes of interest (VOIs) drawn from a single individual. Colors correspond to age categories given in the lower right of the figure. **A:** Mean of degree of anisotropy (DA) for the 11 VOIs plotted against age for each individual. As individuals age, trabeculae become both more strongly oriented within individuals (increasing mean DA) and less variable in strength of orientation between individuals (narrowing spread of data points with respect to the y-axis). **B:** Standard deviation of DA within the 11 VOIs in an individual plotted against age. As individuals age, trabeculae tend to become more uniform in their degree of orientation across the distal tibia within individuals (decreasing standard deviation of DA), and less variable between individuals (narrowing spread of data points with respect to the y-axis). **C:** The angular 95% confidence interval for the mean orientation vector of the primary orientation of trabecular bone across 11 VOIs within an individual ( $\alpha_{95}$ ) plotted against age. As individuals age, direction of orientation of trabeculae across the distal tibia becomes both more uniform within individuals (decreasing  $\alpha_{95}$ ) and less variable between individuals (narrowing spread of data points with respect to the y-axis after 2.7 years of age). **D:** Plot of  $\alpha_{95}$  against mean DA. As trabecular bone anisotropy increases within individuals (increasing mean DA), the direction of the principal material orientations becomes more uniform across the 11 VOIs within each individual (decreasing  $\alpha_{95}$  indicates less variation in the direction of orientations of the primary material axis in 3D space). **E:** Stereonet plot showing





**Figure 5.** Comparison of age-specific variation in standard deviation in mean DA and shank angles (A, at touchdown; B, at midstance). For each age category, the standard deviation is calculated for mean DA among all individuals in that age group (i.e., standard deviation by color for the vertical axis in Figure 3A), and the standard deviation is calculated for the standard deviation of shank angle among all individuals in that age group (i.e., standard deviation by color for the vertical axis in Figure 2C or 2D). Box height and width are proportional to the number of individuals in each age category for the living (kinematic data) and archaeological sample (mean DA), respectively. Older age groups are consistently less variable than younger groups in both mean DA and standard deviation of shank angles, indicating that older individuals tend to converge on more stereotypical steps and that trabecular bone tends to converge on the same (high) degree of anisotropy.

et al., 2012). In the first years of independent walking, major structural changes in the cortico-spinal pathway occur, and continue until 17 years of age (Paus et al., 1999; Eluvathingal et al., 2007; Petersen et al., 2010).

The growth and development of these neural pathways alter patterns of neuromuscular control and lead to muscle activation patterns that differ greatly in young children compared to adults (Petersen et al., 2010). Muscle activation differences link neural development to kinematic variation and therefore to variation in loading patterns throughout ontogeny. For example, at young ages, children often co-contract flexor and extensor muscles at the knee and ankle in ways not seen in adults (Forssberg, 1985). These patterns continue to change and muscle activation patterns do not fully resemble those of adults until the age of 15 (Sutherland et al., 1980). Recently, Petersen et al. (2010) showed that maturation of muscle activation control was correlated with a reduction in intra-individual variance in ankle kinematics, suggesting developmental changes in neurobiology can strongly influence movement and loading patterns.

Because changes in locomotor variation seem tied to the growth and development of neuromuscular control, we argue the ontogeny of trabecular bone may act as a skeletal marker of neural maturation. Changes in trabecular bone in the distal tibia reflect reductions in step-to-step variation in tibial angles with age that are likely due to developmental changes in brain structure and function. While we explored the distal tibia here, similar studies may find other skeletal sites that similarly reflect ontogenetic changes in locomotor patterns. Based on these connections, we suggest that analyses of ontogenetic sequences in the fossil record can provide a unique window into hominin growth and development across evolutionary time.

#### Applications for the hominin fossil record

One way we can use bony markers of motor development in studies of human evolution is to compare the timing of locomotor maturation with developmental milestones that track the overall pace of life history in a given species (e.g., brain growth, molar eruption schedules, etc). Developmental trajectories in fossil hominins remain highly debated (Robson and Wood, 2008; Schwartz, 2012), although a few patterns seem evident from studies of dental ontogeny. First, early hominins, including australopiths, likely had faster developmental schedules than modern humans, while early *Homo* and *Homo erectus* may have had life history schedules somewhere between those of earlier hominins and later members of the genus *Homo* (Smith, 1991; Dean et al., 2001; Schwartz, 2012). However, no matter your pace of growth and development, the challenges of balance in bipedal walking remain high. Thus, we can begin to ask how closely the development of bipedal walking follows differences in ontogenetic schedules.

Two distal tibiae from juvenile individuals (Dikika [Alemseged et al., 2006] and AL333w-43 [Johanson et al., 1980]) may allow a preliminary investigation into the pattern of locomotor development in *Australopithecus afarensis*. Given the possible inclusion of arboreal locomotor patterns in earlier hominins, including australopiths (Stern and Susman, 1983; Stern, 2000), we must first analyze a comparative sample of chimpanzees. However, if chimpanzees follow a similar, albeit accelerated pattern of change in trabecular bone, then analyses of fossil hominins may provide a unique window into development in our ancestors. An overall faster pace of neuromuscular maturation in *Australopithecus*, in line with their faster life history schedule (Robson and Wood, 2008;

the mean orientation vector for the 11 VOIs for each individual (central region of the stereonet delineated by the dotted circle is expanded at right for greater visibility). Mean orientation for each individual is shown as a point which is surrounded by a circle describing the  $\alpha_{95}$  confidence interval for the mean orientation vector. Since each individual has an equal number of orientation vectors (11), variation in  $\alpha_{95}$  between individuals is solely dependent on the degree to which orientation vectors are dispersed around the mean. Vector projections point superiorly such that a point in the lower left of the quadrant indicates that the primary orientation of trabeculae is along an axis that points laterally and posteriorly at its superior end, and medially and anteriorly at its inferior end. Older individuals tend to have a mean orientation that is primarily superoinferior (i.e., points closer to the center of the plot) and to be more uniformly oriented across all VOIs (smaller confidence intervals).

Schwartz, 2012), would result in highly oriented trabecular struts with low variation across the joint in these tibiae from young individuals. However, it is possible that given the challenges to stability, bipedal development may take longer to stabilize relative to an individual's overall developmental schedule. This finding would imply possibly unique challenges for raising bipedal children with a faster overall development schedule, given a long period prior to mastery and full independence of gait. Given the small sample size currently available for early hominins, these kinds of investigations must remain preliminary; however, later taxa provide larger samples of juvenile skeletons allowing for more robust analyses. For example, although Neandertals had slower periods of growth and development than earlier hominins, a study of the relatively large number of immature individuals (e.g., La Ferrassie, Lagar Velho, Skhul 1, Shanidar 10; Ruff et al., 1994; Trinkaus and Ruff, 1996; Cowgill et al., 2007) could reveal subtle differences in locomotor development compared to living humans.

We suggest that determining the pattern of locomotor development across fossil hominins may provide a novel view of the evolution of neural maturation. Hypotheses described above will allow us to assess how the development of motor skills correlates with other life history milestones (e.g., age of weaning, molar eruption schedules, etc.). These types of studies will require a larger sample of fossils than is presently available; however, with the appropriate sample size, analyses of trabecular bone development may provide a new method for analysis of hominin life history patterns.

## Conclusions

Our study provides a framework for exploring how locomotor development and maturation influences the growth of trabecular bone. In bipedal walking, stability is a challenge, and early on children walk with a high degree of step-to-step variation. Our sample exhibited high levels of intra-individual variation in the angle of the tibia at both TD and MS at young ages, which may drive the less oriented and more variable orientation of trabecular struts across the distal tibia within and between individuals, respectively. Maturation of gait, indicated here by the reduced step-to-step variation in tibial angle, is marked by increased organization of trabecular architecture in the distal tibia. Thus, we suggest a bony marker for maturation of gait may be useful in the fossil record for tracking locomotor development across bipeds.

Future research should examine other primate taxa to determine whether similar markers of locomotor development exist. For example, a comparison of chimpanzee and human distal tibiae should help us tease apart differences in the challenges and developmental timing of quadrupedal and bipedal locomotor maturation. Additionally, if this marker of gait is tied to neuromuscular maturation, then this method may provide novel insights into the evolution of brain development and life history in fossil hominins. In the end, the data presented here demonstrate a novel method for exploring ontogeny across taxa, both extinct and extant, to develop new insights into how locomotor maturation relates to overall life history evolution.

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## Appendix A. Supplementary material

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.jhevol.2015.01.003>.

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