Trabecular and Cortical Skeletal Correlates of Locomotor Ontogeny in *Pan* with Comparisons to Skeletal Variation and Adaptation in *Gorilla* and *Pongo*

by

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Dedication

Thank you to my husband, parents, sister, friends, and family for the endless support

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Abstract

The goal of this study was to understand how variability in mechanical loading across ontogeny and between species is reflected in the trabecular and cortical morphology of non-human great ape limb bones. Specifically, this study sought to determine whether trabecular and cortical bone responds to changes in substrate use and positional behavior from infancy through adulthood, using chimpanzees as a model. Chimpanzees are an ideal species for this investigation because they have well documented age-related locomotor shifts.

The second chapter used microCT and pQCT data from the proximal femur and humerus of chimpanzees across ontogeny to identify patterns of trabecular morphological change. It then assessed how trabecular and cortical bone correlate across ontogeny. Trabecular and cortical bone operate as a unit, but the degree to which they both respond to locomotor change is unknown in chimpanzees. Results indicated that femoral bone volume fraction (BV/TV) in infants and juveniles was lower than that in adults and adolescents. In the humerus infants had significantly lower BV/TV than all other ages. There were few predictive relationships between cortical and trabecular bone. These findings indicate that the two bone compartments respond differently to locomotor variation.

The third chapter used pQCT data from the femur and humerus of chimpanzees, gorillas, and orangutans to determine whether changes in the proportion of arboreal and terrestrial locomotion were reflected in cortical bone during development. Infant and juvenile chimpanzees, who move in the trees frequently, were predicted to be more similar to arboreal

orangutans, while adult and adolescent chimpanzees were predicted to resemble terrestrial gorillas. Relative strength ratios were different between infant and adult *Pan*, matching predicted changes. These results tracked changes found in *Pongo* (relatively stronger humerus) and *Gorilla* (relatively stronger femur) . Infant chimpanzees had rounder bones compared to adults who had relatively elliptical bones. These results suggest that cortical bone adapts to changes in behavior across ontogeny.

The fourth chapter assessed whether intraspecific variability in cross-sectional properties of long bones differed in populations of gorillas and chimpanzees. These two species are closely related and live in similar habitats, but are different in terms of their locomotion, body size, and levels of sexual dimorphism. Overall levels of intraspecific variation were similar within each species, with females tending to be more variable than males. Gorillas tended to be more variable than chimpanzees. There were few differences between bones, indicating that intraspecific variation in the morphology of bones is quite constrained.

This research demonstrated the importance of studying both cortical and trabecular bone, and how the complex relationship between the two bone types and locomotion. Future research should be devoted to understanding the differences in magnitude of response to locomotion in cortical and trabecular bone, and how these responses may contribute to overall skeletal variability. This research will allowed for insights into the degree of plasticity of cortical and trabecular bone in response to locomotor variability across ontogeny. These data are critical for understanding how bone responds to varying loads across the lifespan. Creating trabecular proxies for locomotor variability allows for novel applications of modern skeletal data to understand the locomotor evolution of fossil apes and humans. Developing new tools to interpret the preserved internal skeletal anatomy of modern and fossil taxa will allow for better understanding of locomotor evolution in apes, including hominins to be attained.

Chapter 1 Introduction

1.1 Introduction

The degree of arboreal and terrestrial locomotion of fossil taxa is particularly of interest over human and hominoid evolution since all modern apes, except for humans, have some degree of arboreal behavior. Over the course of human evolution, our species moved from arboreal locomotion to habitual terrestrial bipedalism. Identifying arboreal vs. terrestrial substrate use in early hominins, e.g. the degree of arboreality in australopiths, is essential to understanding our evolutionary past (Stern, 2000; Ward, 2013; Ruff et al., 2016). When this change occurred is unclear within the fossil record. Although early hominin evolution coincided with aridification in Africa and rise of possibly more open landscapes (Bobe and Behrensmeyer, 2004), recent work indicates that australopiths were likely engaging in arboreal climbing (Ruff et al., 2016; DeSilva et al., 2018). Thus, substrate use may have varied in early hominins.

Bones are a crucial source of data from the fossil record because bone size and shape reflect both heredity as well as developmental plasticity associated with responses to mechanical loads placed upon them during locomotion. To understand the locomotor repertoire of Miocene hominoids, it is necessary to understand how arboreal and terrestrial locomotion is encapsulated in the skeleton. In primates, correlations between aspects of morphology and locomotor pattern have generally been shown to be strong. In adults, long bone cross-sectional diaphyseal properties are typically consistent with locomotor behaviors (e.g. Ruff, 2002; Pearson and Lieberman, 2004; Ruff et al., 2006), with stereotypical loading patterns usually associated with more elliptical cross-sections and variable locomotion with more circular cross-sections (Ruff and Runestad, 1992; Ruff 2002; Carlson, 2005; Ruff et al., 2006). Similarly, primates using hindlimb dominant postures, such as leaping or bipedal walking, have stronger hindlimbs than forelimbs (Demes et al., 1991; Ruff and Runestad, 1992; Ruff, 2002), and taxa such as orangutans with predominantly forelimb dominated locomotion have stronger humeri relative to femora (Ruff, 2002). In humans, adult femoral and humeral cross-sections reflect differences in activity patterns (Stock and Pfeiffer, 2001). Furthermore, cortical bone has variable responsiveness to loading in humans, with distal elements more correlated with terrestrial locomotion than proximal elements, a pattern which may relate to tissue economy (Stock, 2006). Overall, comparative studies show that different locomotor patterns are reflected in the cortical bone of adults, particularly in limb bones and this pattern may vary across bones.

The distribution and orientation of trabecular bone has also been shown to respond to locomotor positional differences in adults (Kivell et al., 2011; Ryan and Shaw, 2012; Ryan and Walker, 2010; Saparin et al., 2011; Raichlen et al., 2015). Trabeculae of primates with stereotypical locomotor patterns tend to be more uniformly oriented (anisotropic), while primates with more variable locomotor repertoires have more randomly oriented (isotropic) trabeculae (MacLatchy and Muller, 2002; Shaw and Ryan, 2012; Fajardo et al., 2013). Suites of trabecular bone traits in adults can successfully distinguish between locomotor categories, such as arboreal suspensory vs. terrestrial quadrupedal (Ryan and Shaw, 2012; Shaw and Ryan, 2012). Experimentally, trabecular bone responds to changes in locomotor joint angle orientation (Pontzer et al., 2006; Barak et al., 2011; Wallace et al., 2013). Thus, trabecular orientation can be used to distinguish primate locomotor behaviors.

Although previous studies show there is a strong form-function relationship between locomotion and skeletal morphology, most comparative studies assessing properties of bone have focused on adult specimens, even though the majority of skeletal growth occurs earlier in life (Bertram and Schwartz, 1991; Pearson and Lieberman, 2004). Few studies have investigated whether bone is equally plastic in response to loading throughout life or reflects locomotion at different ages (but see Lieberman et al., 2003), yet there is good reason to hypothesize that juvenile locomotion has a strong influence on adult phenotype. Cortical strength development differs in arboreal and terrestrial primates, with arboreal primates demonstrating steeper declines in relative bone strength during early ontogeny compared with terrestrial primates (Young et al. 2010; Ruff, 2003). In humans, cortical bone is most plastic around puberty than before or after (Bailey, 1997; MacKelvie et al., 2002; Pearson and Lieberman, 2004). Studies have found that adolescent humans gain more bone than adults do in response to a given strain stimulus, and that exercise prior to or during puberty has a lasting effect on adult bone shape, strength, and density (Bass et al., 1998; Bailey et al., 1999; Haapasalo et al., 2000; MacKelvie et al., 2002). Thus, locomotor behaviors through ontogeny may have a particularly strong influence on the adult skeletal phenotype.

Consistent with this hypothesis is the key finding that in baboons, infants have stronger humeri relative to their femora (Ruff, 2003). Prior to two and a half years of age, infants are more arboreal than adults and cling to their mothers during travel; after two and a half years, when baboons use almost exclusively quadrupedal locomotion, the femoral and humeral strength ratios are similar (Ruff, 2003). Complementary work on *Pan* ontogeny has shown that as arboreal locomotion decreases and terrestrial behaviors increase, the humerus and femur become more elliptical, and the femur grows stronger (Sarringhaus et al., 2016). Likewise, in *Gorilla beringei beringei,* increases in proportions of terrestrial locomotion result in corresponding increases in hindlimb strength (Ruff et al., 2013). These findings indicate that age-related locomotor changes measurably alter limb bone cortical geometry.

In contrast to cortical bone, trabecular bone responsiveness to age related locomotor changes in non-human primates is less well known. In humans, femoral and tibial trabecular orientation is highly isotropic (randomly oriented) in infants and becomes progressively more anisotropic through adulthood (Raichlen et al., 2015; Milovanovic et al., 2017), likely reflecting both increasing body mass and more stereotypical loading. In addition, human infants have similar trabecular bone architecture in their femur and humerus, but by age three these bones have diverged dramatically in their structure (Ryan et al., 2018). However, the human ilium appears to have an inherent predetermined scaffolding structure that trabecular bone follows (Cunningham and Black, 2009a, b). Likewise, the ovine calcaneus develops a base trabecular framework in utero that then persists through adulthood (Skedros et al., 2007). In non-human primates the distal femoral bone volume fraction (BV/TV) in hominoids (*Homo, Pongo,* and *Pan)* showed significant differences between the medial and lateral condyles while *Papio* did not (Sukhdeo et al., 2020). Sukhdeo et al. (2020) found that both condyles in hominoids differed from those in *Papio*, with higher coefficients of variation and mean BV/TV. Another study found that the differences between *Homo, Pan,* and *Gorilla* were more limited that previously hypothesized (Georgiou et al., 2018).

Among chimpanzees, the pattern of trabecular change across ontogeny varies in the humerus, femur, and tibia (Tsegai et al., 2018). There is limited change in trabecular values as a function of age in the proximal humerus, but in the proximal femur and distal tibia there is a significant effect in BV/TV and trabecular thickness between juveniles and adolescents (Tsegai et al., 2018). This corresponds to a change in both locomotor usage from arboreal to terrestrial dominance, but also an increase in body mass (Sarringhaus et al., 2014). Further, the ratio of femoral to humeral BV/TV increases over time, indicating increased loading on the femur during ontogeny (Tsegai et al., 2018). These results suggest that like cortical bone, trabecular bone development tracks locomotor changes as chimpanzees age.

While there are open questions about the evolution of human locomotion, the locomotor repertoire of fossil hominoids remains to be determined. Extant apes live in forest environments, and this has been assumed to be true of the earliest apes (Andrews et al., 1997). Recent work has shown that *Morotopithecus bishopi* and *Proconsul major* lived in seasonal woodlands/open forests with grasses present (MacLatchy and Kingston, 2016; Driese et al., 2017; Kinyanjui et al., 2017). The extent to which they used arboreal vs. terrestrial substrates is unknown but is of considerable interest as these apes are the oldest known to exhibit distinctive features also found in extant great apes. These include an upright torso, differential use of fore- and hindlimbs (*M. bishopi*), and large body size (~40-80 kg; both taxa) (MacLatchy, 2010). *Proconsul major* is not well represented by postcrania, and locomotor reconstructions have been based on other *Proconsul/Ekembo* species (Ward, 2015). It is possible that it was terrestrial (Nengo and Rae, 1992) given its weight of over 80 kg (Rafferty et al., 1995) and inferred lack of hominoid postcranial apomorphies (MacLatchy, 2004). Reconciling the locomotor behavior of fossil hominoids is required to determine when and in which lineages key hominoid locomotor apomorphies evolved.

Further investigation of non-human apes is necessary to assess the plasticity of trabecular and cortical bone in response to variable locomotor behaviors through ontogeny. This investigation must include study of fine grain aspects of trabecular morphology, which may be indicative of functional changes. While previous studies show trabecular and cortical bone respond to locomotor changes in humans, this relationship appears to vary regionally, although to an unknown extent.

This dissertation will assess how the skeleton responds to variation in arboreal and terrestrial locomotion across the life course by investigating the effect of ontogenetic changes in locomotor behavior on both cortical and trabecular bone in *Pan,* and a complementary analysis of cortical change across ontogeny in *Pan, Gorilla,* and *Pongo.* These data will be used to test hypotheses about locomotor behavior in Miocene fossil hominoids. The varied substrate use in these taxa makes them an ideal comparative data set to use in reconstructing the locomotor behavior of fossil hominoids, including Miocene apes and Pliocene hominins whose arboreal and terrestrial substrate use is unclear (e.g. Stern, 2000; Ward, 2013).

A major strength and innovation of this study is the integrated analysis of existing locomotor information with new data on trabecular architecture and cortical bone strength. Trabecular and cortical bone have vital, complementary roles in force distribution, so analyzing both bone compartments together as a system will provide a comprehensive understanding of skeletal responses to stress-strain environments. Previous work across primate species demonstrates that cortical and trabecular bone do not have the same response to differential loading in the femur and humerus. For example, Shaw and Ryan (2012) have shown that relative trabecular bone volume co-varies with cortical midshaft strength in the humerus but not in the femur across adult primates. This indicates regional differences in responses to loading between bone compartments and within a skeleton. Previous research has also shown that aging humans lose trabecular and cortical bone at different rates in different bones (Parfitt, 1984), illustrating the importance of studying trabecular and cortical change at multiple sites within a skeleton.

In a recent study assessing cortical and trabecular bones, no correlation was found between cortical area, I_{max}/I_{min} (shape parameter), and standardized torsional strength (J) and trabecular bone mineral density (BMD) in the tibiae of human males that participated in different sports (cricket, hockey, running) (Saers et al., 2021). Comparisons between the sports showed that higher BMD was a signal of high activity relative to controls (Saers et al., 2021). Size and shape of the midshaft cortical bone better showed impact and directionality of loading (Saers et al., 2021). While BMD is a good metric for assessing the overall density of trabecular bone, it is a coarse metric do document trabecular morphology. There may be differences in the number, thickness, connectivity, and relative density of trabecular bone that BMD alone cannot show. This study also only included the distal tibiae. Previous studies have shown distal limb elements to be more variable than proximal elements (Buck et al., 2010), but did not assess other regions in the skeleton. This work has yet to be completed in non-human hominoids, which have more variable locomotion than *Homo.*

To understand the effect of transitions between arboreal and terrestrial habitats, it is necessary to study both trabecular and cortical bone through ontogeny. These transitions are important functionally, but also from an evolutionary perspective. A second strength of the study is the inclusion of apes that use a range of arboreal and terrestrial substrates. This furnishes a natural experiment to assess how variability in locomotor behavior is reflected in the skeleton. Understanding the effect of variable locomotor behaviors on the skeleton is essential for behavioral reconstruction in fossil hominoids and hominins, as the extent and nature of substrate use in these taxa are unknown. This dissertation will address this and some of the other issues reviewed above in three chapters.

In Chapter two I ask the following questions: 1) Does trabecular bone respond to age related locomotor change in *Pan troglodytes*? and 2) Are the patterns of cortical and trabecular response to changes in locomotor behavior correlated? Chimpanzees provide a model to examine ontogenetic effects on the skeleton because their use of arboreal ands terrestrial substrate varies during development. Infants and juveniles are more arboreal and use more forelimb dominated behaviors such as torso-orthograde (TO) vertical climbing and forelimb dominated suspensory behaviors than do adolescents and adult chimpanzees. Adolescents and adults are more terrestrial and use more hindlimb dominated behaviors such as terrestrial knucklewalking (Doran, 1992; Doran, 1993; Doran, 1997; Doran and Hunt, 1994; Sarringhaus et al., 2014). Preliminary work has shown that ontogenetic changes in locomotion are reflected by changes in the cortical cross-sectional geometry, strength, and some regions of trabecular bone in some limb bones in *Pan* (Sarringhaus et al., 2016; Tsegai et al., 2018) as well as in *Gorilla* (Ruff et al., 2013). However, it is unclear how extensive this cortical response is or whether trabecular bone exhibits the same magnitude and ontogenetic pattern of responsiveness.

The third chapter investigates whether changes in the proportion of arboreal and terrestrial locomotion in the skeleton of *Pan troglodytes* are reflected in humeral and femoral cross-sectional morphology and if the patterns observed in *Pan* match the morphology of *Gorilla* and *Pongo*. Together, *Pongo* and *Gorilla* bracket *Pan troglodytes* in degree of arboreality with *Gorilla* being more terrestrial and *Pongo* more arboreal. *Gorilla gorilla gorilla* (Western Lowland Gorilla) is more terrestrial than *Pan* but more arboreal than *G. g. beringei* (Remis, 1995; 1998; 1999). Females and juveniles of this subspecies were found to engage in more arboreal behaviors than males, sometimes at similar levels to *Pan* adults (Remis, 1995; 1998). However, although *G. g. gorilla* does engage in arboreal behavior (11% in the wet season), unlike *Pan* and *Pongo,* males rarely use suspensory locomotor behaviors (Remis, 1995). *Pongo* is predominantly arboreal, with a variable locomotor profile ranging from leaping behaviors to slower more cautious climbing, (Thorpe and Crompton, 2005; Thorpe and Crompton, 2006; Thorpe et al., 2009). Importantly, both these species have less dramatic ontogenetic changes in their proportions of arboreal and terrestrial behavior compared to *Pan.* Inclusion of a sample of *Gorilla* and *Pongo* in this study will enable us to determine whether the suites of trabecular and cortical traits that characterize terrestrial vs. arboreal locomotion within a species (*Pan*) can also be applied to other species, both extinct and extant, with different proportions of terrestrial and arboreal behaviors (*Gorilla* and *Pongo*)*.* These species are unlikely to have absolutely similar trabecular and cortical structures. The goal is to identify whether high vs. low proportions of arboreal locomotion lead to the predicted skeletal trends.

The fourth chapter addresses skeletal variability in *Pan troglodytes* and *Gorilla gorilla gorilla* cross sectional bone through an analysis of variation in post-cranial cross-sectional properties of adult bones. I conduct analyses comparing species and sexes within each species to understand the impact of body size and locomotor variability on the skeleton. Specifically, I ask how the long bones of *Pan troglodytes troglodytes* and *Gorilla gorilla gorilla* vary between species, males and females within species, and bones between individuals. The two species are sympatric and can occupy similar niches, yet differ in their locomotion and position behavior, body size, and sexual dimorphism. This study contribute sto our understanding of potential factors that influence skeletal variation, particularly among taxa purported to have varying degrees of sexual dimorphism and potentially sex-based differences in locomotion.

Taken together findings in this thesis demonstrate how variable locomotion across ontogeny impacts the adult phenotype, specifically bone plasticity. My analyses of the relationships between trabecular bone distribution and loading provide novel proxies to reconstruct locomotor behavior in fossil taxa. These, in turn, promise to furnish new insights into the locomotion of fossil hominins and early hominoids, whose locomotor repertoires continue to be debated. This study also contributes to our understanding of the effect of locomotion on the development of cortical and trabecular bone strength during the period between infancy and adolescence. This developmental time window is not well studied, particularly in non-human primates. Finally, trabecular and cortical bone have complementary roles in force distribution, so analyzing both bone compartments together as a system provides a comprehensive understanding of skeletal responses to stress-strain environments.

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Chapter 2 Trabecular and Cortical Correlates of Locomotor Ontogeny in *Pan troglodytes*

2.1 Introduction

Bone is a plastic tissue that responds to its mechanical environment through a principle called Bone Functional Adaptation (Ruff et al., 2006). Bones can modify their structure in response to mechanical loading and unloading, leaving patterns that can be used to assess locomotion and other behaviors (Parfitt, 2003; Martin, 2003; Lieberman et al., 2004). Bone may respond to mechanical loading by stimulating increased bone formation when strain surpasses a maximum threshold and unloading when strain falls below a minimum threshold (Frost, 1987). Although bone size and shape have heritable components, both the amount of bone tissue in a cross-section and its shape can change in response to the magnitude and directionality of applied loads (Martin, 2003). This response has been well documented in cortical bone across mammals (e.g. Carter, 1984; Rubin and Lanyon, 1984; Biewener and Bertram, 1994; Turner, 1998; Pearson and Lieberman, 2004; Ruff et al., 2006), but less work has focused on trabecular bone.

In primates, although both cortical and trabecular bone have been used to reconstruct locomotor behavior of modern and extinct taxa, the majority of research has focused on cortical bone (e.g. Ruff and Hayes, 1982; Ruff et al., 2006; Barak, 2019). Few studies have focused on trabecular bone (e.g. Barak et al., 2011; Kivell, 2016), and even less have included analyses of both. While both tissues display plastic responses to loading independently, how they respond to mechanical loading during development and at different skeletal locations remains largely unexplored.

It is necessary to study trabecular and cortical bone as a unit because they share load bearing, particularly near joints (Eswaran et al., 2006; Sornay-Rendu et al., 2007; Nawathe et al. 2015). External joint morphology is useful for inferring locomotor potential but does not indicate individual activity levels in the same way cortical and trabecular bone do (Ward, 2015). Although trabecular and cortical bone share the loads applied to the skeleton (Eswaran et al., 2006; Sornay-Rendu et al., 2007; Nawathe et al. 2015), the degree to which this affects the growth of each compartment across ontogeny is not well understood. Since it is unknown whether cortical and trabecular bone exhibit similar or differential responses to loading throughout life, it is also unclear which type of bone is most useful for inferring activity history at different stages of development. Critically for studies of hominin evolution, fossils are highly fragmentary, and some fossils only cortical or trabecular bone preserved. Thus, understanding the effect of mechanical loading on both trabecular and cortical bone and their interrelationships will provide a possible means to reconstruct how individuals in fossil taxa moved.

2.1.1 Cortical Bone

Hominoid cortical bone has been shown to vary in response to locomotor differences among adults of different species, within humans whose activity differs, and across ontogeny within species. Since bending moments are greatest at midshaft, bone is expected to have the greatest response to locomotion there (Bertram and Biewener, 1988), and most comparative studies focus on this region. In primates, adult long bone cortical midshaft geometry is generally correlated with locomotor behaviors. For example, stereotypical loading such as leaping or quadrupedalism results in more elliptical bones while more varied loading results in rounder bone cross-sections (e.g., Demes et al., 1991; Ruff and Runestad 1992; Carlson, 2005; Barak et al., 2013). Slow climbers load their bones in a variety of different postures, many of which are in the mediolateral (ML) plane, resulting in a more elliptical bone in the ML direction (Demes et al., 2000). Ruff (2002) found that taxa with forelimb dominated locomotion display more robust forelimb bones relative to leapers or hindlimb dominated taxa, in which the reverse pattern was found. A recent study comparing the relative strength of the femur compared to the humerus (measured at 35%, 50% and 65% of diaphyseal length) across extant hominin species found that these ratios were correlated with positional behavior (Sarringhaus et al., 2022). *Pongo abelii*, as the most arboreal taxon with the greatest locomotor variability, had the lowest ratio of femoral/humeral strength, followed sequentially by increasingly less arboreal taxa: *Pan troglodytes, Pan paniscus, Gorilla gorilla, Gorilla beringei* and finally, *Homo sapiens*.

The effects of differences in habitual loading on cortical morphology have been documented extensively in humans with variable lifestyles and activity patterns. Stock and Pfeiffer (2001) found that the skeletons of Later Stone Age foragers, who were very active terrestrially, had stronger hindlimb bones compared with Andaman Islanders, who were more active swimmers and boaters. By contrast, Andaman Islanders had stronger forelimbs (Stock and Pfeiffer, 2001). Moreover, studies in modern humans have shown that differential activity patterns (e.g., varying training patterns seen in sports), influence skeletal form during adulthood (Turner and Robling, 2003; Turner and Robling, 2005). In a study comparing cricketers and swimmers, cricketers had greater difference in humeral and ulnar strength in their dominant vs. non-dominant arms, while swimmers had less bilateral asymmetry (Shaw and Stock, 2009). When comparing athletes (cricketers, hockey players, and swimmers) compared to controls, all athletes significantly differed from controls (Saers et al., 2021) but there were also variations in bone strength and ellipticity between athletes. Runners had higher tibial J and I_{mas}/I_{min} compared to controls while hockey players had higher J than swimmers (Saers et al., 2021). Taken together, these results suggest that different sports result in varying skeletal phenotypes depending on the loads imposed. There was also substantial inter-individual variation in bone properties (Saers et al., 2021). Among tennis players, both male (Haapsalo et al., 2000) and female adults (Haapsalo et al., 1996) had significantly higher BMC and BMD in the dominant arm compared to the non-dominant arm. In addition, the effects were greater when the individual played tennis before and during puberty than if they started after puberty (Haapsalo et al., 1998; Bass et al., 2002; Ducher et al., 2011). These studies indicate plasticity of the skeletal phenotype is particularly acute during growth.

In *Gorilla beringei beringei* (Ruff et al., 2013), infants exhibit significantly stronger humerii relative to their hindlimbs compared to all other ages. The shift to stronger femora relative to humerii begins after infancy, matching a rapid decrease in overall arboreal locomotion and increase in terrestrial knucklewalking (Ruff et al., 2013). Though typically indicative of locomotion, there have been equivocal results of cortical bone responsiveness to locomotor variability. In one study of *Papio anubis* across ontogeny, cortical bone thickness and bending strength did not change once locomotor independence was established (Cosnefroy et al., 2022). In contrast, Ruff (2003) found that the femoral to humeral strength ratios in *Papio* before 2.5 years old showed increased forelimb strength relative to those after 2.5 years, indicating a shift in skeletal strength that matched locomotor change.

In studies of *Pan troglodytes,* the relative strength of the femur compared to the humerus increased across ontogeny, matching a shift from more arboreal to more terrestrial locomotion (Sarringhaus et al., 2016). In addition, the midshaft of the femur became more elliptical over time (*ibid*.). Infants engaged in the highest level of locomotor variability, with the number of locomotor modes and sub-modes decreasing as chimpanzees became more and more quadrupedal (Sarringhaus et al., 2014). This decrease in locomotor diversity is presumably associated with a decrease in the variability of the loading environment (*ibid*.), leading to femoral shafts that become more reinforced in the anteroposterior over the mediolateral plane.

Chimpanzees are a model organism to explore cortical and trabecular shifts in response to locomotor change, particularly across ontogeny. *Pan* infants and juveniles use significantly more arboreal positional behaviors than adults and adolescents (Sarringhaus et al., 2014). In addition, infants and juveniles spend more time using torso-orthograde suspension and vertical climbing compared to adults and adolescents who use significantly more terrestrial quadrupedal running and walking (Sarringhaus et al., 2014). This results in infants and juveniles using more forelimb dominated behaviors such as clinging and orthograde forelimb suspension, and adults and adolescents using more hindlimb dominated behaviors such as pronograde standing (*ibid.*). The change from forelimb dominated locomotion to terrestrial dominated locomotion is not abrupt, infants are the most suspensory, followed by juveniles, then adults and adolescents, who do not significantly differ from one another (Sarringhaus et al., 2014). In addition to the change in forelimb vs. hindlimb locomotion, infants have more behavioral variability than older ages (Sarringhaus et al., 2014). Overall, cortical bone is responsive to mechanical load variability. Given the effect of mechanical loading on cortical bone, it is probable that trabecular bone also responds to variations in mechanical loading as it is a critical tissue to buffering against joint loads (Eswaran et al., 2006; Sornay-Rendu et al., 2007; Nawathe et al. 2015).

2.1.2 Trabecular Bone

Trabecular bone parameters, including bone volume fraction (BV/TV) and degree of anisotropy (DA) have also been shown to vary with mechanical loading. Rafferty and Ruff (1994) compared trabecular bone morphology in the femoral and humeral head of *Papio, Colobus,* and *Hylobates.* They concluded that the differences in femoral and humeral head bone mass and density corresponded to variation in locomotion. Rafferty (1998) also found that variation in trabecular bone correlated strongly with locomotor type across 21 species of nonhuman primates. In strepsirrhines, MacLatchy and Muller (2002) found significant differences in the trabecular bone of the proximal femur between *Galago* and *Perodicticus. Galago* utilizes a vertical clinging and leaping locomotor style, while *Perodicticus* is a slow quadrupedal walker and climber. There were no differences in trabecular bone volume between these species, but there were differences in DA. *Galago* had much more oriented trabeculae, which fits with its more stereotypical locomotion. *Perodicticus* trabeculae were more randomly oriented and denser. This matches with its locomotor style where the animal is using its limbs in a variety of orientations.

In hominoids, some studies had found that locomotion affects trabecular bone in predictable and quantifiable ways. Other studies, however, suggested that the patterns are complex. For example, trabecular bone volume fraction and DA in the long bones and in the hands and feet of primates consistently varied among species (Kivell et al., 2011; Ryan and Shaw, 2012; Tsegai et al., 2013; Bird et al., 2021). In *Homo,* trabecular thickness, number, and degree of anisotropy changed through the limb in response to differences in loading (Raichlen et al., 2015; Saers et al., 2016).

Interestingly, studies of the femur and humerus independently have shown trabecular morphology to be representative of locomotion. However when tested within the same study there were limited differences between species with different locomotor repertoires (Ryan and Walker, 2010). For example, the humerus was unable to discern between brachiating and quadrupedal species based on trabecular morphology (e.g. BV/TV, trabecular number and thickness, and DA) (Ryan and Walker, 2010). Nevertheless, there was differentiation between femoral and humeral BV/TV in adult *Pan.* Specifically, there was significantly higher BV/TV in the femoral head compared to the humerus. In addition, DA was consistently more isotropic in the humerus than the femur, regardless of locomotor behavior (Ryan and Walker, 2010). In subsequent research Shaw and Ryan (2012) found that primate trabecular bone in the proximal femur correlated more strongly with locomotor type when compared with the proximal humerus. These differences were evident even when cortical diaphyseal changes in the same bone were indicative of locomotor mode (Shaw and Ryan, 2012). This indicates that trabecular bone responds to loading differently than cortical bone, especially cortical bone at midshaft. There may be limited response by BV/TV to changes in locomotion, even when cortical bone adapts. Work by Fajardo and Muller (2001) suggested that DA is a better indicator of positional behavior than BV/TV across adults of different species. In sum, studies have found that degree of anisotropy in trabecular bone was a relatively consistent indicator of locomotion, and would thus be useful in identifying locomotor mode depending on the region of bone studied (Lieberman et al., 2004).

Trabecular bone can also be used to detect skeletal responses to ontogenetic changes in locomotor behavior. In an experimental study in juvenile guinea fowl, differences in trabecular orientation of the distal femur were associated with whether individuals had run on an incline or flat surface (Ponzter et al., 2006). In humans, studies have found that trabecular bone across the skeleton responds to locomotor shifts. In humans, we start with a crawling/non-bipedal phase and an unassisted but unstable bipedal gait period, followed by an adult like bipedal gait (Cowgill et al., 2010). In the human calcaneus, trabecular structure continually remodels due to mechanical load changes linked to gait maturation (Saers et al., 2020). For example, BV/TV initially decreases in newborns, but stops decreasing around age one, which is linked to the onset of unassisted walking (Saers et al., 2020).

There is a wide variety of locomotor positional behavior within and between species of hominoids. For example, *Gorilla gorilla gorilla* is more arboreal than *Gorilla beringei beringei* (Remis, 1995; Doran, 1997; Remis, 1998; Remis, 1999). Ontogenetically in *G. b. beringei,* infants under two years of age are more arboreal than adults, and at two years of age there is a dramatic decline in arboreal locomotion and increase in terrestrial knuckle walking (Doran, 1997). *Pan troglodytes* has a shift from forelimb dominated arboreal locomotion that is greatest in infants (zero to five years) and declines until adolescence (10-15 years) (Sarringhaus et al., 2014). During adolescence hindlimb dominated terrestrial locomotion matches the adult pattern, with juveniles often being intermediate between infants and adults/adolescents

(Sarringhaus et al., 2014). Taken together, these studies predict that trabecular bone will reflect different patterns of arboreal locomotion between species, as has already been demonstrated for cortical bone (Sarringhaus et al., 2022), and across ontogeny.

Trabecular bone in third metacarpal and capitate of *Pan,* had a greater range of change over ontogeny relative to similar ages of *Gorilla gorilla* matching predicted change given the locomotor repertoire of each species (Ragni, 2020). Moreover, the primary orientation of the trabeculae in *Gorilla* became consistent earlier in ontogeny relative to the pattern in *Pan.* This pattern of trabecular orientation followed the locomotor behavioral shifts along ontogeny (Ragni, 2020). However, minimal trabecular bone differences in the third metacarpal were found between *Gorilla gorilla gorilla* and *Gorilla beringei beringei* (Deckers et al., 2022). Since knuckle walking is still the predominant locomotor mode of both species, the differences in arboreality may not have been great enough to engender a locomotor response. It is important to note that the cortical bone midshaft geometry of long bones in both species was able to discern between locomotor repertoires of adults (Ruff et al., 2018; Sarringhaus et al., 2022).

Previous studies examining trabecular ontogeny in chimpanzees have found different patterns depending on skeletal site (Tsegai et al., 2018). For example, BV/TV was significantly higher in adolescents than juveniles in the proximal femur, but there were limited changes in the humerus (Tsegai et al., 2018). Tsegai et al. (2018) also found that the ratio of femoral to humeral BV/TV increased across ontogeny, indicating a greater load placed on the femur relative to the humerus. This pattern matches previous studies of cortical bone along ontogeny in chimpanzees and other hominoids (Ruff et al., 2013; Sarringhaus et al., 2016; Ruff et al., 2018). Additional studies of *Pan* ontogeny found that BV/TV in the calcaneus tracked age-related variation in kinetic data (Saers et al., 2022a). BV/TV in the calcaneus was also found to change its break point, or slope, in models of *Pan* ontogeny at around five years old to match that of adults (Saers et al., 2022a).

2.1.3 Studies of Both Cortical and Trabecular Bone

A few comparative and experimental studies have evaluated cortical and trabecular responses to loading together. In adult primates, Shaw and Ryan (2012) found that the femur and humerus differed in how tightly trabecular and cortical properties were correlated. In the humerus, there were significant correlations between proximal humeral subarticular trabecular bone volume fraction and diaphyseal cortical area and torsional strength (*J*), but in the femur there were no significant associations (Shaw and Ryan, 2012). Interestingly, in cortical diaphyseal morphology, a shift in relative strength from the humerus to the femur reflected the shift from forelimb to hindlimb dominated locomotion, while trabecular morphology did not (Shaw and Ryan, 2012).

Experimentally, mice who were exposed to controlled tibial loading showed that trabecular and cortical bone responses are age-dependent (De Souza et al., 2005). Cortical bone volume increased with loading at all ages studied, but trabecular bone responsiveness to loading was greatest at 8 weeks of age and decreased thereafter (De Souza et al., 2005). In mice housed in linear or curved enclosures, there were no differences in any trabecular characteristics within the distal femur (Carlson et al., 2008). This was similar to results from Carlson and Judex (2007) who found that cortical area did not vary with linear vs. curved enclosures but that ML bending rigidity in the distal femur did. This indicates that there may be differences in cortical and trabecular response to age related locomotor changes.

Further study of the trabecular and cortical relationship across ontogeny is necessary to determine the effects of mechanical loading on trabecular and cortical bone, and to determine if the two bone compartments respond to loading in similar manners. Limited research has been conducted on a single species with known locomotor change, other than humans who have limited change across ontogeny. This study builds on previous work through an analysis of developmental changes in skeletal morphology in *Pan troglodytes*.

2.2 Hypotheses and Predictions

(1) A. If trabecular bone responds to changes in mechanical loading across ontogeny, then it is predicted that infant and juvenile *Pan* will have trabecular bone morphology consistent with forelimb driven arboreality. Infants will have a stronger signal of forelimb driven locomotion followed by juveniles. These will include features such as high isotropy, higher bone volume fraction (BV/TV) in the forelimb, and stronger forelimbs relative to their hindlimbs.

B. Due to increased use of terrestrial hindlimb dominant behaviors with age, adolescents and adults will have more anisotropic trabeculae, higher BV/TV in the femur, and hindlimbs stronger than their forelimbs.

(2) A. If cortical and trabecular bone respond similarly across ontogeny, then cortical and trabecular bone will have similar patterns of strength increase. As terrestrial locomotion increases, both trabecular and cortical bone will become stronger in the hindlimb.

B. Increased arboreal locomotion will result in higher correlations in humeral trabecular and cortical bone of infants and juveniles, with adults and adolescents having higher correlations within the femur.

2.3 Methods

2.3.1 Anatomical and Osteological Data Collection

Standard linear measurements of the humerus and femur from all individuals following figures and descriptions found in Ruff (2002).

2.3.2 Samples

Trabecular scans used wild caught specimens with no visible atrophy or injuries from the American Museum of Natural History (AMNH), Harvard University Museum of Comparative Zoology (MCZ), and the University of Minnesota Gombe Collection (UMGC) (Table 2.1). While previous locomotor work has been performed on chimpanzee subspecies *Pan troglodytes verus* and *Pan troglodytes schweinfurthii* (Doran, 1992; Doran and Hunt, 1994; Sarringhaus et al., 2014), the majority of bone samples from the MCZ are from *Pan troglodytes troglodytes* and *Pan troglodytes ellioti.* Specimens from Gombe are *P.t. schweinfurthii,* and specimens from the AMNH are *P. t. troglodytes.* While *P. t. troglodytes* locomotion has not been studied in the wild, studies of the other two subspecies have shown them to be similar (Doran and Hunt, 1994; Sarringhaus et al., 2014). Locomotor differences cannot be ascribed to subspecies, but are better explained by sex, habitat, and foraging strategies. For example, Sarringhaus et al., (2014) found that *P. t. schweinfurthii* chimpanzees at Ngogo in Kibale National Park vertically climbed and descended trees like *P. t. verus* at Tai compared to members of the same subspecies. *P. t.*

schweinfurthii, at Gombe or Mahale. This likely reflects habitat differences; Mahale and Gombe are woodland habitats and are covered by scant primary forest while Tai and Ngogo are evergreen rainforests (Doran and Hunt, 1994; Struhsaker, 1997). Thus, available evidence suggests all chimpanzees display similar locomotor behavioral repertoires regardless of their taxonomic status and evolutionary relationships. Prior research has also shown that cortical bone changes during development do not vary among different chimpanzee subspecies (Sarringhaus et al. 2016).

2.3.3 Aging

This project required aging chimpanzee skeletal specimens. During development chimpanzees pass through different age classes, including infancy, juvenility, adolescence, and adulthood (Goodall, 1986). Individuals in these age classes display distinct morphological, physiological, and behavioral differences. Individual chimpanzees will be categorized to age class using previously established age categories (Sarringhaus et al., 2016) and their absolute age was estimated to assess fine-grained changes across development. Specimens used in the following analyses were aged based on tooth emergence patterns (Zihlman et al., 2004; Smith and Boesch, 2011; Smith et al., 2013; Sarringhaus et al., 2016) from photographs of the occlusal surface of the maxilla and mandible.

2.3.4 Trabecular Scans

High resolution computed tomography (CT) scans were taken at the proximal humerus and proximal femur in chimpanzee specimens. These scans permitted site-specific and intra-individual comparisons with sufficient precision to document skeletal changes in response to differences in limb loading (Table 2.1 for sample sizes, Table 2.2 for properties collected). Scans were made using a X-Tek HMXST225 MicroCT Scanner (Harvard Center for Nanoscale Systems, Cambridge), GE phoenix v|tome|x s240 (American Museum of Natural History), and a North Star Imaging X5000 high resolution MicroCT (University of Minnesota X-ray CT Lab). Scans were taken at the maximum resolution possible for each individual. This was necessary as younger individuals required higher resolution scans to best detail their trabeculae, while older adults did not necessitate as high resolution scans. Further, adults have larger bones and to scan the entire proximal articular surface required a lower resolution scan. Data analysis used cubic regions of interest (ROI) created in Dragonfly (ORS, Montreal) and were analyzed in BoneJ (Doube et al., 2010).

Region of interest (ROI) size has a strong effect on trabecular morphology (Kivell et al., 2011; Kivell, 2016), thus the largest cubic ROI possible was used for each joint to account for ontogenetic and interspecific size variation (Saers et al., 2016). ROI's in subadults with no epiphyseal fusion were placed in the neck of the femur and humerus due to the variability in size of the humeral and femoral head across ontogeny following Tsegai et al., 2018. The region was placed using the z and y axis views in Dragonfly. To ensure consistency in methodology and placement, the top of each ROI cube was placed at a distance of half the total length of the ROI from the cortical bone of the metaphysis. If a cube was 5mm in size, then the top of the ROI was placed 2.5mm from the articular surface. In adults and subadults with fused epiphyses, the largest cubic ROI possible was placed in the femoral and humeral head, and often encompassed the junction between the neck and head, following an approximation of Tesgai et al., 2018's methodology.

2.3.5 Statistics

All data were analyzed in R (R Core Team, 2020). Relationships were first assessed between age and trabecular variables using linear regressions across estimated ages. Age categories were then created to divide the data. This was done to investigate specific changes predicted to occur during infancy, specifically around the age of two. A prior study found linear relationships between degree of anisotropy (DA) and bone volume fraction (BV/TV) and age in chimpanzees (Tsegai et al., 2018). This finding, however did not compare adults and subadults to one another, which is important for full ontogenetic testing. Linear regressions of BV/TV and DA as a function of age were modelled to test estimated age effects. Kruskall-Wallis tests were then conducted to compare the means of BV/TV and DA across development. In situations where heterogeneity among age classes existed, Wilcoxon-Signed-Rank tests were used to determine where changes occurred between age classes. A post-hoc Bonferroni correction was employed to reduce the probability of committing Type I error.

Linear regressions were used to compare the relationship between cortical and trabecular variables. In these analyses linear regressions between cortical and trabecular variables were employed, with age as a covariate to test whether the relationship between trabecular and cortical variables changed across ontogeny. BV/TV was compared to cortical area (CA) and torsional strength (*J*) as in Shaw and Ryan (2012). DA was compared to Imax/Imin, as both variables demonstrate adaptations to directionality of loading in trabecular and cortical bone, respectively. Spearman correlations were conducted to investigate the relationships of trabecular properties between elements and cortical and trabecular properties across elements. with a Bonferroni correction post-hoc test.

Table 2.1: Sample sizes per age and bone.

Bone	Age	Sample size
Femur	Infant	9
Humerus	Infant	9
Femur	Juvenile	12
Humerus	Juvenile	12
Femur	Adolescent	7
Humerus	Adolescent	7
Femur	Adult	10
Humerus	Adult	9

Table 2.2: Cortical and Trabecular variables selected for analysis.

Feature	Unit	Definition
Cortical Area	CA (mm^2)	Compressive and tensile strength
Relative Cortical Area	CA/TA	Relative amount of cortical bone (bone area) given the total area
Ellipticity	I_{max}/I_{min}	"I" area the second moment of areas, or the ability of a bone to resist bending in one plane. I_{max} and I_{min} are the maximal and minimal strength in either plane. Dividing I_{max} by I_{min} gives an indication of ellipticity of the cross section.
Polar second moment of area	J (mm^4)	Torsional and bending rigidity
Degree of Anisotropy	DA	Distribution of trabeculae in 3D space. Structures that are randomly oriented are more isotropic (value = 0) with more anisotropic (value = 1) structures having higher values.
Bone volume fraction	BV/TV	The proportion of trabecular bone voxels to the total number of voxels in a set region of interest.

2.4 Results

2.4.1 Trabecular Changes Across Ontogeny: Bone Volume/ Total Volume (BV/TV)

To assess the relationship between age and skeletal properties, age categories were used as detailed in (Sarringhaus et al., 2014; 2016). This study did not separate young infant from old infant due to sample size constraints. These age categories match documented locomotor data from *Pan troglodytes* (Sarringhaus et al., 2014). Following previous methodology of *Pan troglodytes* ontogeny, continuous ages were used to compare trends in cortical and trabecular properties across ontogeny (Sarringhaus et al., 2016; Tsegai et al., 2018).

Femoral bone volume fraction (BV/TV) increased linearly with age (Figure 2.1) (r^2=0.51, p=2.83e-07). However, there were ontogenetic changes in the directionality and strength of the relationship between femoral BV/TV and age. When grouped into three categories, young infants, older infants, and juveniles to adults, young infants had a unique pattern of bone loss (Figure 2.2). Young infants had a marked decrease in femoral BV/TV from ~0.4 to ~0.15 during the first year and a half of life (Figure 2.2), which rebounded and then increased from older infancy and juvenility through to adulthood. There was a significant positive slope in BV/TV in the 5-16 year age range, but non-significant negative and slight positive slopes in 0-2 and 2-5 ages respectively (Figure 2.2). The difference shown by young infants between the ages of 0 and 2 and older chimpanzees is illustrated in Figure 2.17.

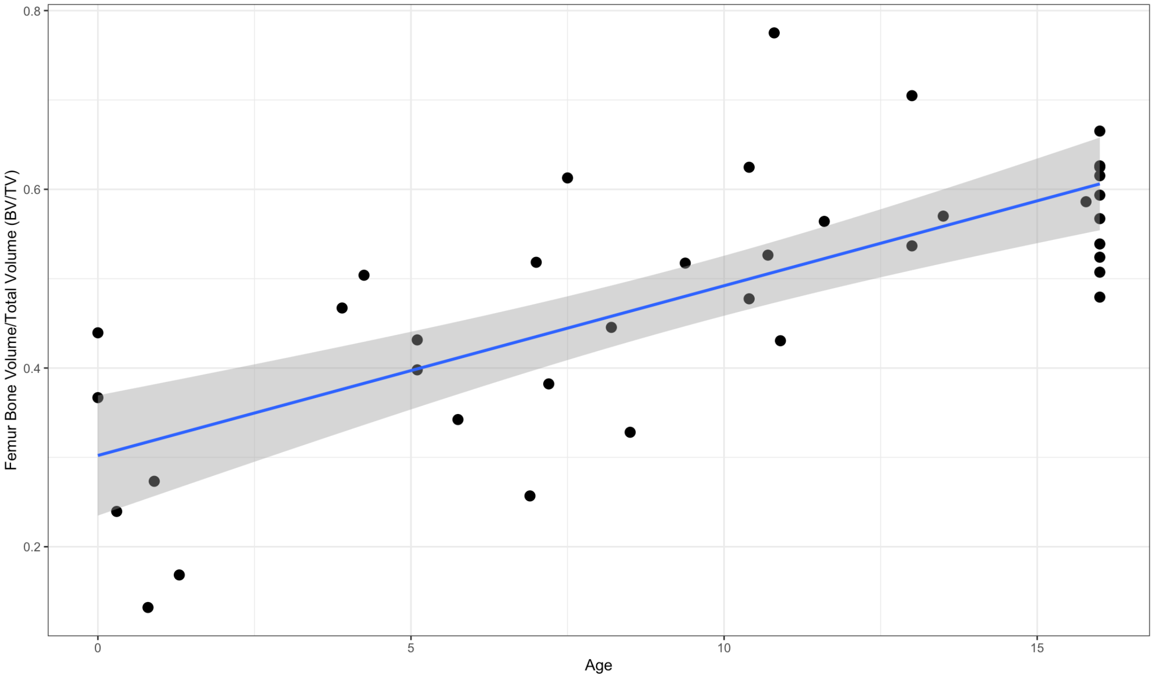

Figure 2.1: Age in years on the x axis, and femur bone volume fraction (BV/TV) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error.

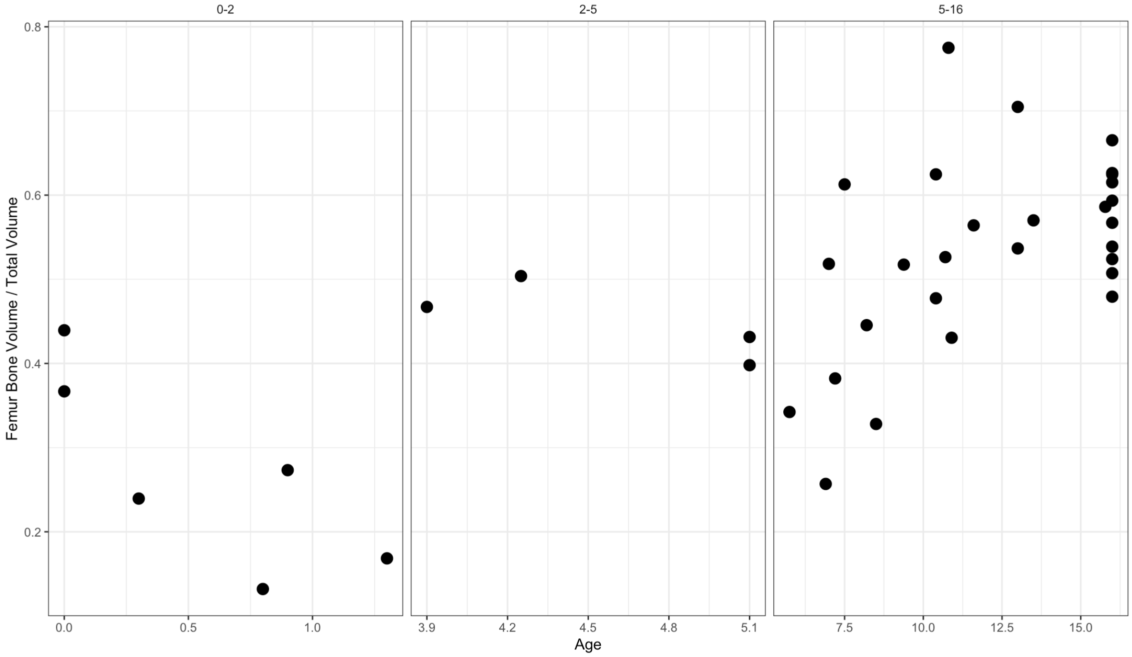

Figure 2.2: Comparison of the femur bone volume fraction change across age from 0-2 years in the left panel, 2-5 years in the middle, and 5-16 years on the right. Age 0-2 years is early infancy, age 2-5 is older infancy and from ages 5-16 is juveniles to adults.

BV/TV in the humerus increased with age (p= 0.02), but the amount of variance explained by age was very low (r-squared = 0.13, Figure 2.3). As in the femur, there appeared to be a drop in bone volume fraction during young infancy (Figures 2.3 and 2.4). There was a slight increase in BV/TV from young infants to older infants and then again from juvenility to adulthood. When chimpanzees were classified into age groups (Figure 2.5), the slight negative relationship between age and BV/TV in the 0-2 and 5-16 age categories. These negative trends were not significant. However, the positive trend during the ages 2-5 category was significant, suggesting an increase in BV/TV during this stage.

In sum, there was a decrease in humeral BV/TV during young infancy, a rebound during older infancy, and another increase from older infancy to juvenility (Figure 2.5). Figure 2.18 reveals these changes in the humerus across ontogeny. These models considered two adolescent individuals who may be outliers. When removed from the model, there was no change in the pattern of relationship between ages 0-2, 2-5, and 5-16 (Figure 2.18).

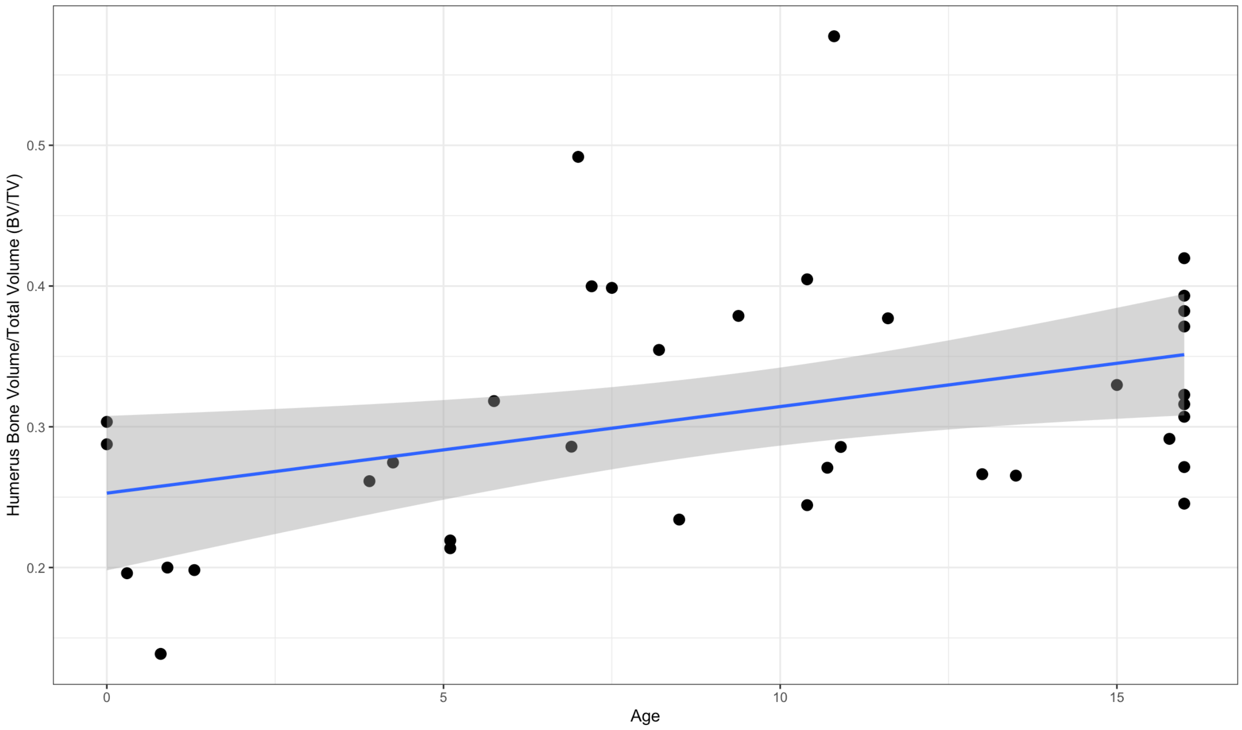

Figure 2.3: Age in years on the x axis, and humerus bone volume fraction (BV/TV) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error.

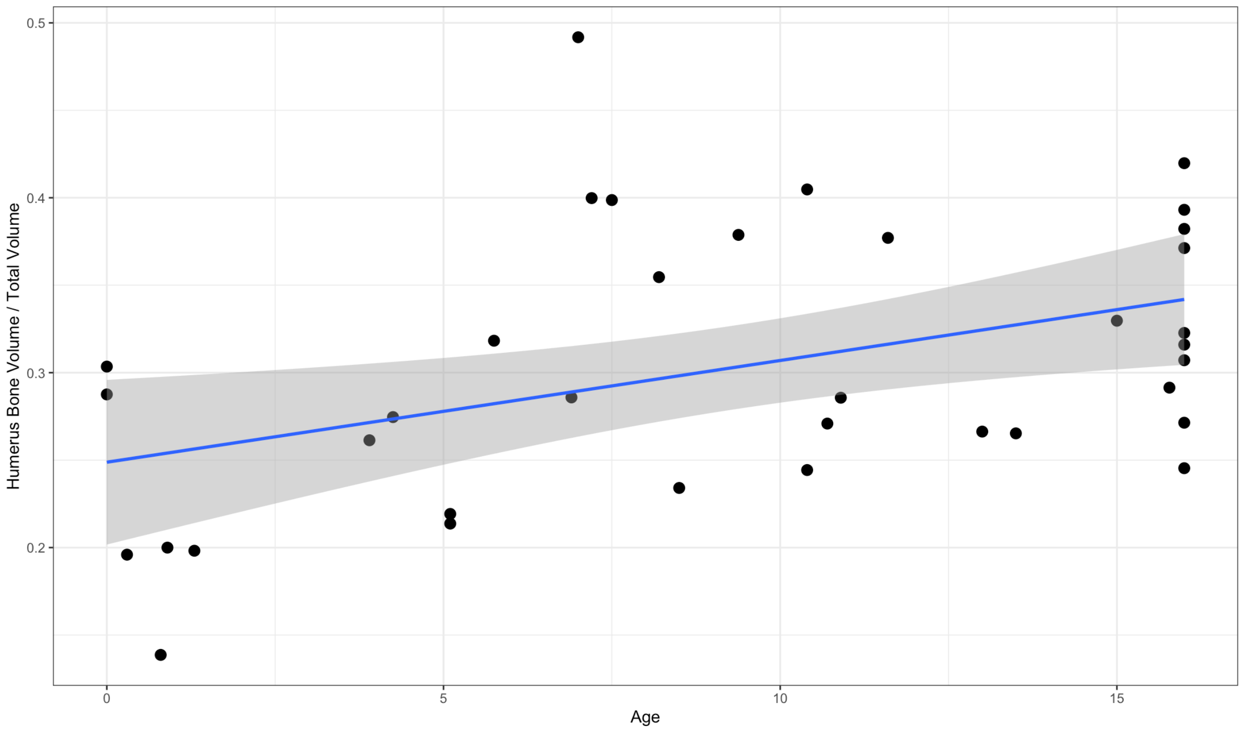

Figure 2.4: Age in years on the x axis, and humerus bone volume fraction (BV/TV) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error. This does not include two adolescents who have relatively high BV/TV.

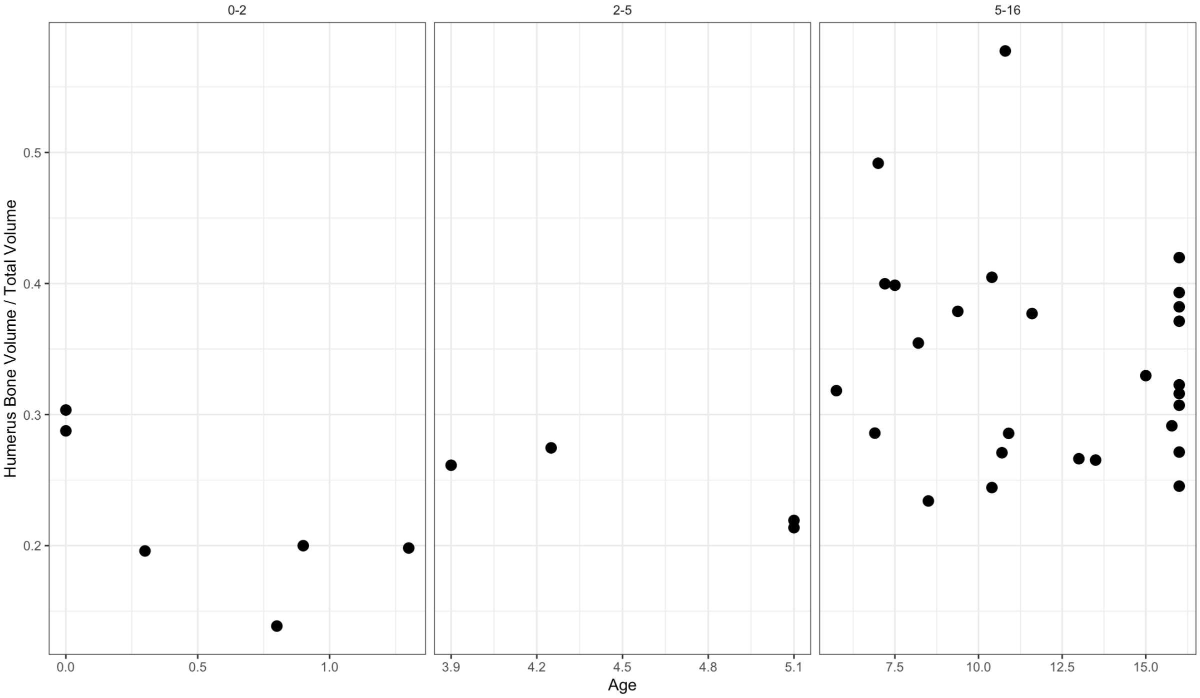

Figure 2.5: A comparison of humerus bone volume fraction change across age from 0-2 years in the left panel, 2-5 years in the middle, and 5-16 years on the right. Age 0-2 years is early infancy, age 2-5 is older infancy and from ages 5-16 is juveniles to adults.

Femoral and humeral BV/TV increased significantly as chimpanzees aged (Femur: KW $\chi 2 = 24.75$, df = 3, p = 1.75e-05; Humerus: KW $\chi 2 = 13.39$, df = 3, p = 0.004) (Figure 2.6). In the femur, infants and juveniles had significantly lower BV/TV values than adults and adolescents (Figure 2.6, Table 2.3). Juveniles had a higher BV/TV relative to infants, but this difference was not significant (Table 2.3). Similarly, adolescents did not differ from adults. In the humerus, infants had significantly lower BV/TV compared to members in all other older age categories (Table 2.3). Juveniles, adolescents, and adults did not differ (Table 2.3).

In sum, BV/TV increased across ontogeny in both bones. There was a more stepwise pattern in the femur, with consistent increases in BV/TV until adolescence when it matched the adult phenotype. In the humerus, there was an increase in BV/TV from infancy to juvenility that was then maintained across the older age categories. The relative change from infancy to adult

BV/TV was greater in the femur than in the humerus (Figure 2.6). The femoral BV/TV ratio increased from a median below 0.4 in infants to a median of nearly 0.6 in adults. Alternatively, the humerus changed from a median of ~0.2 in infants to above 0.3 in adults.

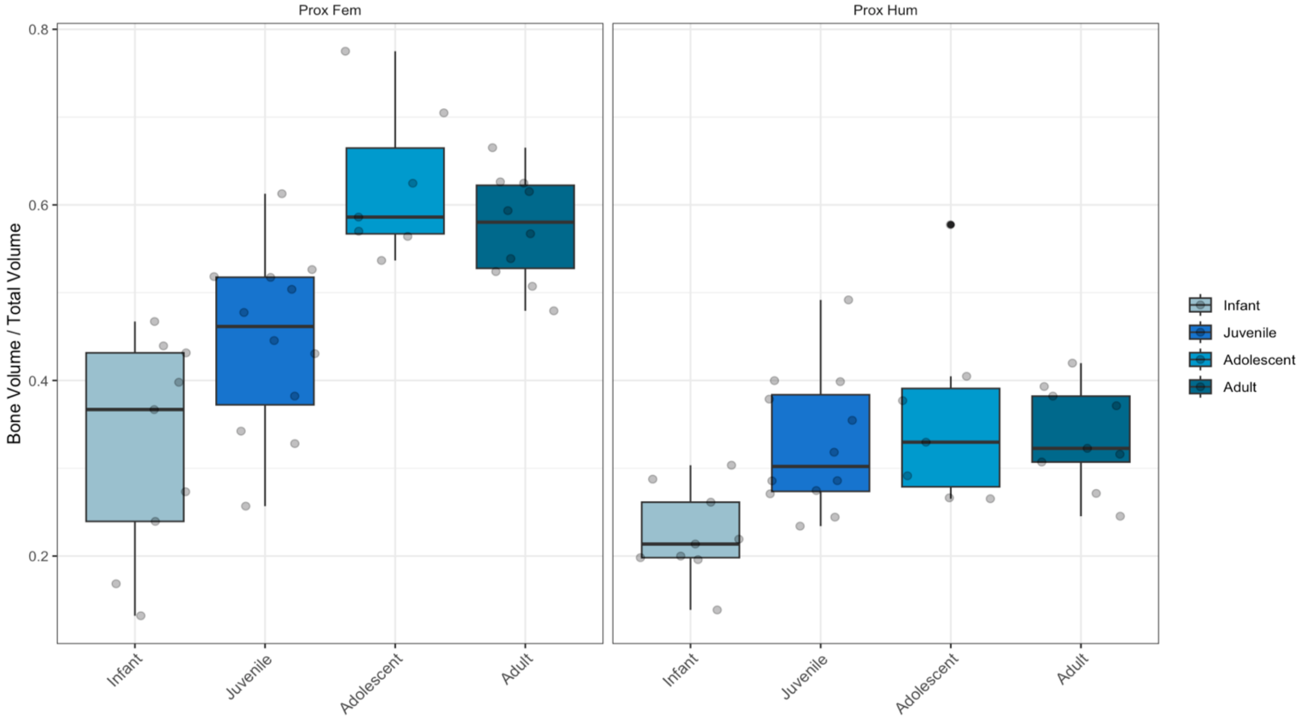

Figure 2.6: Boxplot of bone volume fraction (BV/TV) in infant, juveniles, adolescent, and adult age classes in the femur (left) and humerus (right). BV/TV increases with age.

Table 2.3: P-values from the Kruskal-Wallis comparison of BV/TV values across age categories in the femur and humerus. Significant values are bolded.

Femur	Adult	Adolescent	Infant
Adolescent	0.41732	-	-
Infant	**0.00013**	**0.00052**	-
Juvenile	**0.00228**	**0.00095**	0.04064
Humerus	Adult	Adolescent	Infant
Adolescent	1	-	-
Infant	**0.0047**	**0.0066**	-
Juvenile	0.7813	0.7813	**0.0066**

Finally, the ratio of femoral to humeral BV/TV was compared to assess strength change during development. There was no heterogeneity among members of the different age classes (p=0.13).

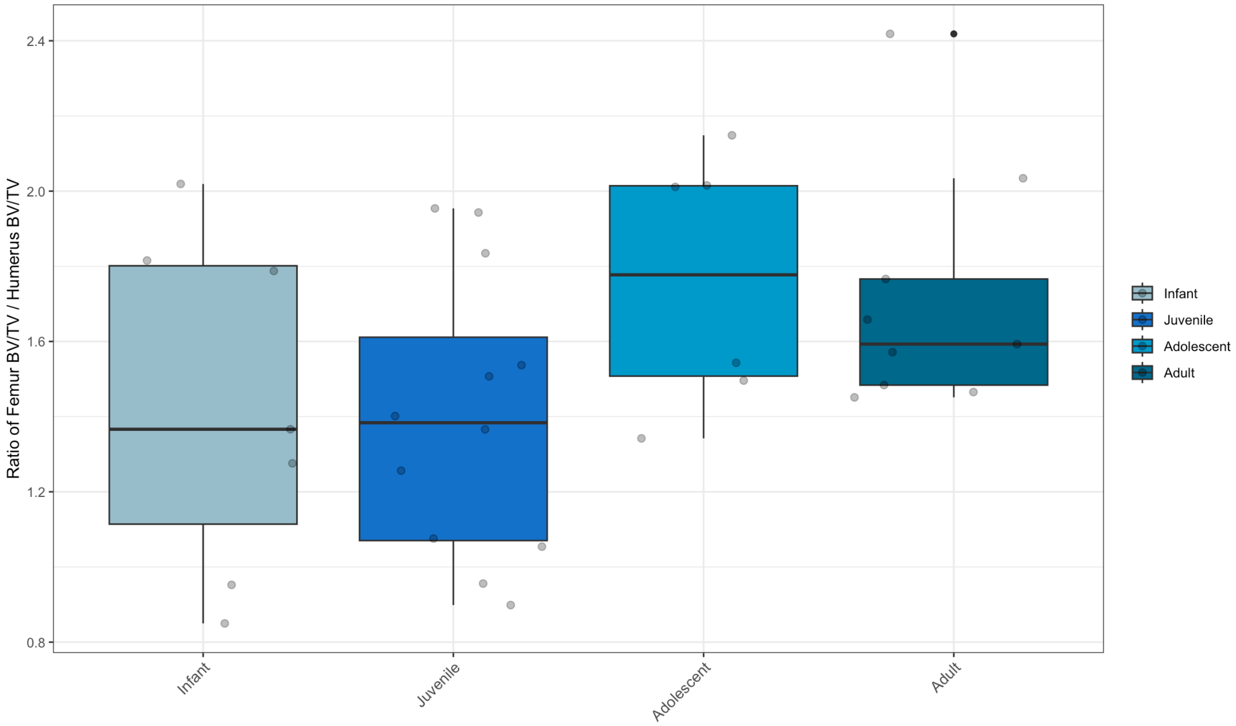

Figure 2.7: Boxplot of the ratio of femoral divided by humeral bone volume fraction (BV/TV) in infant, juveniles, adolescent, and adult age classes.

2.4.2 Trabecular Changes Across Ontogeny: Degree of Anisotropy (DA)

Femoral DA decreased with age (r-squared = 0.22, $p < 0.01$), with the femur becoming increasingly isotropic during development. It was the most anisotropic in newborns and the most isotropic in older infants and adults (Figure 2.9). The difference between the pattern displayed by young infants (0-2) and the pooled sample of individuals of all other ages can be seen in Figure 2.19.

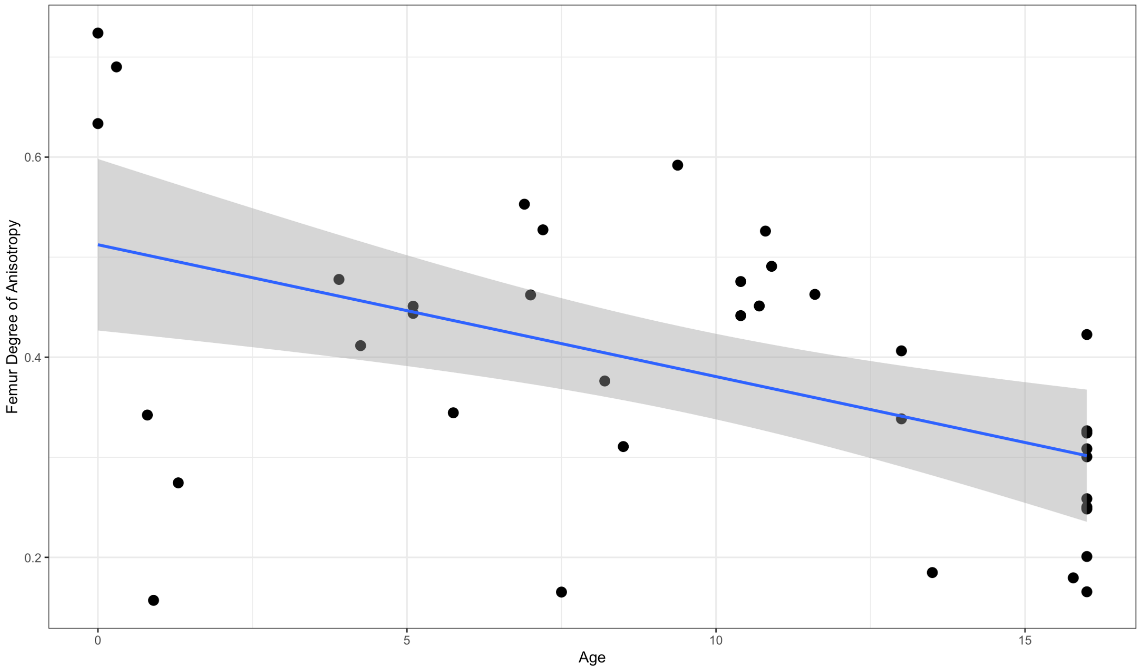

Figure 2.8: Age in years on the x axis, and femur degree of anisotropy (DA) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error.

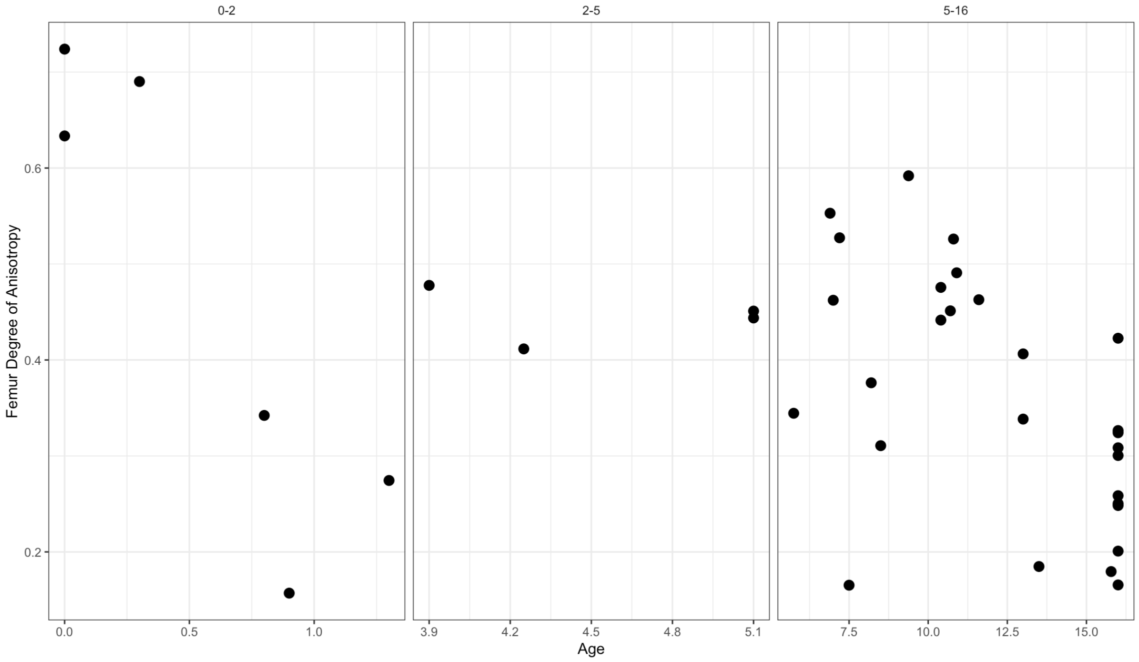

Figure 2.9: A comparison of femur degree of anisotropy change across age from 0-2 years in the left panel, 2-5 years in the middle, and 5-16 years on the right. Age 0-2 years is early infancy, age 2-5 is older infancy and from ages 5-16 are juveniles to adults.

In the humerus, there was a negative relationship between DA and age. DA declined across ontogeny, becoming increasingly isotropic. This result was significant in a linear regression (Figure 2.10) (r-squared: 0.35, p=0.7.11e-05). However, as in the femur, most data points were not encapsulated within the margins of error of the model (Figure 2.10). A second linear regression including age categories had an r-squared of 0.64 (p= 3.67e-08), and found all three age groups had significantly different slopes. As in the femur, younger infants had a steeply negative slope, then older infants had a positive slope from the increasing DA post the young infant drop (Figure 2.11). Amongst the third category the slope was negative, however this was driven by the dramatic change in DA from adolescence to adulthood (Figure 2.11). As with BV/TV, DA did not have a strictly linear relationship with age. The difference between the pattern of young infants (0-2) and all other ages (pooled) can be seen in Figure 2.20.

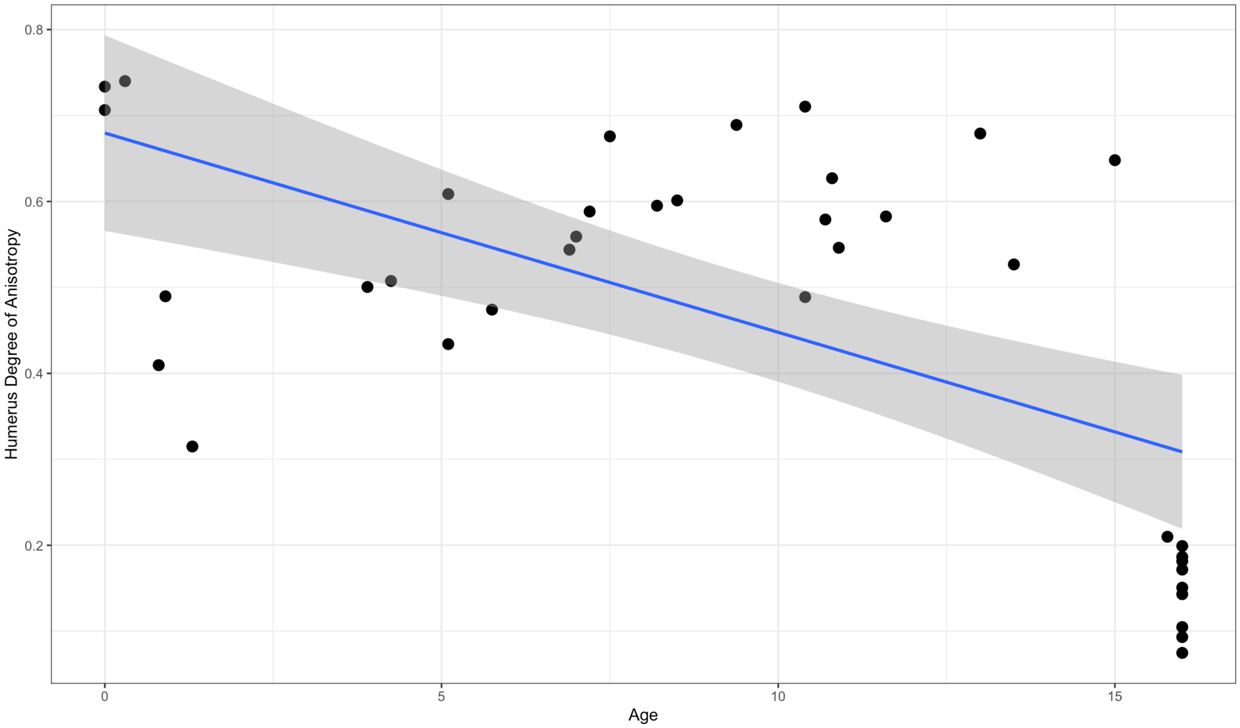

Figure 2.10: Age in years on the x axis, and humerus degree of anisotropy (DA) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error.

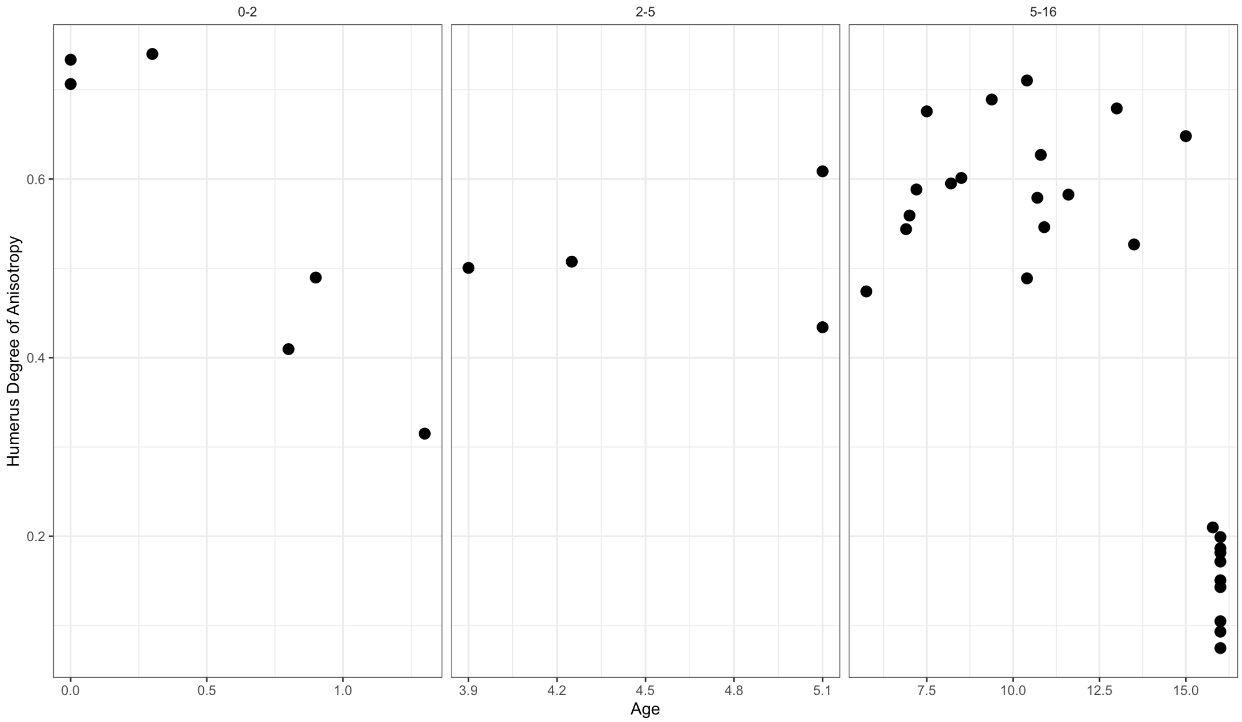

Figure 2.11: A comparison of humerus degree of anisotropy change across age from 0-2 years in the left panel, 2-5 years in the middle, and 5-16 years on the right. Age 0-2 years is early infancy, age 2-5 is older infancy and from ages 5-16 are juveniles to adults.

Due to non-normality of the data, a Kruskal-Wallis test with a Bonferroni correction was used to investigate differences in DA across age categories (Femur: KW $\chi 2 = 9.46$, df = 3, p = 0.02; Humerus: KW $\chi 2 = 20.24$, df = 3, p = 0.0002). DA in the femur was significantly more isotropic in the adults than in juveniles before the Bonferroni correction, but not after (Table 2.4) (Figure 2.12). Values for infants trended in the same direction as the juveniles but were not significantly different from adults (Table 2.4). There were no differences between adults and adolescents in femoral DA. In the humerus, all subadult ages had significantly higher DA compared to adults (Table 2.4), but not from one another. Humeral DA remained consistent across ontogeny, and was relatively anisotropic, until adulthood when it became dramatically more isotropic (Figure 2.12).

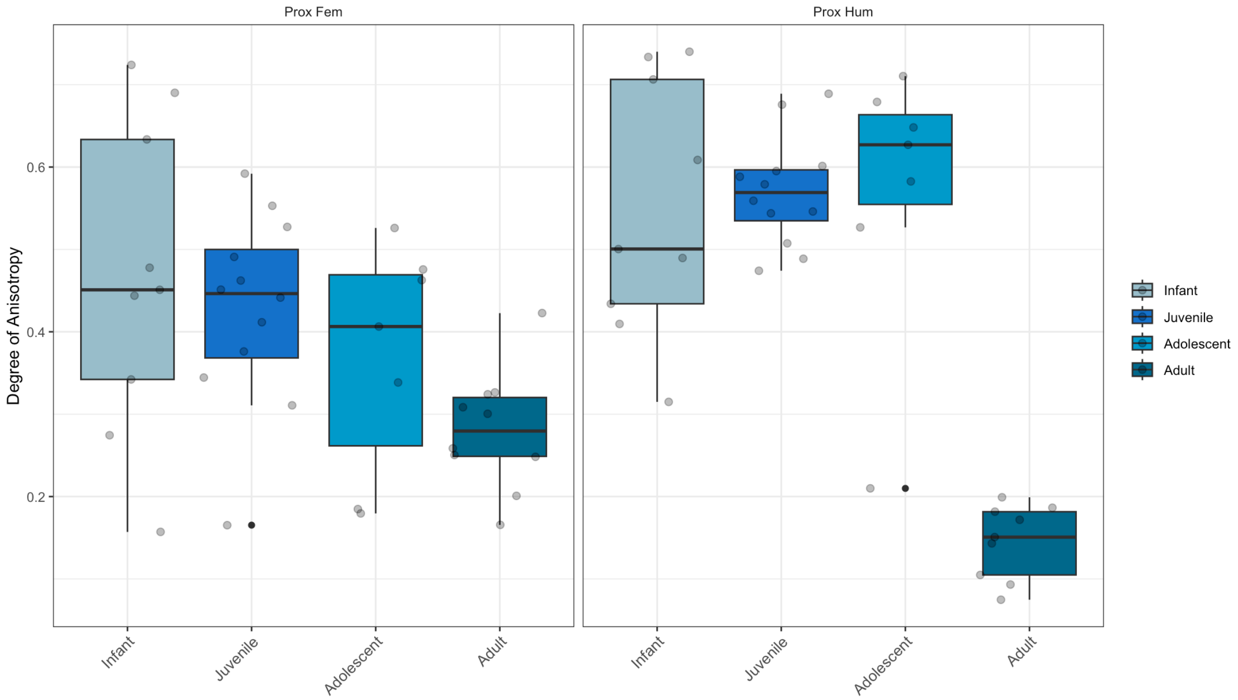

Figure 2.12: Boxplot of Degree of Anisotropy (DA) in infant, juveniles, adolescent, and adult age classes. DA decreases across ontogeny in both bones, however the pattern of change across ontogeny differs.

Table 2.4: P-values from the Kruskal-Wallis comparison of DA values across age categories in the femur and humerus. Significant values are bolded.

Femur	Adult	Adolescent	Infant
Adolescent	0.323	-	-
Infant	0.052	0.579	-
Juvenile	0.016	0.579	0.754
Humerus	Adult	Adolescent	Infant
Adolescent	**0.00035**	-	-
Infant	**0.00012**	0.8078	-
Juvenile	**4.10E-05**	0.57668	0.8078

2.4.3 Cortical and Trabecular Relationship: Bone Volume Fraction (BV/TV) and Cortical Area (CA):

Bone volume fraction (BV/TV) had a significant positive relationship with cortical area in both the femur (R-squared: 0.59, $p << 0.001$) and humerus (R-squared: 0.28, $p < 0.01$). There was no significant effect of age in either the femur or humerus; in other words, the relationship between BV/TV and cortical area was consistent across ontogeny (Figure 2.13). the humerus, there were two adolescents whose residuals were well outside the confidence intervals (Figure 2.14). The analysis was run excluding those two individuals, and the results were consistent with the previous model. Age did not have a significant effect on the relationship between BV/TV and cortical area. There was a significantly positive relationship (p=0.002) between cortical area and BV/TV in the humerus without the two possible outliers (Figure 2.15). Notably, the femur had a more positive slope compared to the humerus (p=1.98e-08) (Figure 2.16): per each unit increase of CA in the femur, BV/TV increased more than it did in the humerus. This can be seen in the slopes of Figures 2.13 and 2.14, and also when comparing the plots of Figure 2.16.

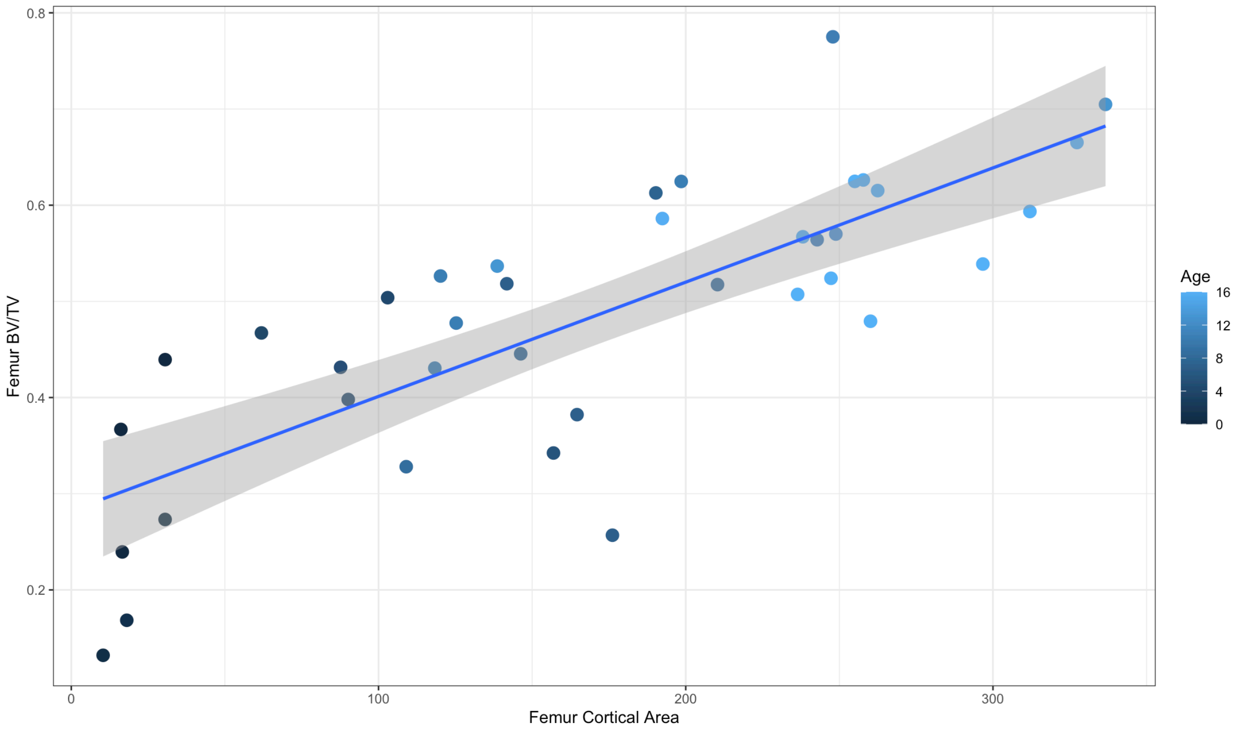

Figure 2.13: Femur cortical area on the x axis, and femur bone volume fraction (BV/TV) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error. Age is colored from darkest (infants) to lightest (adults).

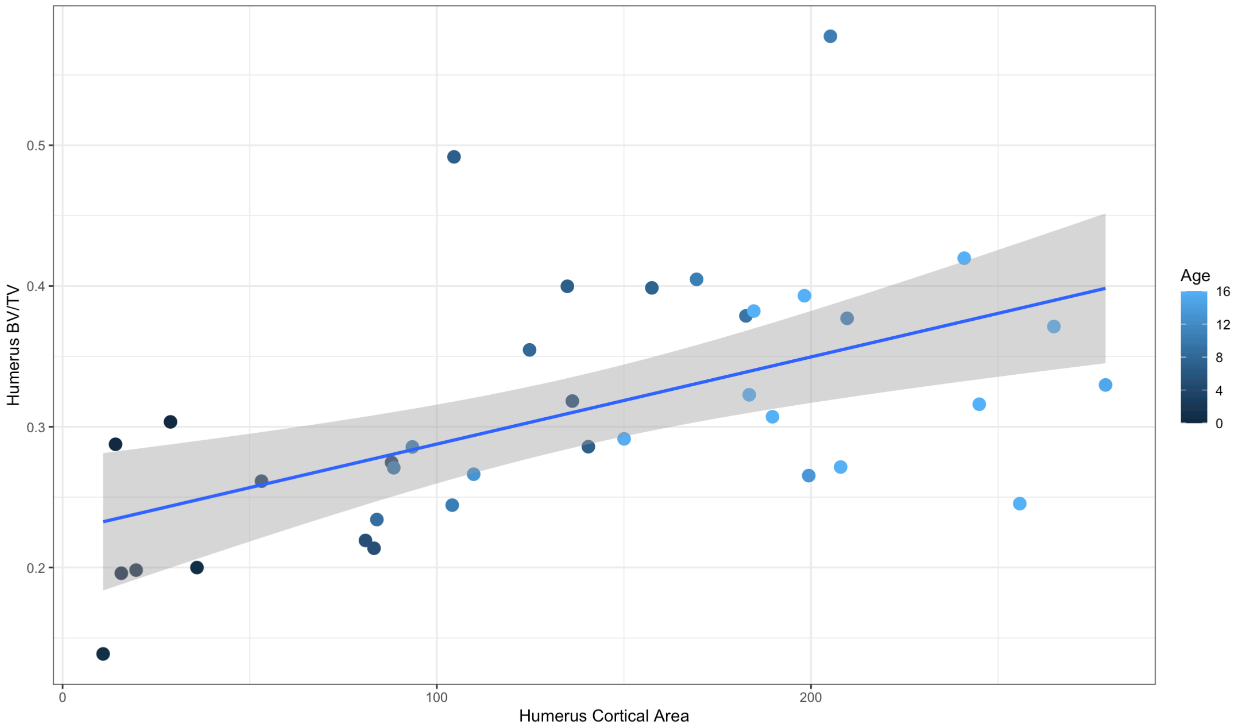

Figure 2.14: Humerus cortical area on the x axis, and femur bone volume fraction (BV/TV) on the y axis. The blue line represents the slope, and the shaded grey areas the standard error. Age is colored from darkest (infants) to lightest (adults).

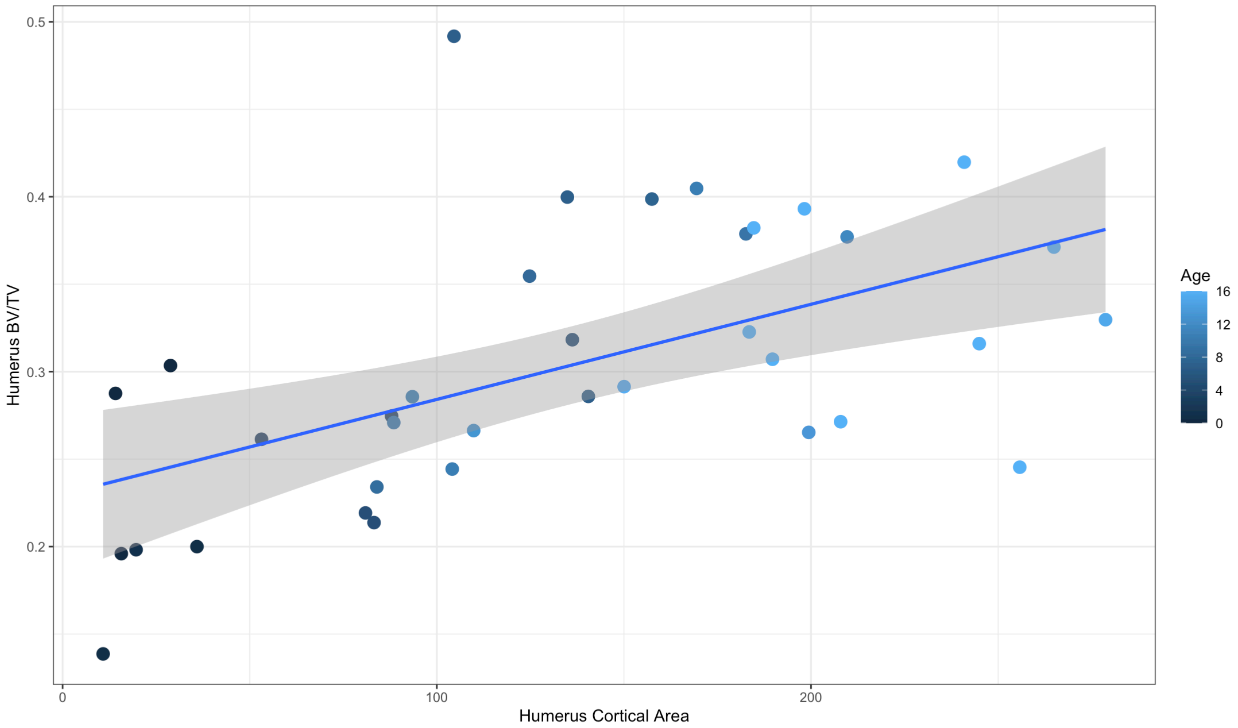

Figure 2.15: Humerus Cortical Area on the x axis, and humerus bone volume fraction (BV/TV) on the y axis. The blue regression line represents the slope, and the shaded grey areas the standard error. This model does not include two adolescents who were outliers

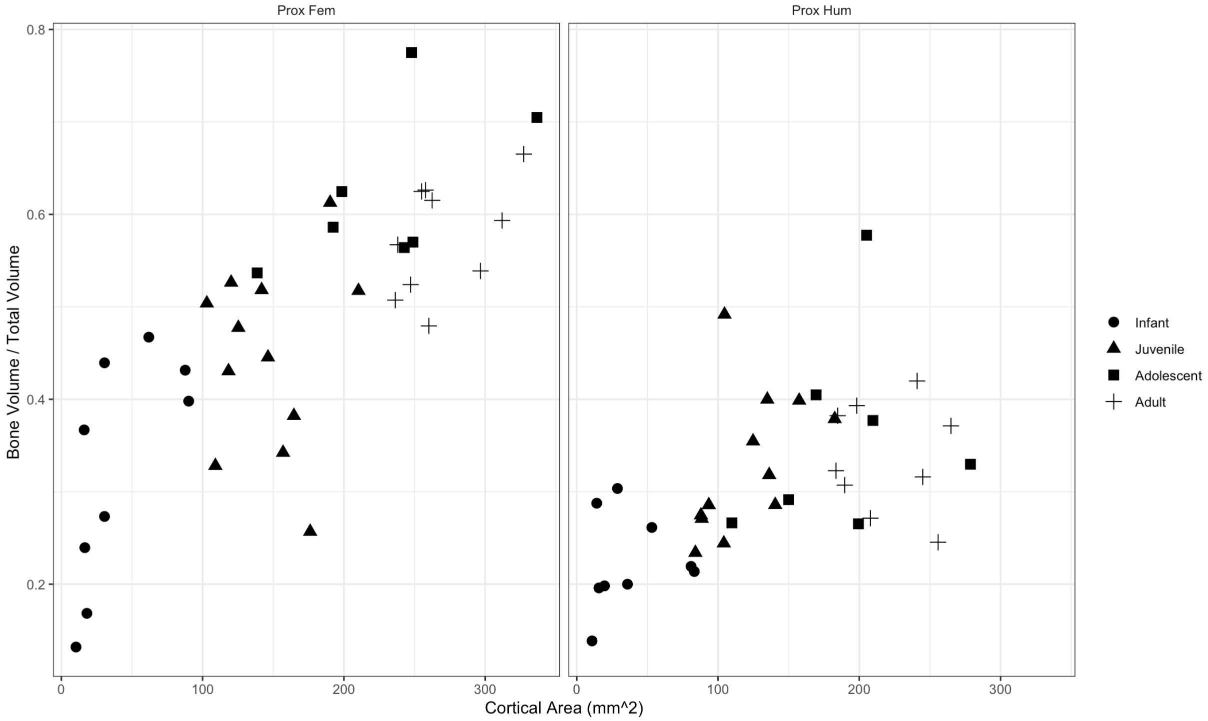

Figure 2.16: Cortical Area on the x axis, and bone volume fraction (BV/TV) on the y axis in both the femur and the humerus. Shape denotes age.

2.4.4 Cortical and Trabecular Relationship: Bone Volume Fraction (BV/TV) and Cortical Torsional Strength (J)

There was no significant relationship between *J* and BV/TV in either the femur or humerus (p = 0.98, p=0.81) (Figures 2.22, 2.23). Age did have a significant effect in both bones; as individuals became older, the relative increase of *J* for a unit of BV/TV increased (Femur: p= 0.0013, Humerus: p= 2.27e-11). This indicates that adult individuals had a higher *J* for a given BV/TV than infants, for example. In both bones, infants tended to have higher variability in both *J* and BV/TV compared to older aged individuals, who had less variation in log-transformed *J* and more variation in BV/TV. However, *J* was not an accurate predictor of bone volume fraction in either the femur or humerus.

2.4.5 Cortical and Trabecular Relationship: Degree of Anisotropy (DA) and Cortical Ellipticity (I_{max}/I_{min})

There were no significant relationships between degree of anisotropy and I_{max}/I_{min} or age in the femur (I_{max}/I_{min} p=0.24 and age p=0.08) (Figure 2.24). In the humerus there was no significant effect of I_{max}/I_{min} on DA (p= 0.11) (Figure 2.25). There was a negative relationship between I_{max}/I_{min} and DA across age (p=0.00025). When considered by age category, DA values for a given I_{max}/I_{min} were higher in all subadults than in adults (Table 2.9). Adults had both lower DA values (meaning more isotropic trabecular orientations), and more elliptical cross-sections compared to the subadult age categories.

2.4.6 Correlations in Trabecular Properties Across Age and Bone

The relationships between BV/TV in the femur and humerus across ontogeny (Table 2.5) and DA in the femur and humerus across ontogeny (Table 2.6) were tested using a Spearman's rank correlation test. There were no significant correlations between the femur and humerus for BV/TV or DA at any age category. Cortical and trabecular correlations (CA vs. BV/TV and *J* vs. BV/TV) were also tested within each bone. There were no significant correlations between cortical or trabecular properties at any age stage post-Bonferroni correction in either the femur (Table 2.7) or humerus (Table 2.8).

Table 2.5: Spearman's Rho comparing BV/TV correlations in the femur and humerus within age. Significant values are bolded.

Spearman's Rho	Humerus Infant	Humerus Juvenile	Humerus Adolescent	Humerus Adult
Femur Infant	0.75			
Femur Juvenile		0.273		
Femur Adolescent			0.714	
Femur Adult				0.383

Table 2.6: Spearman's Rho comparing DA correlations in the femur and humerus within age. Significant values are bolded.

Spearman's Rho	Humerus Infant	Humerus Juvenile	Humerus Adolescent	Humerus Adult
Femur Infant	0.75			
Femur Juvenile		0		
Femur Adolescent			0.714	
Femur Adult				0.633

Table 2.7: Spearman's Rho comparing trabecular and cortical correlations in the femur and within age. Significant values are bolded.

Spearmans Rho	Infant CA	Juvenile CA	Adolescent CA	Adult CA
Infant BV/TV	0.733 (p=0.03)			

Juvenile BV/TV		0.077		
Adolescent BV/TV			0.536	
Adult BV/TV				0.442
Spearmans Rho	Infant *J*	Juvenile *J*	Adolescent *J*	Adult *J*
Infant BV/TV	0.467			
Juvenile BV/TV		0.147		
Adolescent BV/TV			0.321	
Adult BV/TV				-0.321
Spearmans Rho	Infant I_{max}/I_{min}	Juvenile I_{max}/I_{min}	Adolescent I_{max}/I_{min}	Adult I_{max}/I_{min}
Infant DA	0.25			
Juvenile DA		-0.098		
Adolescent DA			-0.786 (p 0.048)	
Adult DA				-0.018

Table 2.8: Spearman's Rho comparing trabecular and cortical correlations in the humerus and within age. P-values are in brackets when significant pre-correction.

Spearmans Rho	Infant CA	Juvenile CA	Adolescent CA	Adult CA
Infant BV/TV	0.35			
Juvenile BV/TV		0.685 (p=0.017)		
Adolescent BV/TV			0.393	
Adult BV/TV				-0.183
Spearmans Rho	Infant *J*	Juvenile *J*	Adolescent *J*	Adult *J*
Infant BV/TV	0.133			
Juvenile BV/TV		0.378		
Adolescent BV/TV			-0.214	
Adult BV/TV				-0.567
Spearmans Rho	Infant I_{max}/I_{min}	Juvenile I_{max}/I_{min}	Adolescent I_{max}/I_{min}	Adult I_{max}/I_{min}
Infant DA	-0.417			
Juvenile DA		-0.182		
Adolescent DA			0.143	
Adult DA				0.4

2.5 Discussion

This paper asked two questions: does trabecular bone respond to age related locomotor change in *Pan,* and are the patterns of cortical and trabecular response to changes in locomotor behavior correlated? It was predicted that infants would have trabecular BV/TV and DA changes that reflect the forelimb dominated locomotion during those periods of ontogeny, such as high isotropy, higher bone volume fraction (BV/TV) in the forelimb, and stronger forelimbs relative to their hindlimbs. Juveniles would be intermediate between infants and adults and adolescents. Adults and adolescents were predicted to have more anisotropic trabeculae, higher BV/TV in the femur, and hindlimbs stronger than their forelimbs. Cortical and trabecular properties were predicted to correlate more strongly in the humerus during infancy and juvenility with stronger correlations in the femur during adolescence and adulthood.

Results of this study indicated that trabecular responses to age-related locomotor changes in *Pan* were limited. BV/TV increased across ontogeny, with the exception of young infants who had a dramatic decrease in bone volume in both the femur and humerus between ages 0-2. BV/TV did not differ between adolescents and adults and was higher in the femur than in the humerus. Femoral BV/TV was lower in infants and juveniles compared to adolescents and adults. Infants did not differ in their BV/TV values from juveniles in their femurs. By contrast, infant humeral BV/TV values were significantly lower than those in juveniles, adolescents, and adults. The ratio of femoral to humeral BV/TV did not vary across age categories. These results did not match predictions, although there were clear differences in BV/TV acquisition between the femur and humerus.

DA also did not match predictions; DA was predicted to increase over ontogeny, but results showed a non-significant stepwise decline in the femur. Humeral DA values remained relatively high during infancy, juvenility, and adolescence before decreasing in adulthood. Finally, cortical and trabecular properties did not seem to be linked. The exception to this was that cortical area was modestly predictive of trabecular bone volume.

2.5.1 Trabecular Properties Across Ontogeny

Trabecular BV/TV and DA did not match predicted phenotypes given locomotor ontogeny. Although the ratio of BV/TV in the femur vs. humerus were lower in infants and juveniles compared to adolescents and adults, indicating a relatively stronger humerus compared to the femur, this difference was not significant, indicating relative BV/TV did not change with age (Figure 2.7). It should be noted that mechanical loading is only one mechanism that can influence trabecular bone morphology, and other factors such as hormonal changes (Kalu et al., 1989; Ohlsson et al., 1998; Compston, 2001; Cauley, 2015) and life history events such as reproduction (Mallinson et al., 2013; Hyde et al., 2017; Goshtasebi et al., 2018; O'Brien et al., 2021), stress (Mori et al., 2014; Wippert et al., 2019) and limited energy availability (Karlsson et al., 2000; Misra et al., 2008; Papageorgiou et al., 2018) may also play a role. However, across ontogeny it is likely that the greatest contributors to plasticity in trabecular bone morphology is mechanical loading, body size, and canalized or developmental controls.

2.5.2 Locomotion

There were limited significant effects of locomotion on trabecular bone volume fraction across ontogeny. The closest signal found was in the pattern of relative femoral to humeral BV/TV: infants and juveniles had relatively stronger humerii compared to their femora, while adults and adolescents had relatively stronger femora compared to humeri (Figure 2.7). Previous studies found similar patterns, with nonsignificant trends of increased femoral to humeral BV/TV ratio across ontogeny (Tsegai et al., 2018). Although Tsegai et al. (2018) did not compare adult to subadult morphology, our findings from limb elements indicated limited response of trabecular bone to age-related locomotor signal within *Pan troglodytes*.

DA became more anisotropic across ontogeny in both the femur and humerus, which did not fit the predictions of this study. It was predicted that DA in the humerus would become more isotropic across ontogeny. This finding is consistent with modern human femoral trabecular orientation, which is highly isotropic in infants, and becomes progressively more anisotropic through life, resulting in increased bone strength in the face of an increasing body mass and increasingly stereotypical loading (Ryan and Krovitz, 2006; Raichlen et al., 2015; Milovanovic et al., 2017). Humans exhibit regional patterns of variation in trabecular orientation of the proximal femora through ontogeny (Milovanovic et al., 2017), although it is not clear if these are predetermined or develop due to loading. Similarly, DA changes through ontogeny in the human distal tibia, marking the shift from kinematic instability while learning to walk to kinematic stability once bipedal walking is learned (Raichlen et al., 2015). However human locomotion is not as variable as in *Pan*, and increasingly anisotropic DA fit what was predicted from stereotypical loading of human bipedality.

In *Pan,* DA did not significantly differ in the femur or humerus across subadult age categories in a previous study (Tsegai et al., 2018). This is in conflict to results from this current study, which found that DA changed significantly in the humerus between subadults and adults. Other elements from non-human hominoids, for example the carpals and tarsals, showed mixed trabecular response to locomotor behavior across ontogeny. In a principal components analysis (PCA) of trabecular morphology in the capitate and third metacarpal, DA separated juvenile and adult *Pan* (Ragni, 2020). However, when DA values were compared statistically across age categories there were no significant differences (Ragni, 2020). Trabecular orientation did vary over ontogeny, with *Pan* maintaining more variable orientation compared to *Gorilla* of the same age category. During the juvenile stage in *Pan*, trabecular orientation was more proximo-distally oriented, matching the directionality of tensile stress during suspension, but during adulthood the orientation of trabeculae in both the capitate and third metacarpal changed to an anterio-posterior orientation, matching predicted orientations for terrestrial knucklewalking (Ragni, 2020). Similar to the carpal and metacarpal, trabecular bone morphology in the *Pan* calcaneus somewhat follows locomotor ontogenetic changes, with the best explanatory model including body mass, percent of adult brain size, and locomotor onset (Saers et al., 2022a). Further, mean ground reaction forces are strongly correlated with BV/TV in the calcaneus of *Pan* (Saers et al., 2022a). However, the change in morphology stops at age 5, the shift from infancy to the juvenile stage, which the authors attribute to onset of adult locomotion Saers et al. (2022a). However, this is not quite accurate, as there is still a dramatic decrease in the proportion of suspensory behavior and increase in terrestrial quadrupedalism in adolescents (age 10-15) compared to juveniles (age 5-10) (Sarringhaus et al., 2014, 2016). These results demonstrate that hand and foot position across ontogeny in *Pan* is reflected in trabecular bone, unlike what is seen in the femur and humerus.

A necessary further step to understand locomotor effects on trabecular bone across ontogeny in limb bones is the addition of more infants (age 0-5 years). This time period is critical, particularly ages 0-3 when trabecular bone volume decreases dramatically and then rebounds (Figure 2.3). This trend of decreasing BV/TV and DA in the 6 months to one year post birth, followed by a steady increase in BV/TV and DA until age 5, is shown in many species, such as humans (Ryan and Krovitz, 2006; Acquaah et al., 2015; Colombo et al., 2019; Saers et al., 2022a), macaques (Saers et al. 2022b), Neanderthals (Chevalier et al., 2021), and now chimpanzees (this paper). This loss may reflect the limited mobility of infants during this time; in *Pan,* infants begin showing signs of independent movement at around five months of age (Doran, 1992; Sarringhaus et al., 2014). For example, during the first five months of life, one infant only engaged in sitting, lying, or clinging (Sarringhaus et al., 2014). However, even during this period of limited independent mobility, infants are still grasping onto their mothers and loading their humerus, and previous studies have posited that muscle contractions would be sufficient to increase trabecular bone strength (Lam and Qin, 2008; Acquaah et al., 2015). Thus, the rate of loss that occurs in both the femur and humerus is surprising, and it remains to be determined whether this rapid loss of bone followed by steady gains is genetic or developmental. Although the underlying mechanism is unclear, the prevalence of the pattern amongst different species across primates indicates a degree of developmental canalization.

The type of mechanical loading may influence the degree of trabecular bone response (Judex and Carlson, 2009), and different trabecular parameters may have varying sensitivities to changes in loading regime. Rubin et al. (2001) found that low magnitude, high frequency vibration in sheep was sufficient to generate a significant increase in trabecular bone mineral density within the proximal femur (34.2% increase relative to controls). In terms of DA, a study of juvenile guinea fowl found that only 10-15 minutes per day of inclined treadmill activity altered trabecular bone orientation compared to controls (Pontzer et al., 2006). However, Carlson et al. (2008) found that cortical bone was more responsive in mice when given linear vs. curved housing enclosures, and found no differences in trabecular bone. The data presented here do not support the prediction that relative BV/TV would change between the femur and humerus following the marked change from arboreal to terrestrial (forelimb vs. hindlimb) dominated locomotion, and DA decreased only after this locomotor shift had occurred. Thus it may be that BV/TV is restricted by developmental or genetic constraints, or that the changes in loading regime were insufficient to generate a change in BV/TV or DA.

Studies of baboon cortical bone changes across ontogeny suggest a similarly nuanced response to mechanical loading. Baboons increase the proportion of terrestrial locomotion across ontogeny, from 60.2% at 0.5 years years of age (Druelle et al., 2016) to 98.7% of locomotor behavior in adults (Hunt, 1992; Druelle and Berillon, 2013; Hunt, 2016). While a nearly 64% increase in quadrupedalism is not a small change, the limited ontogenetic differences could be attributed to a thresholding effect on cortical thickness. Bone responds best to loads that are high frequency, high magnitude, high duration, and variable (Parfitt, 2003; Martin, 2003). Loads that are sufficient to generate strains that exceed the typical physiologic zone tend to be osteogenic, while underloading leads to no osteogenic response and/or bone resorption (Martin, 2003). The limited differences observed here could indicate that the changes in loading resulting from *Pan* locomotor changes are insufficient to cause a change in skeletal phenotype. Infants show a transient decline in BV/TV in both the femur and humerus, while juveniles have significantly less BV/TV compared to adults and adolescents in the femur. Although juveniles engage in more forelimb dominated behaviors compared to adults and adolescents, there may be enough arboreal behaviors in adults and adolescents to maintain subadult patterns of trabecular bone in the humerus.

2.5.3 Body Size

Across mammals (Doube et al., 2011) and within primates (e.g., MacLatchy and Muller, 2002; Ryan and Shaw, 2013), trabecular morphology shows a mixture of positive and negative allometric scaling with body mass depending on the variable of interest. Within primates, trabecular thickness, spacing, and BV/TV are absolutely greater with higher body mass (Ryan and Shaw, 2013), but when scaled to body mass, struts are thinner and more tightly packed (Doube et al., 2011; Barak et al., 2013; Ryan and Shaw, 2013). This pattern is maintained across mammals and birds as well (Doube et al., 2011). Larger-bodied mammals and birds have absolutely fewer trabeculae that are thicker and more spaced than do smaller-bodied mammals and birds, but relative to body mass, the trabeculae are thinner and more tightly packed (Doube et al., 2011). Primate vertebral trabecular bone scales isometrically with body size (Fajardo et al., 2013). Within *Pan,* body size and sex do not contribute significantly to trabecular morphological variation among adults (Tsegai et al., 2018). For example, trabecular bone volume does not correlate with total joint size or femoral head height (as a proxy for body mass) in the femur, tibia, or humerus (Tsegai et al., 2018). Further, BV/TV and DA do not significantly differ within subadults, indicating limited differences in overall strength and orientation from infancy to adolescence (Tsegai et al., 2018). This also suggests that some of the sex-specific (and thus possibly size-related) differences in behavior, such as a higher degree of quadrupedalism among male vs. female chimpanzees (e.g. Sarringhaus et al., 2014) may be too subtle to pick up in samples that combine subspecies.

The limited difference in trabecular BV/TV across varying body sizes may be due a change in trabecular bone structure, for example a shift from more rod-like to plate-like struts (Ryan and Shaw, 2013). Another explanation could be that trabecular bone volume is constrained by the need to maintain adequate surface area for calcium deposition and release (Kerschnitzki et al. 2013). Alternatively, trabecular morphological change may be limited by constraints required for osteocyte density due to the structure of trabecular bone (Christen et al., 2015). Body size is inversely related to metabolic rate, and subsequently bone cell density and number also have an inverse relationship with mass (Christen et al., 2015). This hypothesis is supported by previous studies showing larger animals have longer bone remodeling cycles (Reinwald and Burr, 2008) and also have fewer osteocytes than smaller animals do (Mullender et al., 1996). These results led to the conclusion that metabolism, rather than body size or mechanical loading, constrained trabecular remodeling and morphology (Mullender et al., 1996).

2.5.4 Canalization and Development

The limited response of trabecular bone to changes in mechanical loading across ontogeny may be due to genetic and/or developmental constraints. Further, the difference between the femur and humerus can be attributed to canalized traits (Ryan and Walker, 2010; Ryan and Shaw, 2012). For example, femoral BV/TV is consistently higher amongst primate adults compared to the humerus traits (Ryan and Walker, 2010; Ryan and Shaw, 2012). While this relationship has not been well studied in non-human apes, evidence from humans and from experimental models indicates that trabecular bone response could be tempered through genetic or developmental effects.

Trabecular bone morphology has been shown to be highly site specific. A study of two lines of mice, C3H and B6, found that C3H mice had enhanced cortical structure in the femur but conversely had less trabecular structure in both the proximal femur and lumbar vertebrae (Turner et al., 2000). Judex et al. (2004) found that in a study of three inbred mouse lines, there was no single strain load that consistently demonstrated similar morphological change across two trabecular and four cortical regions of interest. For example, in the epiphysis and metaphysis regions there were no consistent patterns of change in trabecular response to strain among the two regions, within or across the three lines of mice (Judex et al., 2004). This finding demonstrates that there are specific genetic and developmental restrictions on response, even amongst regions that are close to one another anatomically. Similarly, Judex et al. (2013) showed that in mice subject to hindlimb unloading, BV/TV, Tb.Th, and BMD increased upon re-ambulation, while Conn.D, Tb.N, and Th.Sp decreased. During the unloading phase, nearly 20% of variation in BV/TV was linked to six chromosomal locations, while during re-ambulation BV/TV was linked to only one, demonstrating the complex genetic basis of trabecular bone sensitivity to altered mechanical signals (Judex et al., 2013). Relative cortical area of a lumbar vertebra in AXB/BXA recombinant mice was found to have a slight negative correlation with relative bone volume (Tommasini et al., 2009). In the path analysis, trabecular mass and mineralization were dependent on cortical traits. When total tissue mineral density, relative cortical area, and BV/TV were included in analysis, 88% of the correlation length were explained. This indicates functional integration between trabecular and cortical traits, and how changes in one bone compartment will impact the other (Tommasini et al., 2009).

In baboons, BV/TV is strongly influenced by genetics, with a heritability score of 0.55 and total variation due to genetics estimated at 48% (Havill et al., 2010). Age and sex accounted for less than 12% of variation in bone mechanical properties, though the study used only adults of primarily early to middle ages (Havill et al., 2010). Interestingly, there was a limited effect of sex, which was unexpected given the levels of sexual dimorphism in baboons. Studies in rats reported similar results, with at least eight different quantitative trait loci (QTLs) linking the femur and lumbar vertebrae (Alam et al., 2005). Many of these points were homologous between rats, mice, and humans, including locations that have been associated with hip and femoral BMD levels (Beamer et al., 2005; Alam et al., 2005). In a study of trabecular bone in the human ilium, results demonstrated an inherent predetermined trabecular structure (Cunningham and Black, 2009a,b), further supporting the impact of developmental canalization on trabecular morphology.

In human infants there is a dramatic decrease in BV/TV within the femur, humerus, and tibia (Gosman and Ketcham, 2009; Acquaah et al., 2015; Milovanovic et al., 2017). This phenomenon was not described in non-human apes before (Tsegai et al., 2018), but was noted in Neanderthal infants (Chevalier et al., 2021). This decrease in infant BV/TV has been attributed to sculpting of bone due to overproduction during gestation (Acquaah et al., 2015). The trabecular structure is continually modified through childhood (Acquaah et al., 2015). In the spine, trabecular bone in the final stages of gestation becomes isotropic, then anisotropic post-loss, and the mass is never recovered (Acquaah et al., 2015). These results demonstrate that during infancy and childhood there is a developmental or genetic link with trabecular BV/TV that impacts the ability to reconstruct locomotion from trabecular bone, at least in individuals before three years of age.

DA appears to be more developmentally plastic than BV/TV and hasn't been associated as strongly with genetic factors. It therefore has been hypothesized as a better reflection of mechanical loading (Fajardo and Muller, 2001). In experiments assessing differential load on sheep femora, DA was found to be consistent even when other mechanical properties of bone changed (such as BV/TV) (Mittra et al., 2006). Studies across ontogeny in humans found that DA in the femur and tibia both stabilized at an adult-like pattern at around six to eight years of age which is after an adult like gait is achieved (Ryan and Krovitz, 2006; Gosman and Ketcham, 2009). However, in the carpals and tarsals of non-human hominoids, DA and trabecular orientation shifted more consistently with locomotor repertoire (Ragni, 2020; Saers et al., 2022a,b). Overall the findings indicate that early in ontogeny there were changes in the DA that were not associated with locomotor shifts, but later in ontogeny DA shifts more consistently with locomotor variation.

Lovejoy et al. (2003) hypothesized that bone patterning is the result of positional information from mesenchymal cells in early growth, which suggests limited trabecular response across ontogeny, because patterns would be set very early in development. This prediction is consistent with data from the ilium of humans, where trabecular bone orientation appears to be established early in development (Cunningham and Black, 2009a,b). Skedros et al. (2007) found similar patterns in the ovine calcaneus. Conversely, there are many studies that demonstrate that human trabecular bone does respond to locomotion at multiple sites within the tarsals and long bones (e.g. Raichlen et al., 2015; Saers et al., 2016; Saers et al., 2020; Saers et al., 2022a,b; Figus et al., 2022). Overall, these results demonstrate that the magnitude of trabecular bone responses is complex and dependent on bone, skeletal location, and age.

2.6 Limitations and Further Directions

Future studies seeking to understand the relationship between cortical and trabecular bone need to include larger sample sizes, particularly of young infants. For trabecular focused studies, an ontogenetic series that uses newborns as the starting point of bone morphology may not be the best for studies interested in skeletal responses to locomotion. Rather, it may be worthwhile for future studies to begin their ontogenetic series post-bone loss in infants. Impacts of locomotion on trabecular morphology are better reflected post-loss, and thus while inclusion of younger infants is important to best reconstruct overall ontogenetic trends, post-loss is a better indicator of the 'starting' morphology that locomotion impacts. Future studies should consider maintaining homologous trabecular regions of interest to sample. It is necessary to try to maintain a homologous location within the bone at the largest ROI possible so that all ages can be compared equivalently. Among adults the primary trabecular analysis methodology has taken cubic or volumetric regions of interest from the head of the femur and humerus (Kivell, 2016; Tsegai et al., 2018). However, infants and juveniles either lack an epiphysis or have an extremely small one. This results in difficulties sampling regions that reflect the same area across different age groups. To avoid sampling the epiphysis in adults and adolescents, and metaphysis in infants and juveniles it would be better to sample the femoral neck and anatomical neck in the humerus. This would result in homologous regions of interests, but smaller regions of interest in adults. While achieving the largest possible ROI is important (Kivell, 2016), homology amongst sampling sites is paramount.

2.7 Conclusion

This study sought to understand how trabecular bone responds to mechanical loading changes across ontogeny, and to investigate cortical and trabecular bone exhibit similar responses to loading. Overall, while cortical and trabecular bone work together to ensure the strength of bones and joints, mechanical loading appears to have differential effects on each. Trabecular bone morphology did not match predictions given locomotor variability across ontogeny in *Pan.* Furthermore, there were no correlations between trabecular and cortical bone, indicating these tissues differ in responsiveness to age-related locomotor changes. Trabecular bone morphology has been shown to be very useful in discerning interspecies differences in locomotion, and its usefulness for inferring individual loading history may be limited by developmental constraints. Additional high resolution study of skeletal plasticity across ontogeny, the functional significance of bone traits, and the extrinsic and intrinsic factors that influence them is necessary to accurately reconstruct fossil locomotion.

2.8 Appendix

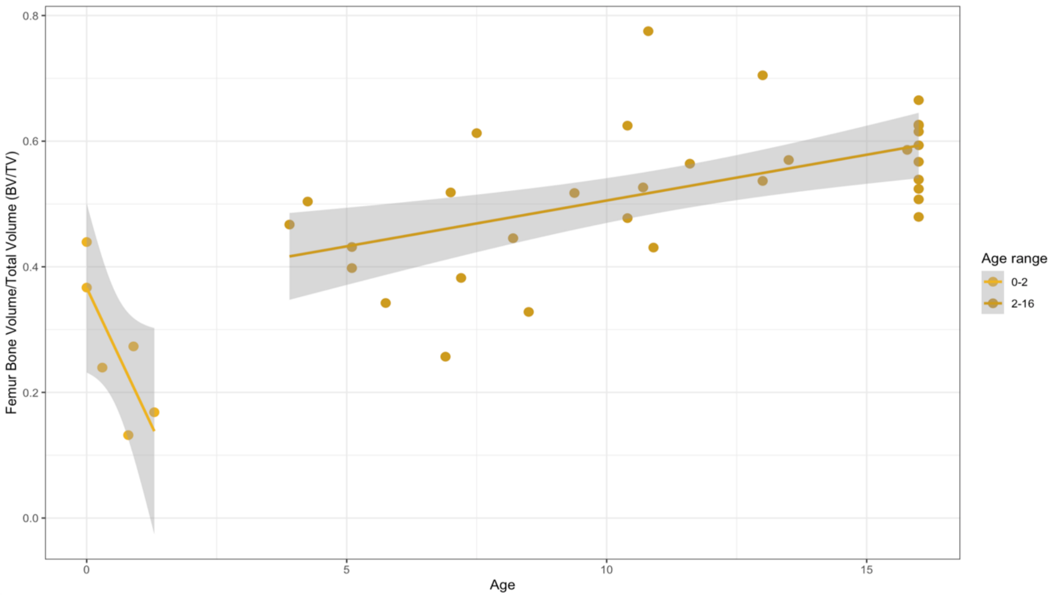

Figure 2.17: Comparison of BV/TV across age in the femur with two age groupings, young infants (0-2 years) and all other ages (2-16 years). Young infants are predominately reliant on their mother and spend a large proportion of their time gripping and holding onto her for transportation relative to other ages. There is a change in slope between BV/TV and Age in the femur during infancy. From ages 0-2 there is a steep negative slope, indicating loss of BV/TV over age. Around age 4 this bone loss has recovered and steadily increases across ontogeny.

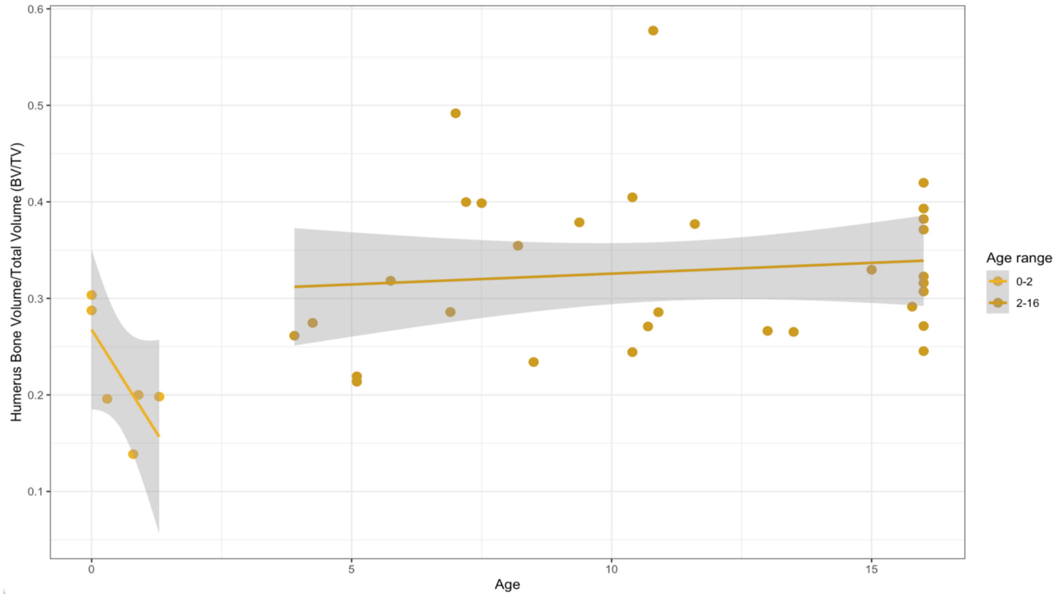

Figure 2.18: Comparison of BV/TV across age in the humerus with two age groupings, young infants (0-2 years) and all other ages (2-16 years) Young infants are predominately reliant on their mother and spend a large proportion of their time gripping and holding onto her for transportation relative to other ages. There is a change in slope between BV/TV and Age in the femur between young infants and older infants. From ages 0-2 there is a steep negative slope, indicating loss of BV/TV over age. Around age 4 this bone loss has recovered and has a slight increase across ontogeny.

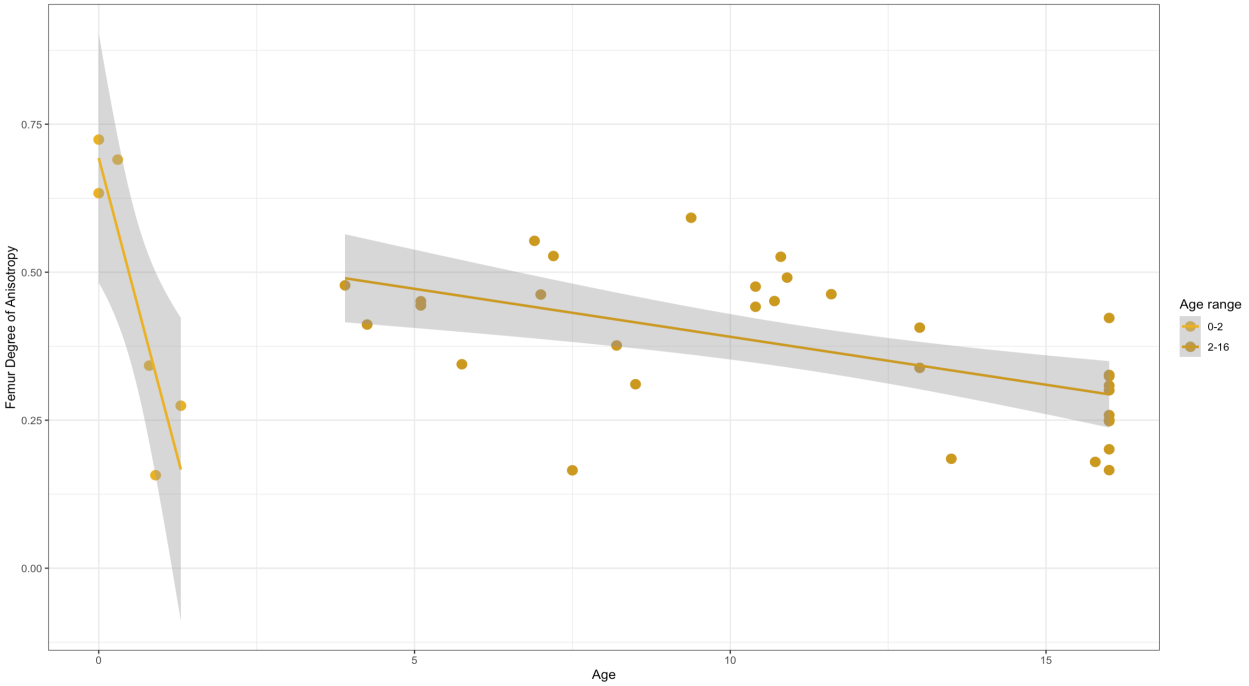

Figure 2.19: Comparison of DA across age in the femur with two age groupings, young infants (0-2 years) and all other ages (2-16 years). From age 0-2 there is a steep negative slope resulting in a decrease in anisotropy. Between ages 2-4 there is an increase in anisotropy, then from age 4 until adulthood DA steadily decreases again. Across ontogeny there is a trend to increasing isotropy. =

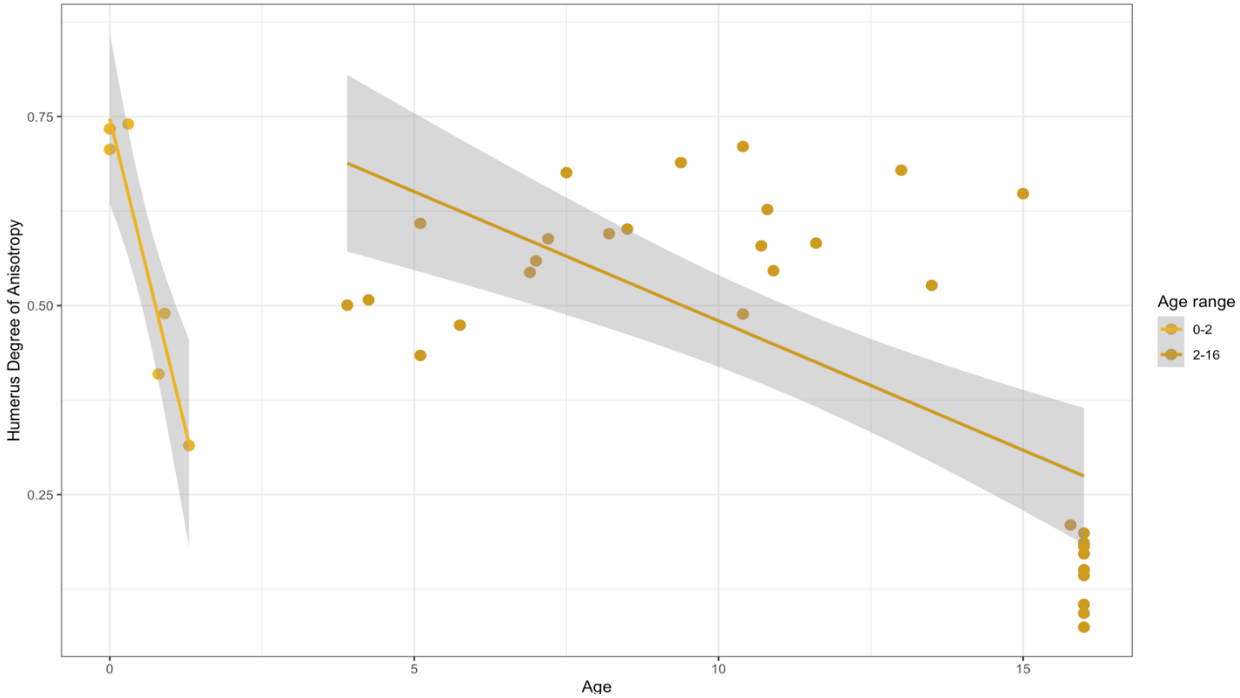

Figure 2.20: Comparison of DA across age in the humerus with two age groupings, young infants (0-2 years) and all other ages (2-16 years). From age 0-2 there is a steep negative slope resulting in a decrease in anisotropy. Between ages 2-4 there is an increase in anisotropy, which remains stable through juvenility and adolescence. DA becomes highly isotropic during adulthood.

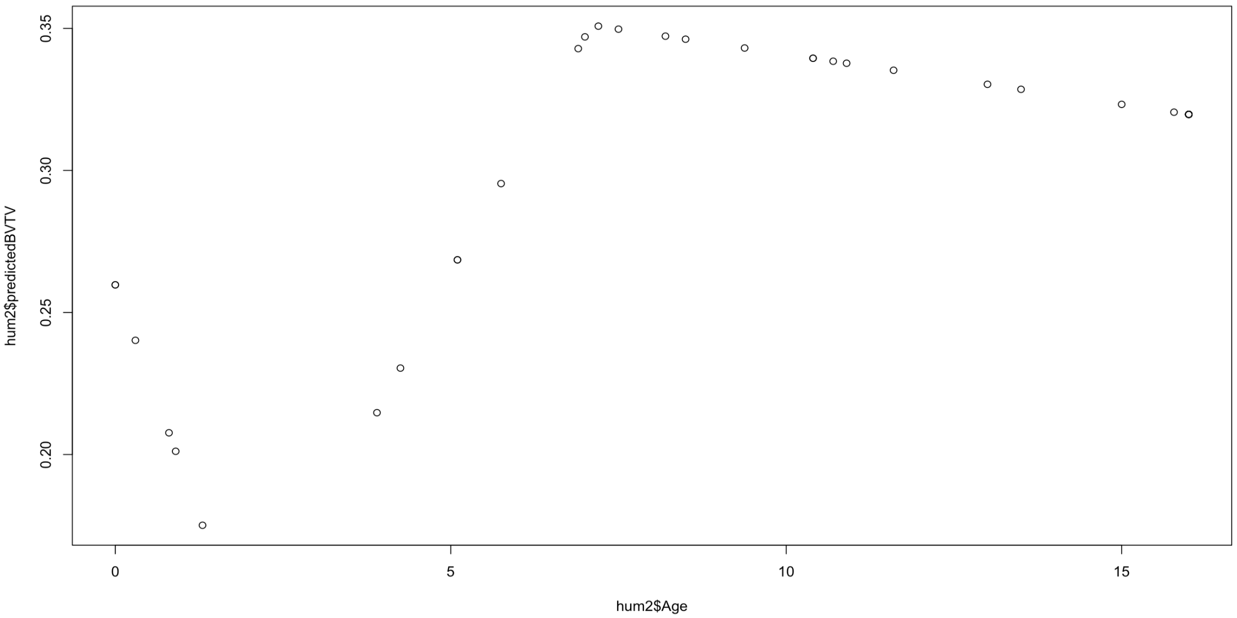

Figure 2.21: Predicted values from the model considering age groupings of 0-2 years, 2-5. years, and 5-16 years when bone volume fraction in the humerus is regressed on age.

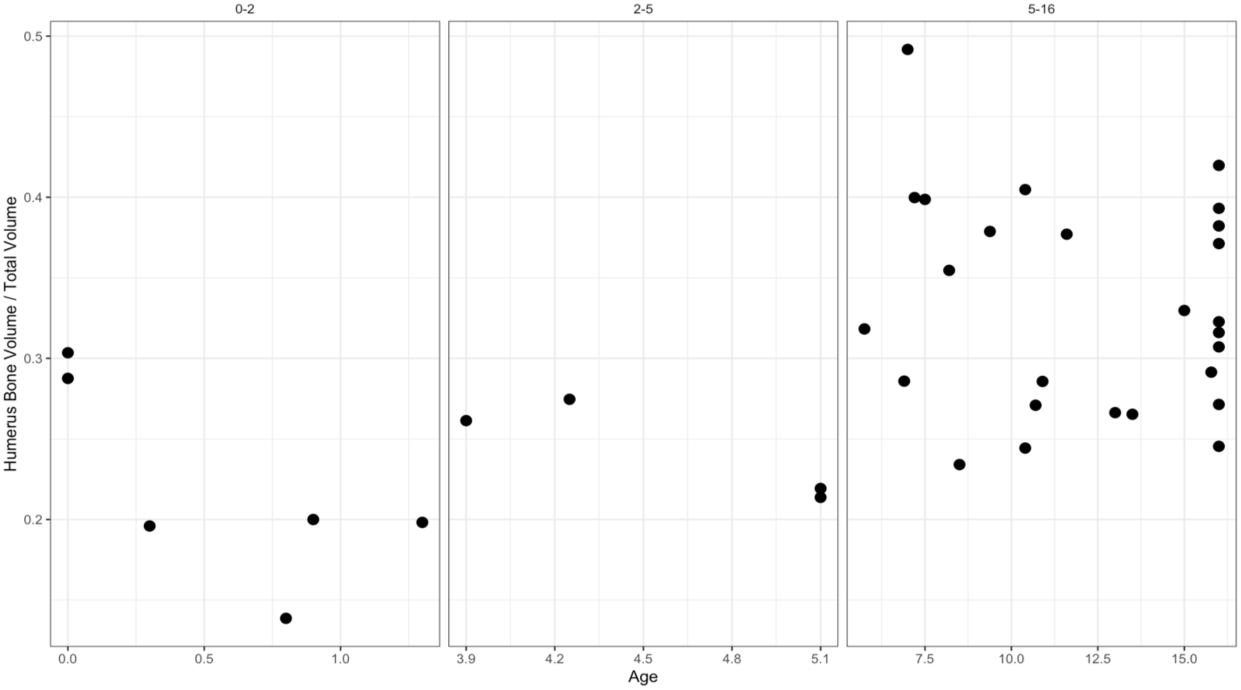

Figure 2.22: Humerus bone volume fraction (BV/TV) across three age categories, 0-2 years, 2-5 years, and 5-16 years demonstrates specific patterns at key age stages. Ages 0-2 have a negative slope, ages 2-5 have a significant positive slope indicating increasing bone volume fraction, and finally a slight negative (non-significant) slope from juvenility to adulthood (5-16 years old).

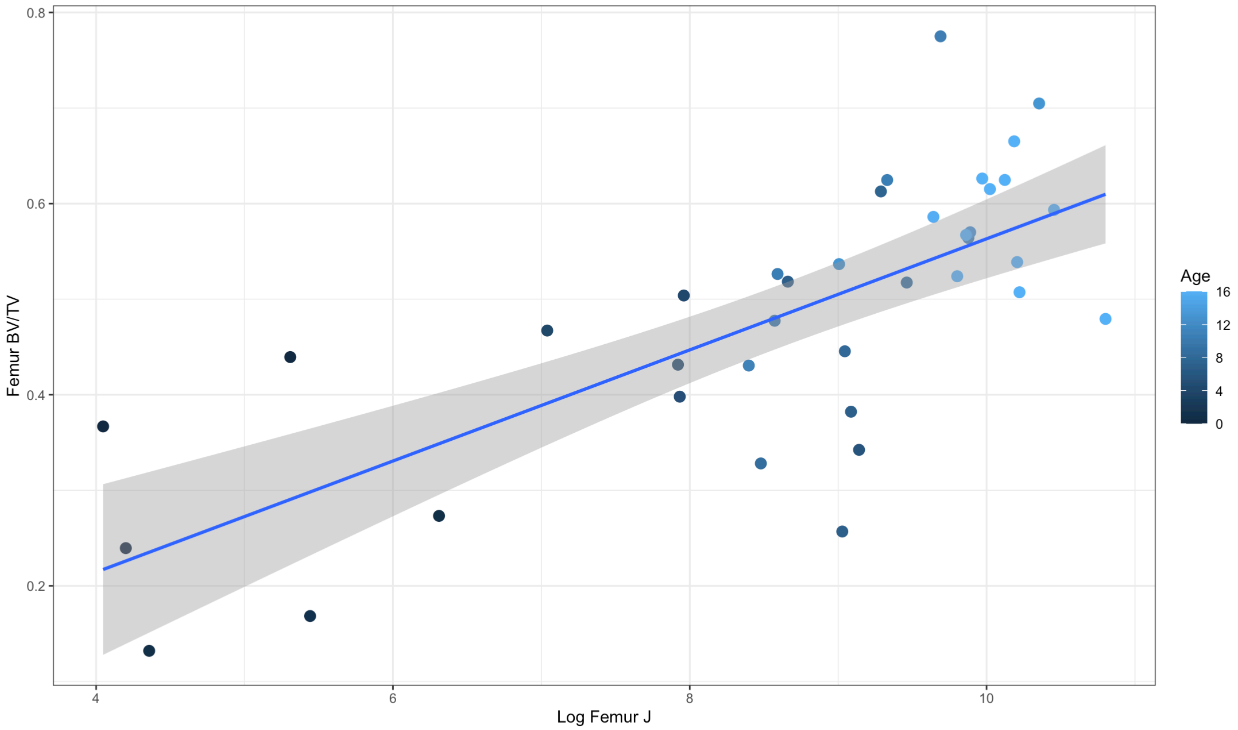

Figure 2.23: Log femoral J on the x-axis, with the bone volume fraction (BV/TV) of the femur on the y-axis. The blue regression line represents the slope, and the shaded grey areas the standard error. Age of individuals is denoted by the color map.

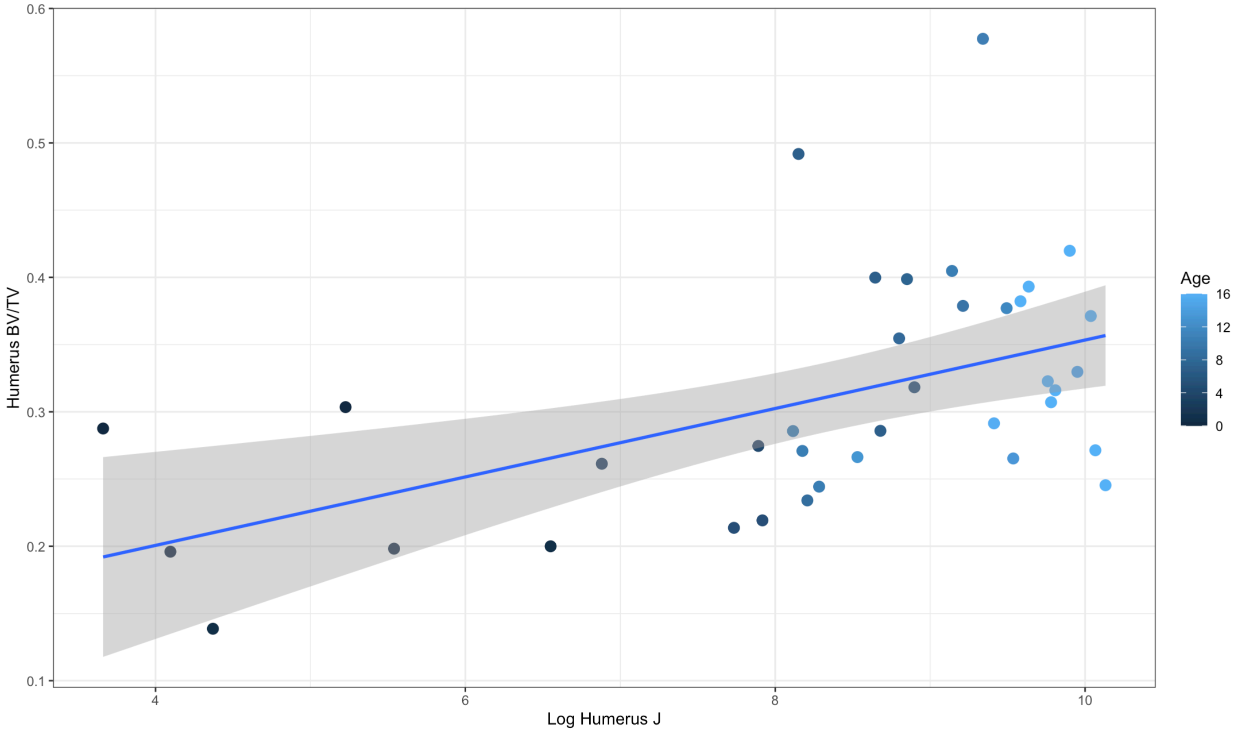

Figure 2.24: Log humeral J on the x-axis, with the bone volume fraction (BV/TV) of the humerus on the y-axis. The blue regression line represents the slope, and the shaded grey areas the standard error. Age of individuals is denoted by the color map.

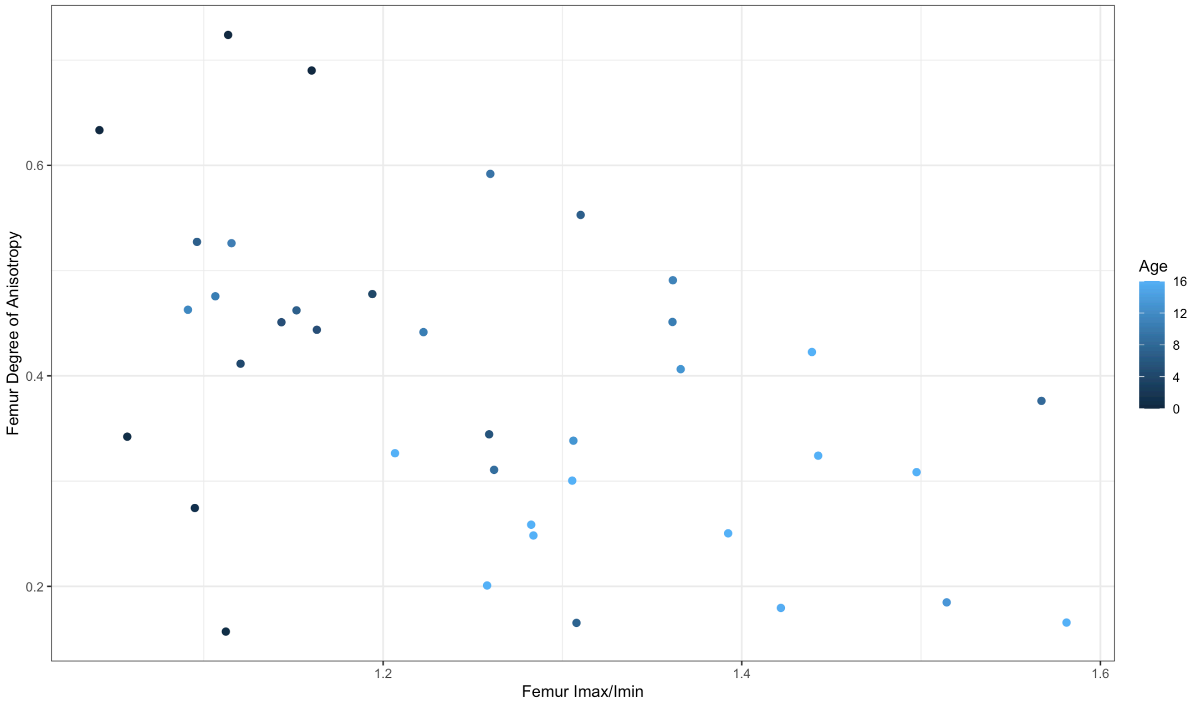

Figure 2.25: Femur I_{max}/I_{min} on the x-axis, and femoral degree of anisotropy (DA) on the y-axis. Age of individuals is denoted by the color map.

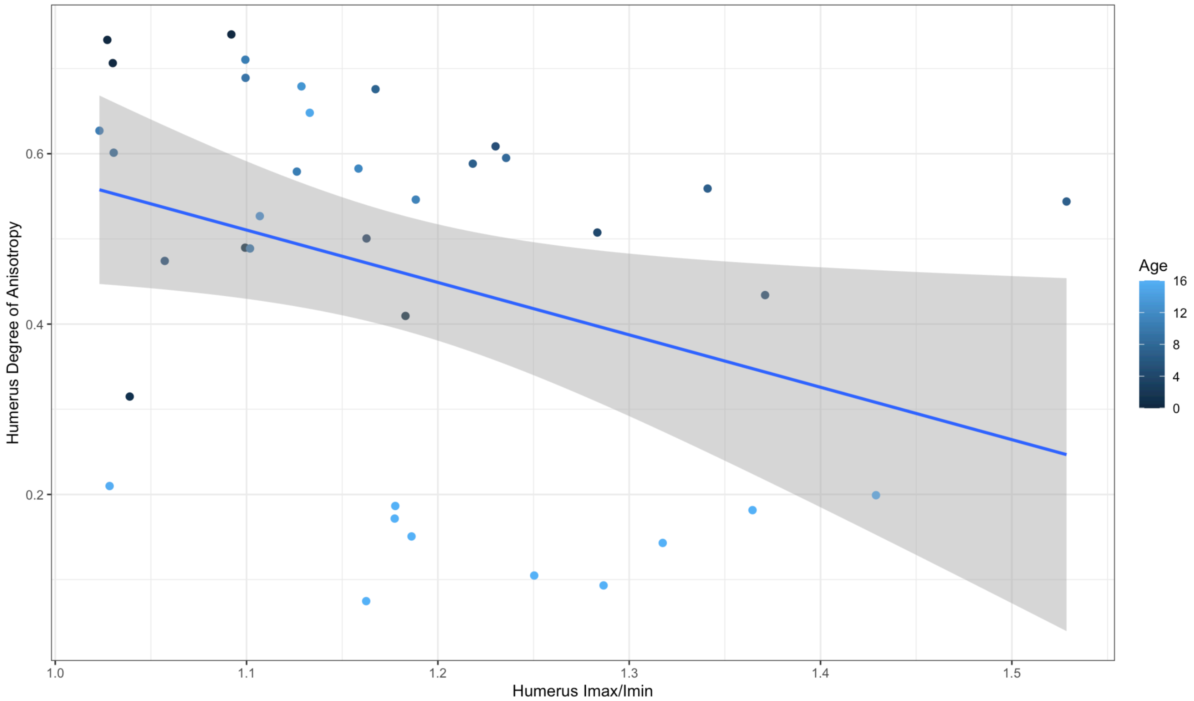

Figure 2.26: Humerus I_{max}/I_{min} on the x-axis, and humeral degree of anisotropy (DA) on the y-axis. Age of individuals is denoted by the color map.

Table 2.9: Regression coefficients comparing the effect of I_{max}/I_{min} on DA across age categories in the humerus. Significant values are bolded.

Model: hum$DA ~ hum$Imax.Imin + Age.Cat Coefficients:				
	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.21677	0.23296	0.931	0.359
Hum Imax.Imin	-0.05685	0.18215	-0.312	0.757
Adolescent	0.41468	0.06552	6.329	**4.21E-07**
Infant	0.39647	0.05902	6.718	**1.38E-07**
Juvenile	0.422	0.05227	8.073	**3.22E-09**

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Chapter 3 Cortical Ontogeny Among Large Bodied Non-Human Apes

3.1 Introduction

Ontogenetically mediated positional behavioral transitions, such as from arboreal to terrestrial substrate use, have been shown to be correlated with changes in cortical bone size and shape in primates (Ruff et al., 2013; Sarringhaus et al., 2016). The promise of this research in reconstructing hominoid behavioral evolution is particularly evident, as 1) some extant taxa undergo distinct developmental changes in positional repertoires, while 2) extinct taxa remain enigmatic in terms of their repertoires. For example, the extent to which large-bodied Miocene hominoids such *Morotopithecus bishopi* and *Proconsul major* used arboreal vs. terrestrial substrates is unclear but is of interest as these apes are the oldest known to exhibit distinctive anatomical features also found in extant great apes, including a dorsoventrally stable lower back (*M. bishopi*), and large body size (~40-80 kg; both taxa) (MacLatchy, 2010). In addition to these features, *Morotopithecus* is thought to share a knee that is capable of variable postures and a mobile hip joint with extant apes (MacLatchy, 2004; Ruff, 2002). *Proconsul major* is not well represented by postcrania, and locomotor reconstructions were historically based on generalizations stemming from analyses of other species in the *Proconsul/Ekembo* family (Ward, 2015). However given its weight of over 80 kg (Rafferty et al., 1995), it is possible that it was terrestrial or possessed derived arboreal adaptations not yet documented in the fossil record (Nengo and Rae, 1992). Thus, using modern taxa to evaluate the impact of ontogeny on skeletal phenotype may lead to the discovery of new form-function relationships that will aid behavioral reconstructions of fossil hominoids.

3.1.1 Bone as a Plastic Tissue

Bone is a plastic tissue, responsive to the mechanical loads placed upon it during locomotion. This responsiveness results in the ability to attribute osteological phenotypes to specific locomotor behaviors. Among adults there is ample research demonstrating cortical response to variable behaviors across primates (e.g. Demes et al., 1991; Ruff and Runestad, 1992; Ruff, 2002; Pearson and Lieberman, 2004; Ruff et al., 2006) and in humans that participate in different sports (e.g. Nikander et al., 2006; Nikander et al., 2010; MacIntosh et al., 2019; Saers et al., 2021). In humans, cortical bone has greater developmental plasticity around puberty than before or after (Bailey, 1997; MacKelvie et al., 2002; Pearson and Lieberman, 2004). Adolescent humans gain more bone than adults do in response to a given strain stimulus, and exercise initiated prior to or during puberty has a greater effect on adult bone shape, strength, and density than does exercise initiated after puberty (Bass et al., 1998; Bailey et al., 1999; Haapasalo et al., 2000; MacKelvie et al., 2002). Thus, the positional behaviors that are performed during ontogeny may have a particularly strong influence on the adult skeletal phenotype. This relationship is critical to understanding skeletal development in nonhuman great apes, as African apes use varied positional behaviors across ontogeny, with adults and subadults showing differences in positional repertoires. When adult locomotion differs from younger subadults, it is necessary to study the skeleton at all ages to understand how variable locomotion at different ages is reflected so that researchers can better identify patterns of behavior. This becomes particularly important when extrapolating modern form-function relationships to the fossil record.

Data from several nonhuman primates are consistent with the hypothesis that age related changes in positional behavior are reflected in the cross-sectional properties of the limb bones. In baboons, limb bone strength reflected locomotor changes with age. Prior to 2.5 years of age, infants were more arboreal than adults and clung to their mothers during travel and had stronger humeri relative to their femora (Ruff, 2003). After 2.5 years, when baboons use almost exclusively quadrupedal locomotion, femoral and humeral strength ratios were similar (Ruff, 2003). Similarly, in mountain gorillas (*Gorilla beringei beringei)* increased proportions of terrestrial locomotion resulted in corresponding increases in hindlimb strength (Ruff et al., 2013). These findings indicate that age-related changes in positional behavior alter limb bone cortical geometry.

3.1.2 Hominoid Locomotor Versatility and Ontogeny

This study investigates the effects of changes in positional behavior during ontogeny on the skeleton. Chimpanzees create a predictive model for how differences in the proportion of arboreal vs. terrestrial behaviors affect trabecular and cortical bone growth patterns. Infants and juveniles utilize more upper limb dominated behaviors, including arboreal orthograde suspensory behaviors, but gradually become hindlimb dominant through terrestrial quadrupedalism as they reach adulthood (Doran, 1992; Doran, 1993; Doran and Hunt, 1994; Sarringhaus et al., 2014). Even when arboreal, adult and adolescent chimpanzees use hindlimb dominated locomotion, while infants' and juveniles' locomotion is significantly more suspensory and forelimb dominated (Doran, 1992; Sarringhaus et al., 2014). Previous study across *Pan* ontogeny has shown that cross-sectional properties such as relative strength and ellipticity track age related locomotor changes (Sarringhaus et al., 2016). To build off these results, the chimpanzee model can be useful to generate hypotheses about how differing levels of arboreal and terrestrial locomotion are reflected in the skeletons of other apes such as *Gorilla* (who is highly terrestrial) and *Pongo* (who is highly arboreal). If the proportions of arboreal and terrestrial behavior practiced by hominoids are evident in the skeleton, this will generate insights into the potential locomotor repertoires of fossil Miocene hominoids, whose locomotion is debated.

Compared to *Pan*, *Gorilla* is generally more terrestrial throughout life, but still exhibits transitions. Doran (1997) documented that *G. b. beringei* under three years engaged in more arboreal suspensory and climbing behaviors compared to individuals over three years of age (although levels of suspension tended to be well below those of subadult *Pan* (Doran, 1997; Sarringhaus et al., 2014)). This change in arboreal behavior was echoed in long bone structure (Ruff et al., 2013). *G. g. gorilla* uses more arboreal behaviors than *G. b. beringei* (Remis, 1995; 1998; 1999). Females and juveniles were found to engage in more arboreal behaviors than males, sometimes at similar levels as *Pan* adults (Remis, 1995; 1998). Although locomotor ontogeny in *G. g. gorilla* is understudied, it is likely that a similar development shift occurs. The shift to greater proportions of terrestriality may be more prolonged in *G. g. gorilla* compared to *G. b. beringei* but should follow the same general pattern. This locomotor inference is supported by morphological data that demonstrates *G. g. gorilla* is distinguishable from *G. b. beringei* in cross-sectional morphology (Sarringhaus et al., 2022).

Both *Pongo* species (*Pongo abelli* and *Pongo pygmaeus*) are much more arboreal than *Pan* (Thorpe and Crompton, 2005; 2006). *Pongo* also has the most variable locomotor profile, ranging from leaping behaviors to slower more cautious climbing (Thorpe and Crompton, 2005; Thorpe et al., 2009). *Pongo* is unique among great apes due to its usage of torso-pronograde as well as torso-orthograde suspensory postures (Thorpe and Crompton, 2005; 2006). *Pongo* locomotion appears to be relatively consistent across age categories (Thorpe and Crompton, 2005; Manduell et al, 2011).

Studying all three taxa will enable comparisons between suites of cortical traits that characterize terrestrial vs. arboreal locomotion within a species *(Pan)* and across species with different proportions of terrestrial and arboreal behaviors (*Gorilla* and *Pongo* respectively*).* Based on existing literature, *Gorilla* and *Pongo* have more rapid locomotor development across ontogeny and less significant locomotor change compared to *Pan*, providing an opportunity to test whether the proportion of arboreality can be detected skeletally in apes.

The three genera in this study were chosen to include a range of positional behaviors to validate arboreal vs terrestrial skeletal signals. This was essential for testing whether skeletal indicators of substrate use are consistent across species as opposed to representing a signal of predetermined developmental change. It was not expected that each species will be identical in its starting morphology or in its response to ontogenetic changes in arboreal and terrestrial substrate usage. Indeed, there may be underlying differences in skeletal mechano-sensitivity and bone formation rate within and among species. However, this project sought to assess whether, despite such variation, there are predictable anatomical shifts that track documented behavioral transitions. The results of this study are necessary for enhancing the understanding of postcranial adaptations in extant hominoids, as they provide the foundation for studying extinct hominoid morphology and reconstructing locomotor repertoires.

3.2 Questions and Predictions

This study asks whether changes in the proportion of arboreal and terrestrial locomotion in the skeleton of *Pan troglodytes* is reflected in humeral and femoral cross-sectional morphology. It investigates whether these changes match the morphology of *Gorilla* and *Pongo*. A previous study found that humeral and femoral midshafts reflect age related changes (Sarringhaus et al., 2016). This study investigates whether these patterns can be replicated in an another sample of *Pan*, and then apply the results to additional samples of *Gorilla* and *Pongo*.

The preceding considerations generate the following predictions:

(1) Cortical features associated with forelimb dominated arboreality will be found in infant and juvenile *Pan,* as these individuals engage in significantly more arboreal behavior than do adolescents and adults (Sarringhaus et. al., 2014). Specifically, infants and juveniles will have relatively higher *J* (strength; see Table 3.1) in the forelimbs vs. hindlimbs compared to adults and adolescents. These traits will be consistent across *Pongo* life history. They will shift earlier in *Gorilla* compared to *Pan* because *Gorilla* infants have been shown to shift to increased terrestrial locomotion earlier in ontogeny and commit to increased terrestrialism from juvenility through adulthood (Doran, 1997).

(2) It is also predicted that overall strength relative to the amount of cortical bone (*J*/CA; see Table 3.1) will be higher in the humerus, but lower in the femur of *Pan* infants and juveniles compared to adolescents and adults. A trend of relative femoral strength overtaking relative

humeral strength will occur earlier in *Gorilla,* and the humerus will maintain greater humeral strength in *Pongo.*

(3) More circular midshaft cross sections in the femur and humerus will be prevalent in infant and juvenile *Pan.* The circularity (ratio of I_{max}/I_{min}) will shift to increasing ellipticity across ontogeny, but adults and adolescents will have more elliptical bones. The shift to elliptical bones will occur earlier in *Gorilla,* and later in *Pongo.* Overall, the cross-sectional morphology of younger chimpanzees will resemble that of *Pongo* instead of *Gorilla,* while the reverse will be true for older *Pan*.

3.3 Methods

3.3.1 Anatomical Measurements

All osteological measurements were taken using digital calipers, following the methodology in Ruff (2000). Midshaft location was calculated by measuring maximal bone length using an osteometric board, and dividing this length in two. For the femur, bone length was measured with both femoral condyles flat along the osteometric board and the most superior point on the proximal femur indicating the maximal length. To calculate maximal bone length in the humerus, the capitulum and trochlea were placed flat along the back of the osteometric board.

3.3.2 Cortical Bone Scanning

Cortical variables (Table 3.1) from the femur and humerus in all three species (See Table 3.2 for sample sizes) were gathered using a portable 2000L peripheral quantitative computer tomography (pQCT) scanner from the Department of Orthopedic Research at the University of Michigan (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany) and table-top pQCT (SA Research+, Stratec Medizintechnik, Pforzheim, Germany) scanner from the Bouxsein lab at the Beth Israel Deaconess Medical Center. Single slices were taken at the highest resolution possible, 100 microns. Similar resolutions have already been shown to be adequate for analyzing infant *Gorilla* specimens (Ruff et al., 2013). Slices were taken at the midshaft, typically the site of highest bending force along the diaphysis during locomotion (Biewener et al., 1983).

Cross sectional geometric properties were analyzed using the MacroMoment plugin for ImageJ, and cortical mineral density was analyzed in ImageJ. To measure anterior-posterior and medio-lateral ratios of the cross-sectional shape, a secondary analysis was conducted in BoneJ (Doube et al., 2010). Slice geometry was used to calculate I_x and I_y, and to generate slice geometry images with principal axes labelled. These axes were then measured, and the linear measurement for the I_x or I_y in the AP or ML direction was recorded.

3.3.3 Aging

To allow for comparison across ontogeny, specimens were aged to the following categories: infant, juvenile, adolescent, and adult. Specimens were aged using tooth emergence, root formation, and dental wear (Zihlman et al., 2004; Smith and Boesch, 2011; Smith et al., 2013; Sarringhaus et al., 2016) using photographs of the occlusal surface of the maxilla and mandible. These age categories represent approximations as the life histories and developmental rates of these genera differ, but this approach allows for comparison between similar developmental stages.

3.3.4 Samples

Wild caught specimens with no obvious pathologies from all three species were scanned from the Cleveland Museum of Natural History and from the Harvard Museum of Comparative Zoology. Among adults, individuals were prioritized who had known sex. Among infants sex information was highly limited, and thus sex was only considered for adults. Bones from the left side were primarily selected unless the bone was damaged or missing. All samples from subspecies of *Pan troglodytes* were combined in this analysis, though majority of the sample is *Pan troglodytes troglodytes*. The positional behavioral repertoire of *Pan troglodytes verus* and *Pan troglodytes schweinfurthii* has been studied in the wild and found to be similar (Doran and Hunt, 1994; Sarringhaus et al., 2014). While *P. t. troglodytes* locomotion has not been studied in the wild, thus far, documented subtle locomotor differences among *P. t. verus* and *P. t. schweinfurthii* do not map onto subspecies categories, but rather are better explained by sex, habitat, or foraging strategies (Doran, 1992; Doran and Hunt, 1994; Sarringhaus et al., 2014). In addition, prior research has shown that cortical bone changes during development do not vary among different *Pan troglodytes* subspecies (Sarringhaus et al. 2016), nor are there differences between subspecies in adult femoral and humeral cortical properties (Sarringhaus et al., 2022). This provides a provisional justification for combining all *Pan troglodytes* samples in this study.

All Bornean and Sumatran *Pongo* (*Pongo pygmaeus* and *Pongo abelli*) samples were also combined. Feeding and travelling have been found to have the greatest effect on locomotor mode, with both *Pongo* species occupying variable habitats including peat swamps, and mixed dipterocarp forest (Manduell et al., 2011; Thorpe and Crompton, 2005). Any differences between the locomotion of the subspecies are likely due to habitat type, predator prevalence, and support size rather than phylogeny (Manduell et al., 2011). In addition, there are limited samples of *Pongo* located in museum collections with an accessible CT-scanner. *Gorilla* samples used in the study are viewed by some as belonging to the same species, *Gorilla gorilla gorilla*.

3.3.5 Statistics

To determine changes across ontogeny in the relationship between cortical variables, such as between femoral and humeral *J* and *J* and cortical area in both bones, a linear regression was used. To select the most appropriate statistical model, a null model and models with combinations of predictor variables were created. These were then tested against one another using an Akaike's Information Criterion (AIC) which penalizes models depending on the number of parameters included (see Anderson and Burnham, 2002). When testing regression patterns across all species, the predictors were: Species, Age, and Sex. When conducting regressions within species the predictors were: Age and Sex. This analysis was used when comparing femoral and humeral *J* changes across ontogeny, patterns of femoral and humeral *J* compared to cortical area (CA), and patterns of change in CA and total area (TA).

To assess the difference in means of cortical properties across ages and species, Kruskal-Wallis' were used because the data was not distributed normally. When significant, a Wilcoxon-Signed-Rank test was used for pairwise comparisons in all mean-based statistical analyses followed by a post-hoc Bonferroni correction. Models were run with both age and sex as covariates. If there were no significant differences between sexes, a second model was run with just age, as this allowed for fewer pairwise comparisons and larger sample sizes within ages. This analysis was used to test the change in femoral and humeral *J* ratios, femoral and humeral *J*/CA ratios, I_{max}/I_{min} ratios in both bones, and ellipticity direction (AP/ML) ratios in both bones.

Table 3.1: Variables measured.

Feature	**Unit**	**Definition**
Cortical Area	CA (mm^2)	Area of cortical bone only, which assesses ability to withstand compressive and tensile loads.
Total Area	TA (mm^2)	Total area of the cross section, including the cortical bone and medullary cavity.
Ellipticity	I_{max}/I_{min}	"I" area the second moment of areas, or the ability of a bone to resist bending in one plane. I_{max} and I_{min} are the maximal and minimal strength in either plane. Dividing I_{max} by I_{min} gives an indication of ellipticity of the cross section.
Polar second moment of area	J (mm^4)	Strength, which is the sum of I_{max} + I_{min} and assesses torsional and bending rigidity.
Ellipticity direction	AP/ML	Primary anterior-posterior length divided by the medio-lateral length. This provides directionality when considering elliptical cross sections.

Table 3.2: Sample sizes per bone for each age category by group.

Bone	Genus	Infant	Juvenile	Adolescent	Adult
Femur	Pan	11	21	7	30
Humerus	Pan	11	21	7	29
Femur	Pongo	3	6	2	9
Humerus	Pongo	3	8	2	7
Femur	Gorilla	5	9	2	15
Humerus	Gorilla	5	11	2	14

3.4 Results

3.4.1 Forelimb vs. Hindlimb Strength

To compare forelimb and hindlimb strength, femoral *J* was regressed on humeral *J* and log transformed for visualization purposes. When comparing individuals in all species and ages multiple models were run and the model with the best AIC score was used. The best fit model when testing across all three species included age, sex, and species as covariates with age and sex having an interaction effect together (Figure 3.1). There were no significant differences across species, age, and sex within the model, indicating that the significant positive relationship of femoral and humeral strength across species and ontogeny was consistent (r-squared = 0.9475, p< 2.2e-16). Figure 3.1 demonstrates the pattern across all three species, age categories, and sex. *Pongo* maintained higher humeral *J* values relative to the femoral *J*, though these were not significantly different. Comparatively, *Gorilla* maintained stronger femora relative to their humerus across ontogeny. *Pan* displayed intermediate values, with infants falling near the line representing equal femoral and humeral *J* Figure 3.1). Some young juvenile *Pan* also had equivalent strength values between the bones, but as chimpanzees age their *J* values start to show a bias toward stronger femora (Figure 3.1).

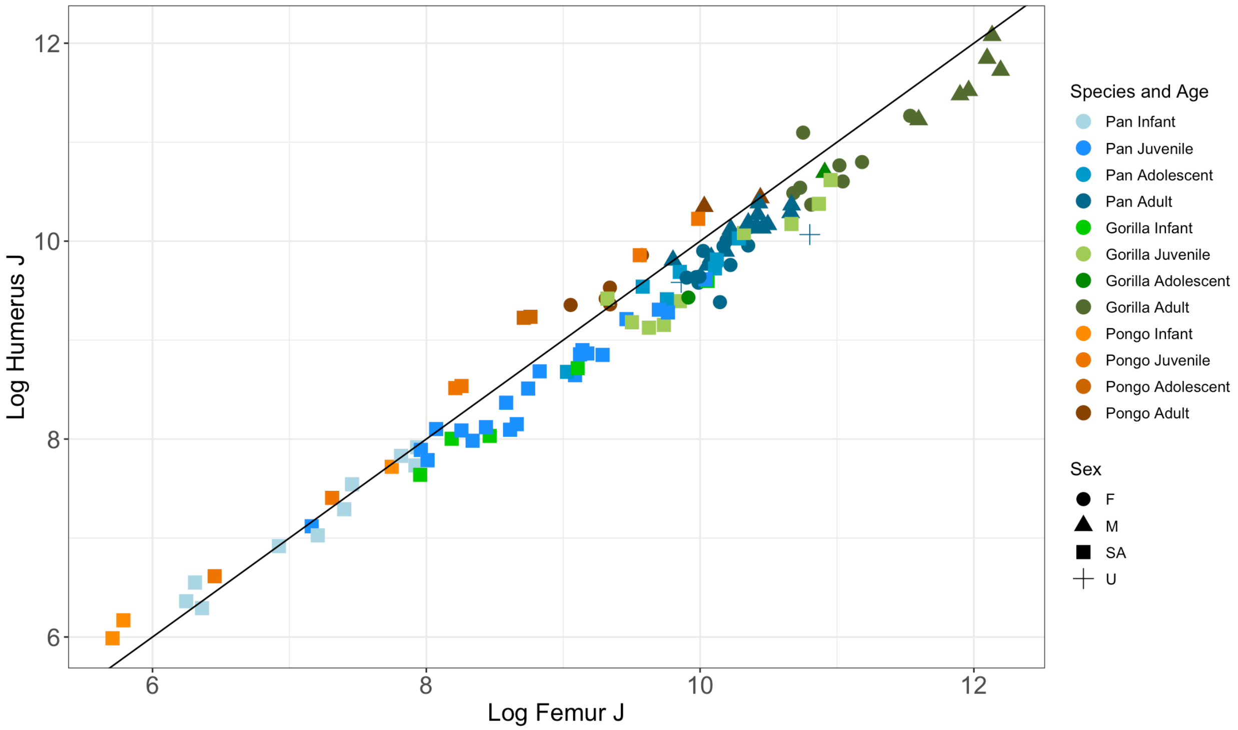
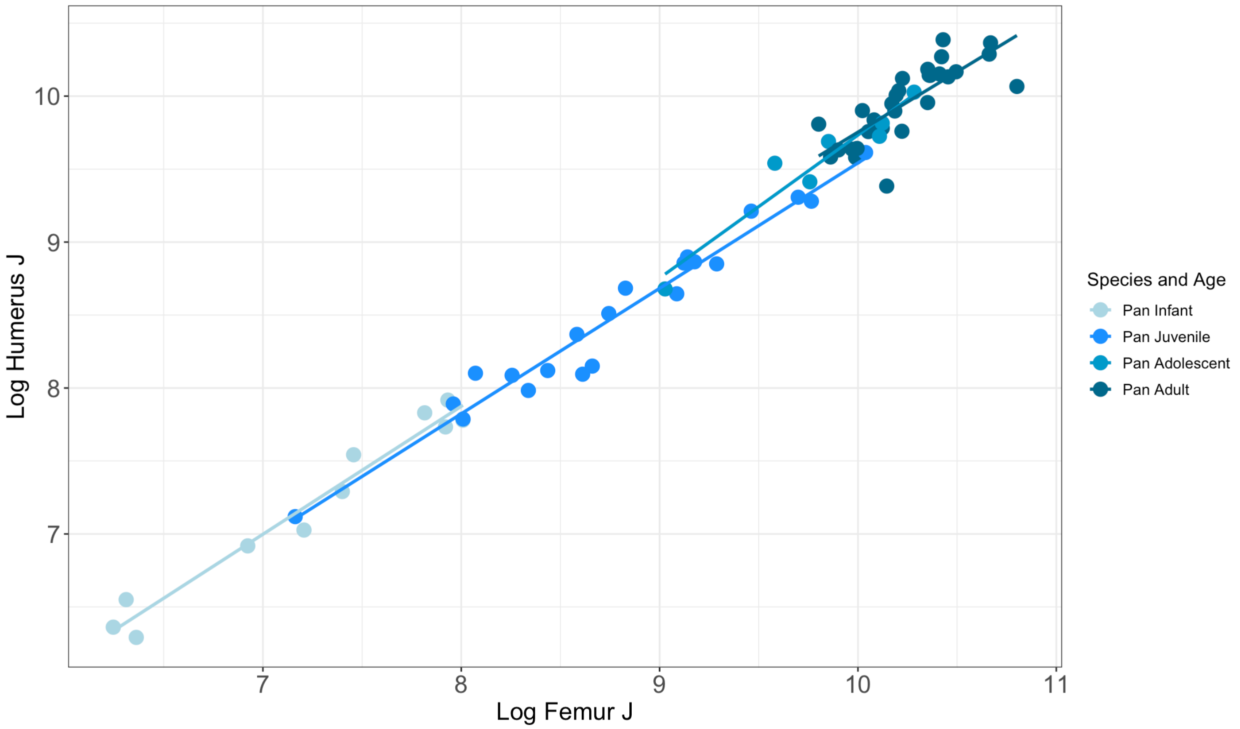


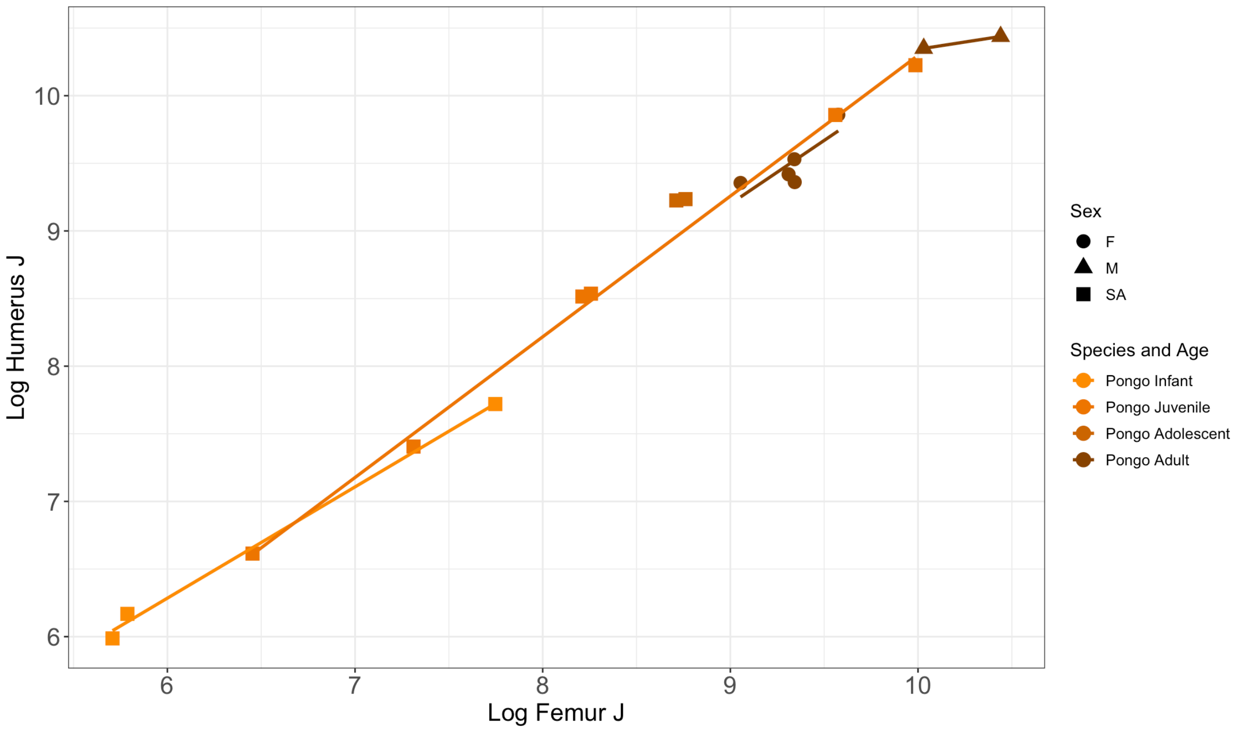
Figure 3.1: Comparison of Log Femoral J on Log Humeral J across age and sex categories. The black line represents isometry.

To investigate the effect of variable locomotion on the relationship between femoral and humeral strength across ontogeny, individual species were compared to determine intraspecific trends. Model fit was tested using AIC scores and included age, sex, and age and sex with an interaction.

In *Pan,* the best fitting model with the lowest AIC score did not include sex or age (r-squared =0.9281, AIC =1262.84, p = <2.2e-16). Though the AIC indicated the best fitting model did not include age, this study was interested in age effects and a second model including age was run. According to the AIC this was the second best model. There was a significant relationship between femoral and humeral *J* (r-squared = 0.9335, AIC = 1260.37, p = <2.2e-16). Per unit increase in femoral strength, the humerus has a slightly greater increase in strength in infants and juveniles relative to adults (infants p = 0.017, juveniles p=0.006). In adults and adolescents the opposite pattern occurs, where per unit increase in femur the humerus does not have as large an increase (relatively stronger femora compared to humerus) (Figure 3.2A). This indicates that the humerus is at its greatest relative strength compared to the femur during infancy and juvenility (Figure 3.2A). However, the effect of age is limited, with only a 0.0054 increase in the r-squared value.

In *Gorilla* the best fitting model included an interaction between age and sex and had a positive linear relationship (r-squared 0.92 p =9.6e-13). There was a significant predictive effect of femoral *J* on humeral *J,* but this did not significantly differ across age and sex (Figure 3.2B). In *Gorilla* the femur was stronger than the humerus consistently across ontogeny. In *Pongo,* the best fitting model included both age and sex as covariates (r-squared 0.95, p = 4.1e-08). *Pongo* was the only species that maintained a stronger humerus relative to femur across ontogeny (Figure 3.2C). Per unit increase in the femur, there was a greater increase in the humerus across ontogeny. There was no significant difference in the relationship between femoral *J* and humeral *J* across any of the age categories or sexes, indicating a consistent relationship over time (Figure 3.2C).


Log Humerus J
10
9
8
7
7
8
9
10
11
Log Femur J
Species and Age
Pan Infant
Pan Juvenile
Pan Adolescent
Pan Adult


Log Humerus J
10
9
8
7
6
6
7
8
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10
Log Femur J
Sex
F
M
SA
Species and Age
Pongo Infant
Pongo Juvenile
Pongo Adolescent
Pongo Adult

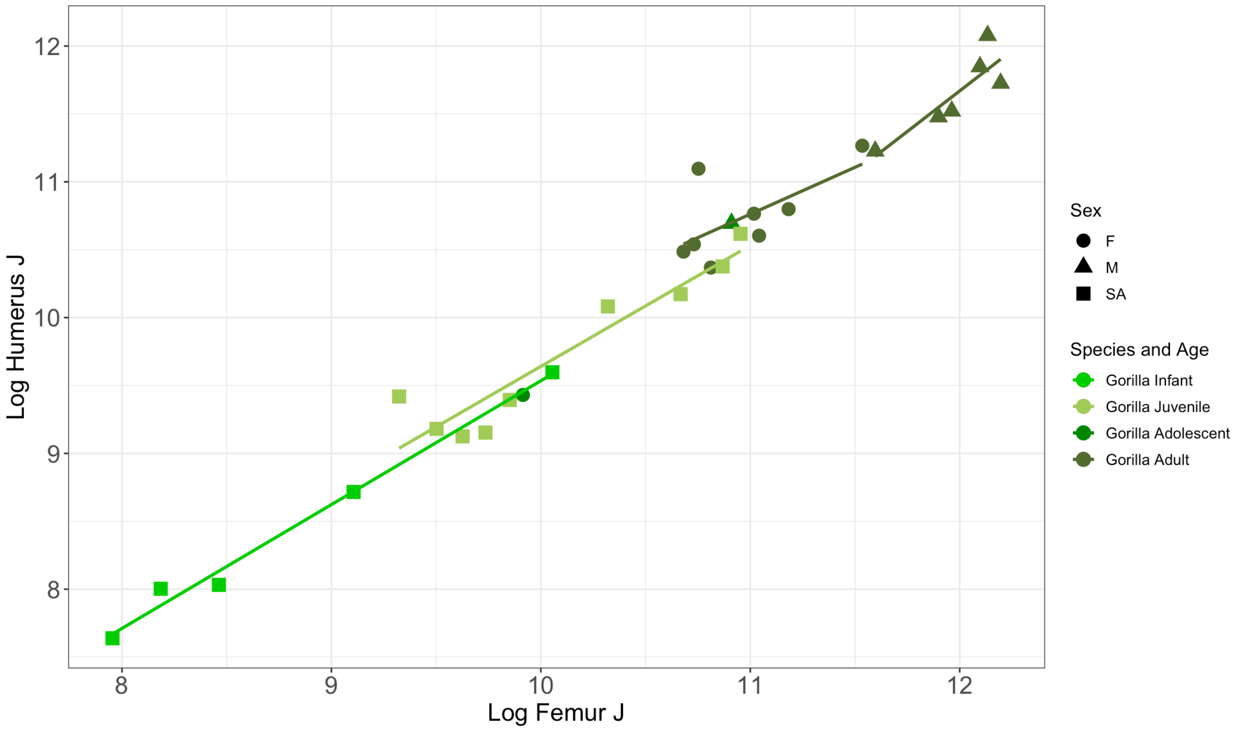

Figure 3.2: (A) Relative strength of Pan during ontogeny. (B) Relative strength of Pongo during ontogeny. (C) Relative strength of Gorilla during ontogeny. Colors denote ontogenetic age categories and shapes denote sex. F = female, M = male, and SA = subadult.

To determine whether the relative strengths of humerii and femora varied during development within species a Kruskal-Wallis model was used. If significant, a series of pairwise tests followed by a post-hoc Bonferroni correction was used. The ratio of femoral to humeral *J* across ontogeny in *Pongo* (p=0.16) or *Gorilla* (p= 0.40) did not differ among age categories (Figure 3.3). In *Pan,* a model was first run including sex to determine if there were any differences within the adult age categories (male, female, and unknown). There were no significant differences in the ratio of femoral to humeral *J* (p = 0.10). A second model was then used, where all adults were binned into one category for statistical robusticity. These showed that *Pan* infants had significantly stronger humerii relative to their femora compared to juveniles, adults, and adolescents (Table 3.3) (Figure 3.3). Additional pairwise comparisons indicated that there were no differences between adults, adolescents, or juveniles. Infants had significantly higher humeral strength relative to femoral strength (Figure 3.1), which resulted in their ratio value being lower (closer to one) (Figure 3.3).

Table 3.3: Statistical p-values from Kruskal-Wallis pairwise comparison of Femoral J/Humeral J across age. Significantly differences post-Bonferroni adjustment are italicized.

Pan troglodytes	Adolescent	Adult	Infant
Adult	0.93773	-	-
Infant	*0.0083*	*0.00021*	-
Juvenile	0.90638	0.90638	*0.00046*

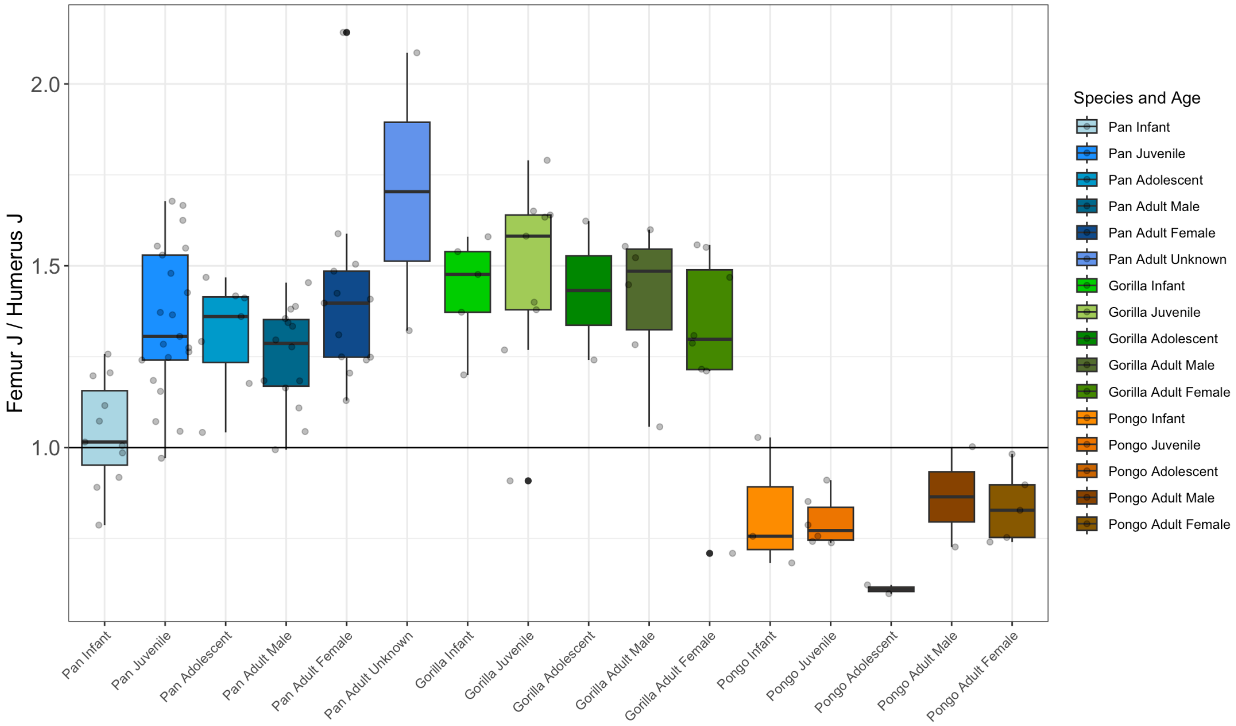

Figure 3.3: Ratio of femoral to humeral strength across species, age, and sex categories. The line demonstrates if the ratio was 1, or if the values were equal. Above the line denotes a relatively stronger femur, below indicates a relatively stronger humerus.

3.4.2 Strength Relative to Cortical Area (J/CA)

To determine how strength changed as a function of amount of bone, as a proxy for size, the ratio of *J*/CA was calculated (Figure 3.4), and *J* was then regressed on CA (Figure 3.5). When comparing the ratio of *J*/CA in the femur, *Pan* adults did not significantly differ from one another, regardless of sex (Table 3.6). Adults had significantly higher ratios than infants (Figure 3.4). Infants had the lowest ratios, indicating that given the amount of cortical bone they have, the strength of their bones is low. Juveniles had significantly lower ratios compared to adults (Table 3.6). Adolescents did not differ from either of the adults, although they overlapped with juveniles in their ratios (Figure 3.4). Though, juveniles had significantly lower ratios than adolescents (Table 3.6). Infants and juveniles had significantly lower ratios in the humerus than male and female adults (Table 3.7). Adults did not significantly differ from one another, nor did they differ from adolescents. Infants had significantly lower ratios than juveniles (Figure 3.4).

Though both models of the femur and humerus in *Pongo* were significant (Femur: KW $\chi2$ = 11.32, p=0.02; Humerus: KW $\chi2$ = 10.77, p = 0.03), there were no significant differences in the ratio of *Pongo* across age or sex (Figure 3.4). There were significant differences in both age and sex in the femur of *Gorilla* (KW $\chi2$ = 24.95, p =5.14e-05), with infants and juveniles having significantly lower ratios than either adult males or females (Table 3.6). Adult males had significantly higher ratios than adult females, and represented the highest ratios of any age or species (Figure 3.4). In the humerus, there were significant differences between juveniles and adult males, where adult males had a significantly higher ratio (p = 0.0016). There were no other significant differences post-Bonferroni correction (Table 3.7).

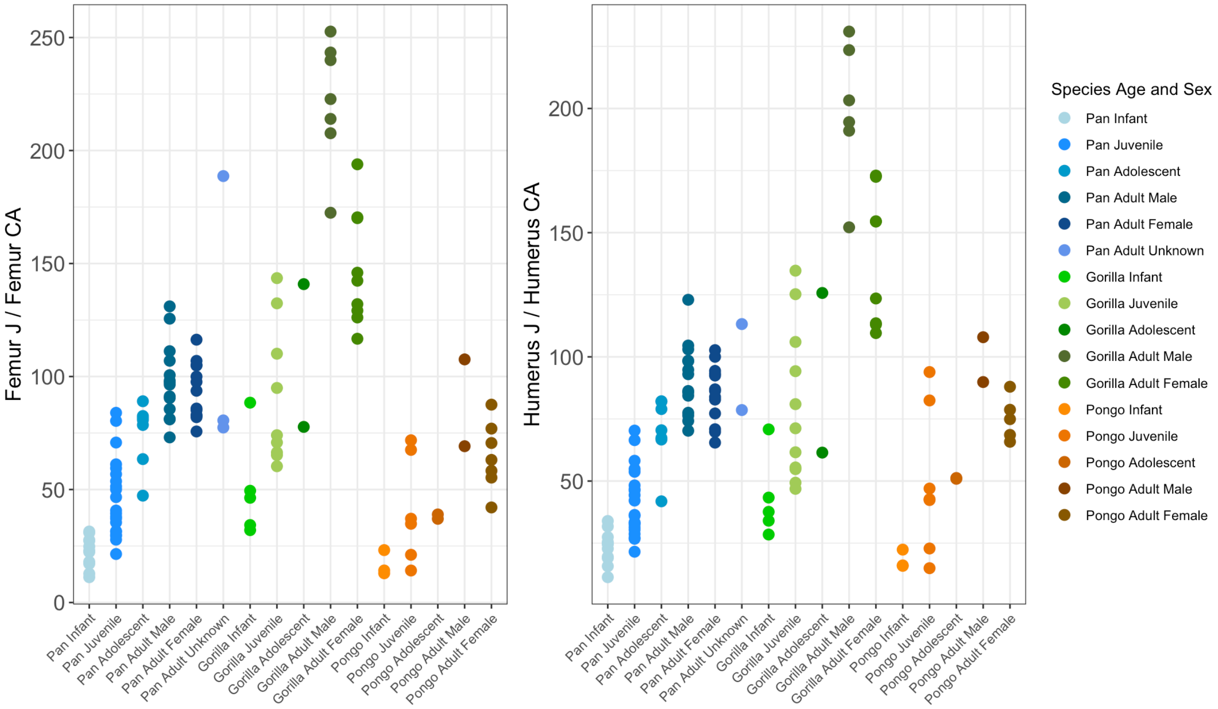

Figure 3.4: Ratio of femoral (left) and humeral (right) torsional strength (J) over cortical area (CA).

All models of *J* regressed on CA found significantly positive relationships across all species and both bones (Figure 3.5). The best modeling according to the AIC did not include age, species, or sex in the regression model of *J* on CA in the femur (r-squared = 0.8787, AIC = 2642.53, p = <2.2e-16). For the humerus the best fit model did not include age, species, or sex, (r-squared = 0.877 , AIC = 2524.980, p= < 2.2e-16) but the second best model had an AIC very close to the best model (r-squared = 0.8798, AIC = 2524.167, p=<2.2e-16). In the second best model, *Pan* significantly differed from *Gorilla* (p=0.03). *Pan,* per unit increase in CA, had less increase in *J* than *Gorilla.* There were no significant differences between *Pongo* and *Gorilla* (p=0.15). This indicates that the humerus of *Gorilla* is stronger, given its cortical area, than in *Pan* (Figure 3.5).

Within *Pan* in both the humerus (r-squared= 0.91, p= <2.2e-16) and femur (r-squared= 0.86, p= <2.2e-16) the best model according to the AIC did not include age or sex, indicating that the relationship between *J* and CA was consistent across ontogeny. *Gorilla* demonstrated the same pattern, with the simplest model that did not include age or sex being chosen by the AIC in both the femur (r-squared=0.94, p= <2.2e-16) and humerus (r-squared=0.94, p= <2.2e-16). In *Pongo,* the best humerus model did not include any covariates (r-squared = 0.91, p=1.46e-10) but the femur included age but not sex (r-squared= 0.86, p=6.63e-7). When age was included, there were no differences across the age categories within the model. Overall, this indicated that there were no differences in the relative strength of bone compared to the cross-sectional area across ontogeny in either bone and within any species.

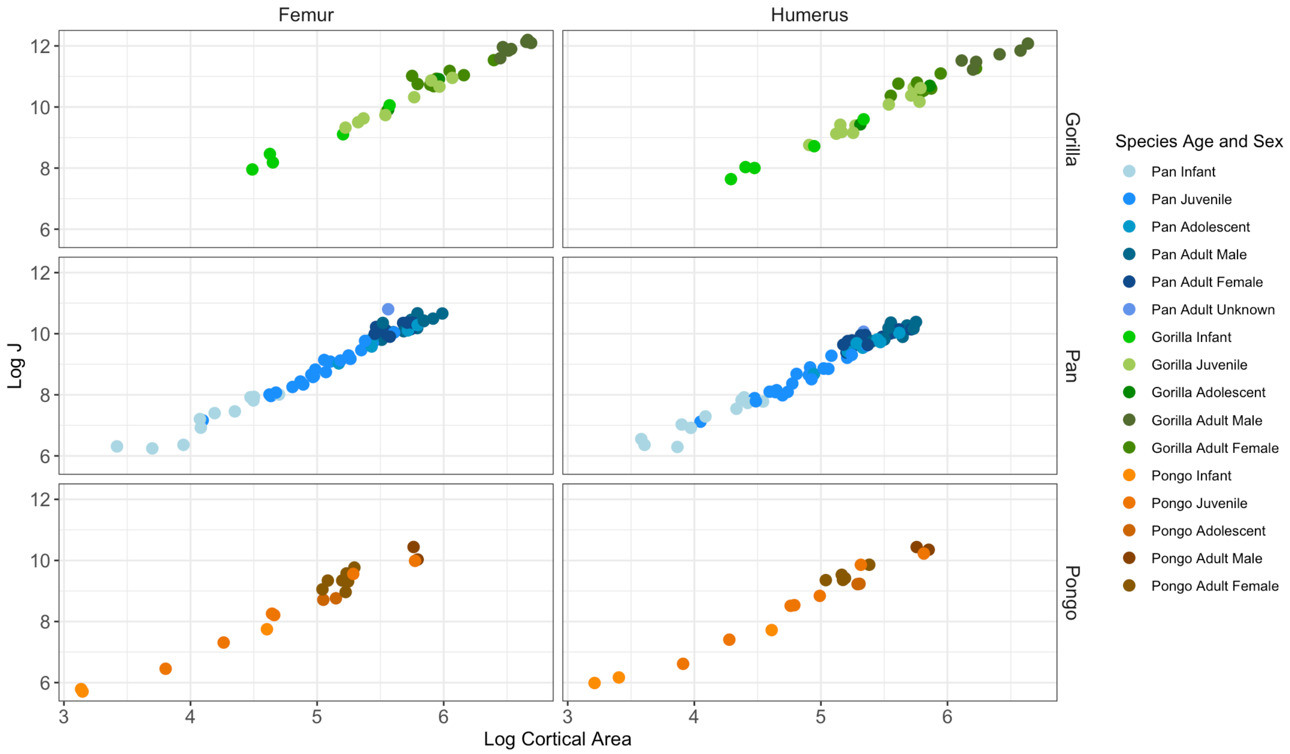

Figure 3.5: Relationship between Log Cortical Area and Log J in the femur (left) and humerus (right) across Gorilla (top), Pan (middle), and Pongo (bottom). Age, sex, and species are color coded.

3.4.3 Cortical Area vs. Total Area

To determine the relationship between total area and cortical area, a regression was run with total area as the independent variable in each species (Figure 3.6). In the femur of *Pan,* the model with the highest AIC score did not include age or sex (r-squared = 0.86, p = 2.2e-16, AIC = 693.98). However, the effect of age was of interest to estimate skeletal response to locomotor change across ontogeny. The model including age but not sex was the second best model according to the AIC (r-squared = 0.88, p = 2.2e-16, AIC = 686.57), though age added limited explanatory power in the model (r-squared of 0.86 vs. 0.88). In this model infants and juveniles had significantly more cortical area given their total area than in adults or adolescents (Figure 3.6). In the humerus of *Pan* the best model according to the AIC included age, though there were no significant differences between any ages (Figure 3.6). *Pongo* femora and humerii had a significantly positive relationship between CA and TA (Figure 3.6), but there were no significant differences in the relationship across ontogeny (femur r-squared = 0.85, p =1.01e-06; humerus r-squared = 0.89, p=2.20e-07). In the humerus adolescents neared statistical significance when compared with adults (p-value=0.0501). However, with a limited sample size in adolescent *Pongo* the statistical robusticity of this difference is not strong. *Gorilla* had a significantly positive relationship between CA and TA but there were no significant differences across ontogeny in either the femur or humerus (femur r-squared = 0.94, p = 2.2e-16; humerus r-squared = 0.90, p = 1.17e-13). This indicates that CA scales consistently across ontogeny in both *Pongo* and *Gorilla*, but there are minimal differences across *Pan* in infants and juveniles compared to adults when age is considered in the model.

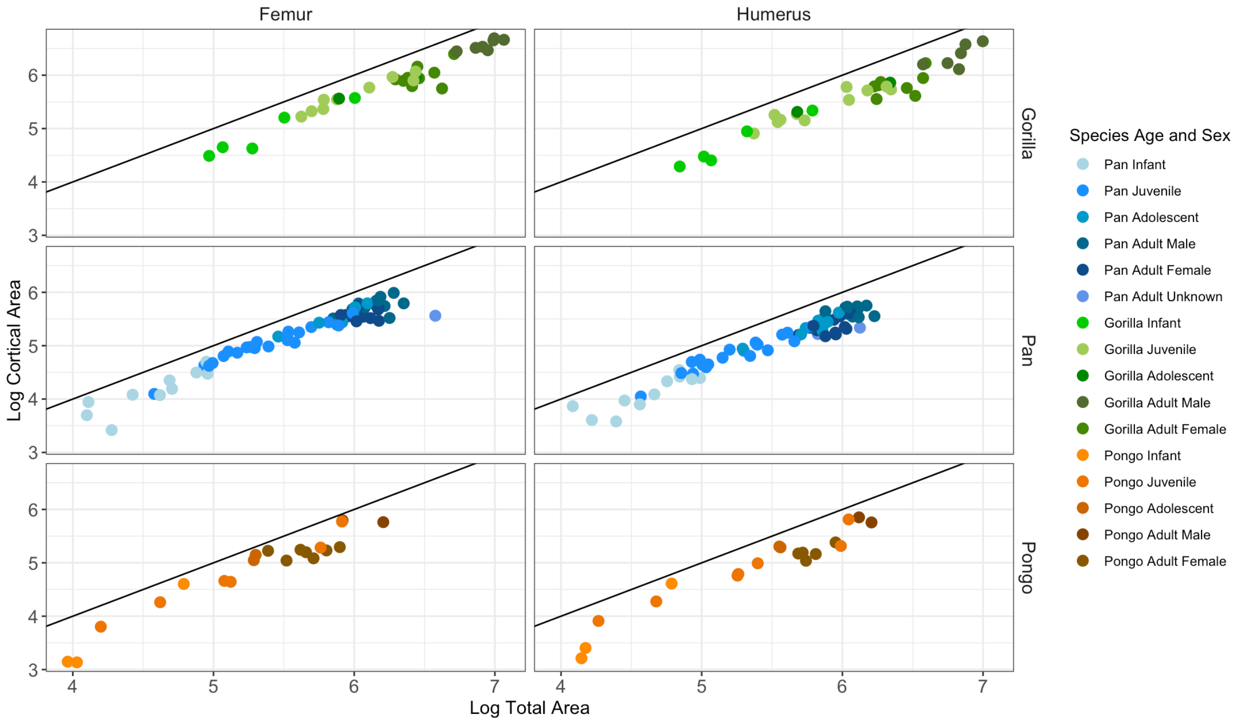

Figure 3.6: Relationship between Log Total Area and Log Cortical Area in the femur (left) and humerus (right) across Gorilla (top), Pan (middle), and Pongo (bottom). Age, sex, and species are color coded. The black line indicates equivalency between CA and TA.

3.4.4 Ellipticity (Shape)

To determine the change in ellipticity across ontogeny in each species independently, the ratio of I_{max}/I_{min} was compared using a Kruskal-Wallis test. If the model was significant a Wilcoxon-Signed Rank Test was used for pairwise comparisons with a post-hoc Bonferroni correction. Each model was run considering age and sex, if sex did not significantly differ among adults then it was removed and the model was run with only age. Male and female adults did not differ in their ellipticity values in any species, and their values were combined in all of the following analyses. The humeral I_{max}/I_{min} values of members of *Pan* age classes did not differ (KW $\chi2$ = 3.31, p = 0.35). Pairwise comparisons showed that only infant femora differed from adults (Table 3.4) (Figure 3.7).

In *Pongo* there were no significant differences in either the femur (KW $\chi2$ = 2.46, p = 0.48) or humerus (KW $\chi2$ = 5.36, p = 0.15) (Figure 3.7). Finally, the *Gorilla* humerus did not vary across ontogeny (KW $\chi2$ = 6.18, p = 0.10). In the femur (KW $\chi2$ = 10.64 p = 0.01), there were significant differences between infants and juveniles (Table 3.4), but no other ages significantly differed from one another. *Gorilla* adult females, though not significantly different, had lower I_{max}/I_{min} than adult males and were the closest in range to that of infants (Figure 3.7).

Table 3.4: Statistical p-values from Kruskal-Wallis pairwise comparison of I_{max}/I_{min} across age in the femur of Pan and Gorilla. Significant differences post-Bonferroni correction are italicized.

Pan Femur			
	Infant	Juvenile	Adolescent
Juvenile	0.075	-	-
Adolescent	0.296	0.756	-
Adult	*1.90E-06*	0.012	0.207
Gorilla Femur			
	Adult	Adolescent	Infant
Adolescent	0.522	-	-
Infant	0.018	0.19	-
Juvenile	0.522	0.522	*0.006*

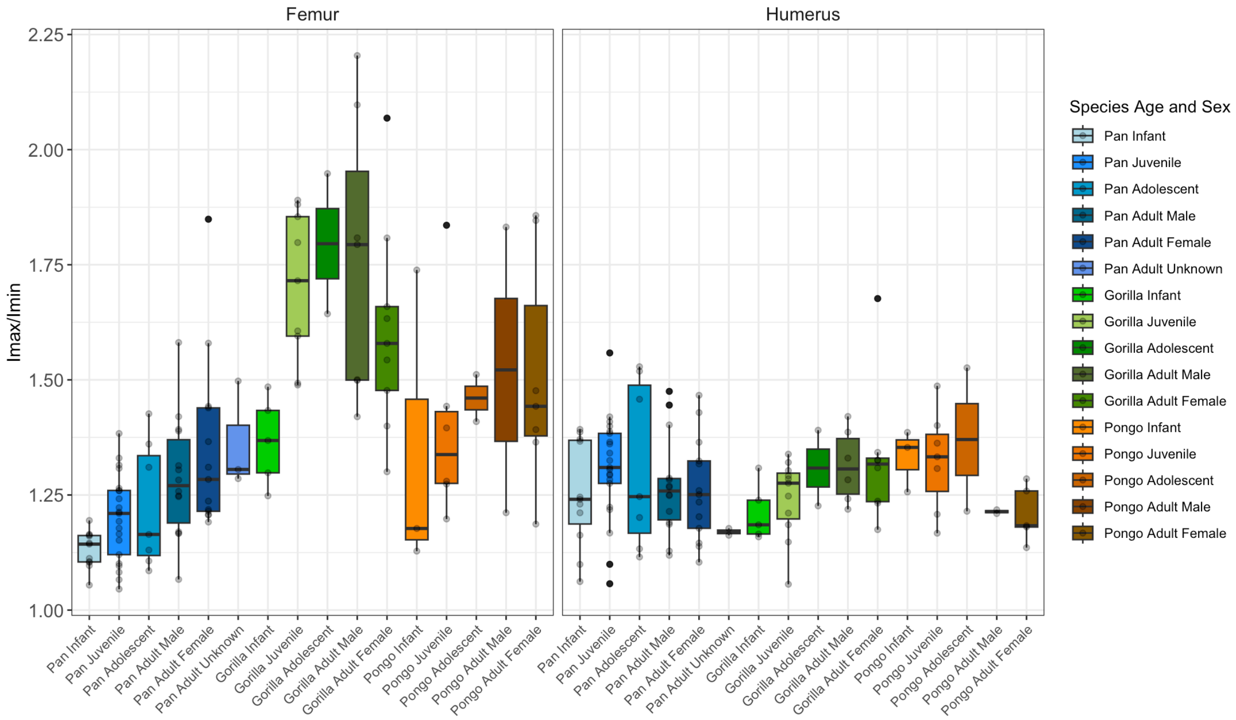

Figure 3.7: Ellipticity, or I_{max} divided by I_{min}, during ontogeny in all species with adults separated by sex. Femoral data is the left panel, and humeral data is the right panel.

3.4.5 Ellipticity Directionality

The directionality of the ellipticity was examined using linear measurements taken from the I_x/I_y of each bone scan. Directionality is not perfectly aligned in the AP and ML plane, but rather in a relative direction within those planes. To test the directionality a similar method was used as in the ellipticity comparison. Overall, there were limited differences when comparing change in AP/ML ratio across ontogeny in each species. *Pan* had no significant differences in the femur model (KW $\chi2 = 7.04$, $p = 0.07$) or humerus model (KW $\chi2 = 3.41$, $p = 0.33$). There were no significant differences in *Pongo* (femur: KW $\chi2 = 3.91$, $p = 0.27$; humerus: KW $\chi2 =6.68$, $p =0.08$). The model including age and sex was significant in *Gorilla* femora, but there were no significant differences post Bonferroni correction between ages (Table 3.5). In the *Gorilla* humerus sex did not have an effect, but age had a significant effect on AP/ML. However, like the humerus, there were no significant differences in the pairwise comparisons (Table 3.5). In all three species the midshaft of the femur became increasingly medio-laterally expanded relative to the antero-posterior length (Figure 3.8). This pattern was a more stepwise decline in *Pan* compared to in *Gorilla* where there was an abrupt shift between infancy and juvenility (Figure 3.8). In *Pongo* the shift was gradual except in adult males. The humerus remained more stable across ontogeny in all three species (Figure 3.8).

Table 3.5: Statistical p-values from Kruskal-Wallis pairwise comparison of Ix/Iy across age in the femur of Pan and Gorilla. Significant differences post-Bonferroni correction are italicized.

Femur	Adolescent	AF	AM	Infant
AF	0.758	-	-	-
AM	0.988	0.758	-	-
Infant	0.238	0.031	0.101	-
Juvenile	0.758	0.758	1	0.031
Humerus	Adolescent	Adult	Infant	
Adult	0.641	-	-	
Infant	0.19	0.017	-	
Juvenile	0.641	0.641	0.019	

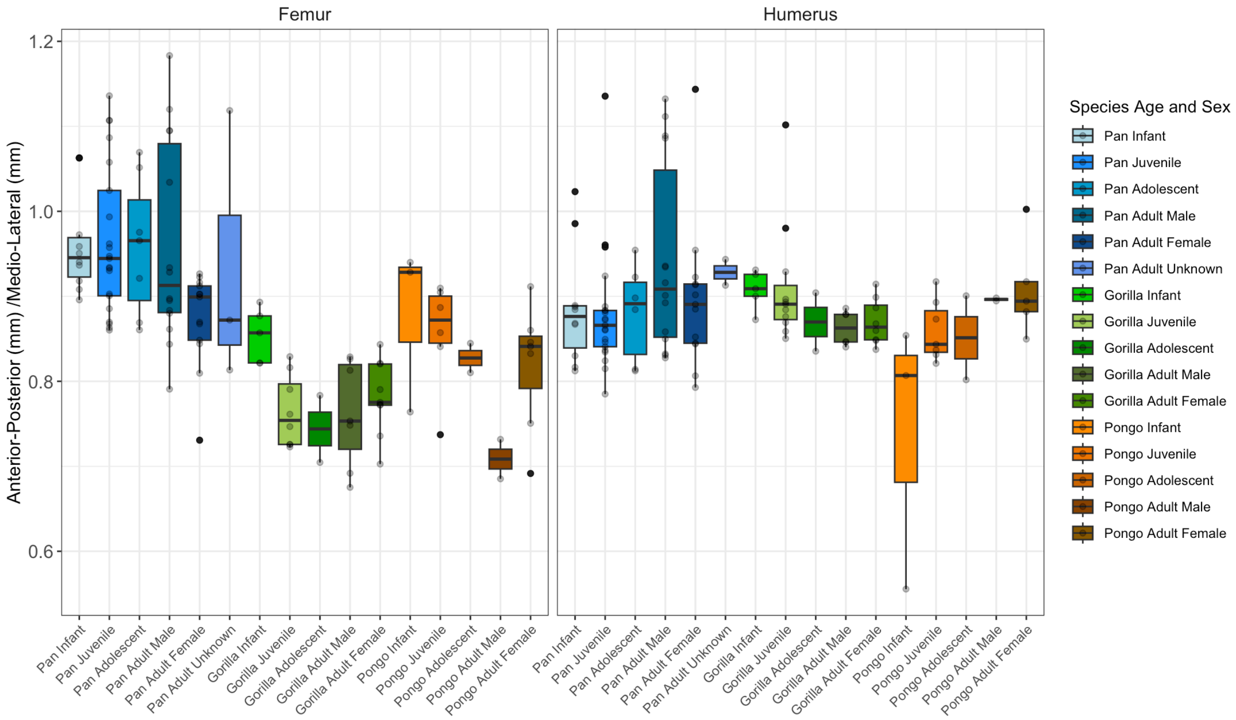

Figure 3.8: AP/ML, or the Ix and Iy in the anterior-posterior and medio-lateral directions divided by one another, during ontogeny in all species with adults separated by sex. Femoral data is the left panel, and humeral data is the right panel.

3.5 Discussion

This study investigated whether the patterns of arboreal and terrestrial locomotor change across ontogeny were detectable in *Pan troglodytes,* and if the changes seen in *Pan* matched the morphology of *Gorilla gorilla gorilla,* and *Pongo* across ontogeny. Overall results indicated mixed patterns of skeletal change. Infant and juvenile *Pan* tended to differ from adults and adolescents with relatively stronger humerii, rounder cross-sections, and increased cortical area relative to the total area. There were limited differences during ontogeny between age categories in both *Gorilla* and *Pongo*, which is supported by locomotor data indicating limited ontogenetic changes across age classes in both species (Doran, 1997; Thorpe and Crompton 2005; Manduell et al., 2011). Developmental differences in *Gorilla* tended to occur between infants and individuals of all other ages classes. This was consistent with evidence from *G. beringei beringei* showing that infants are more arboreal than other age categories. Results from all three species provided partial support for the predictions that cortical features associated with forelimb dominated arboreality was more prevalent in *Pan* infants and juveniles as both of these ages more closely matched *Pongo* in relative forelimb vs. hindlimb strength. Contrary to predictions, there were no effects of ontogeny on *J*/CA values in any species. In terms of midshaft shape, infant *Pan* had rounder cross-sections than adults but not adolescents in the femur. The humerus revealed no ontogenetic effects. Interestingly both *Gorilla* and *Pongo* had more medio-laterally expanded midshaft shapes that were more consistent across ontogeny. This fits the locomotor data but did not fit predictions as *Pongo* was expected to have rounder cross-sections. The *Pan* femoral/humeral strength regression trend was intermediate between *Gorilla* and *Pongo,* matching locomotor predictions.

3.5.1 Femoral and Humeral Strength

It was predicted that infant and juvenile *Pan* would have relatively higher forelimb (humerus) *J* than. The hindlimb (femur) *J,* and that this pattern were shift in adults and adolescents. The pattern of relative strength was predicted to be stable in *Pongo* with the forelimb maintaining greater relative strength across ontogeny. In *Gorilla* it was predicted that the hindlimb would have a relatively higher strength than the forelimb, and that the shift to increasingly stronger hindlimbs relative to forelimbs would occur earlier than in *Pan.* When humeral *J* was regressed on femoral *J* across all three species, there were no specific species trends. This demonstrated a consistent change across ontogeny when all species were included in a model.

In species specific regressions, there were no changes in the relationship between *J* in either bone in *Gorilla* and *Pongo.* In *Pan,* when the second best model was considered, there was a significant difference in infants and juveniles compared to adults and adolescents, with infants and juveniles having relatively stronger humerii given their femora when compared to adults and adolescents (Figure 3.2A). When statistically comparing the difference in the ratio of femoral to humeral strength, there were significant differences. Infant *Pan* had more equivalent strength between the two bones, indicating that post-infancy the femur increases at a greater rate than the humerus. This matched the predictions and previous results which demonstrated that femoral/humeral strength increase with age and that infants had relatively stronger humerii compared to their femora (Sarringhaus et al., 2016). However, in this previous study, there was an adult like femoral and humeral strength pattern by juvenility (Sarringhaus et al., 2016). By contrast, in this study, juveniles grouped more with infants, providing support for a locomotor behavioral stage in juveniles that is intermediate between infants and adolescents/adults and groups more with infants (Sarringhaus et al., 2014). The results of the skeletal regression shows a gradual shift to relatively stronger femora in juveniles, though juveniles still significantly differed from adults. This shift skeletally matches locomotor data indicating a high degree of variability in juvenile behavior relative to adults and adolescents (Sarringhaus et al., 2014). This greater diversity in behavior among juveniles compared to adolescents and adults (albeit lower than in infants) coincides with the period of rapid growth in *Pan*; peak body mass gains (kg/year) occur between 5-10 years, solidly within the juvenile age period (Leigh and Shea, 1996). It is reasonable to expect that during the peak growth during juvenility, there will be higher variability in rate and timing of maturation within the age category, leading to higher variability of the data in this age group (Figure 3.2A).

Pongo maintained relatively stronger humerii compared to their femora throughout ontogeny, and there were no significant differences in the ratio of J_H/J_F across any age category (Fig 3.2C). These results match the predictions, as there is limited evidence for major changes in locomotion across the age categories. All ages are highly arboreal and spend limited time terrestrially with the exception of *Pongo pygmaeus* flanged adult males, many of whom move terrestrially (Manduell et al., 2011). The major positional behavior differences between the ages is substrate usage, diameter of branches, and the size of the gap crossed between trees (Thorpe and Crompton, 2005; Chappell et al., 2015). Given the similarity in locomotion across ontogeny, a plausible explanation for the changes in skeletal morphology (although limited) in *Pongo* cross-sectional properties relative to other apes may be due to differences in the life history of *Pongo* compared to *Pan* and *Gorilla.*

Members of genus *Pongo* have the slowest life history of all the non-human great apes. The two species have slightly different life histories. For example, *Pongo abelli* having longer interbirth intervals (9.3 years, the longest of any ape; Wich et al., 2004) vs. *Pongo pygmaeus* (7.0-7.7 years; Galdikas and Wood, 1990, Knott, 2001). This is still longer than either *Pan* or *Gorilla. Pongo pygmaeus* has an M1 eruption of 4.6 years, vs. *Pan troglodytes* at 4 years and *Gorilla gorilla* at 3.8 years (Kelley and Schwartz, 2010). However, weaning does not occur until 5-6 years in *Pongo*, which is later than that of *Pan* and *Gorilla* (van Noordwijk et al., 2009). Critically, *Pongo* females are not reproductive until around 15 years old, relative to 12 years in

Pan and 10 in *Gorilla* (Knott et al., 2009; Thompson et al., 2007; Robbins et al., 2006). Locomotor independence occurs at around 4-5 years in *Pongo pygmaeus* and as early as 3 years but more definitively at 6 years in *Pongo abelli,* however this appears highly impacted by environment (van Adrichem et al., 2006; Mendonça et al., 2017). These ages are similar to *Pan*, in which complete locomotor independence is achieved at the transition to juvenility (Sarringhaus et al., 2014). Ultimately the results matched the predictions of the present study, with limited change across ontogeny and relatively stronger humerii compared to their femora. The predictions were further supported because *Pan* infants closely resembled the *Pongo* pattern, which was inferred to be caused by increase forelimb loading through arboreal locomotion (Figure 3.1, Figure 3.3).

Gorilla had consistently stronger femora relative to their humerii across all age categories, as predicted. *G. b. beringei* is predominantly terrestrial, with the exception of during infancy (Doran, 1997), and this pattern may also hold for *G. g. gorilla,* although studies of their locomotor ontogeny are limited (Remis, 1995; 1998; 1999) The results of the present study demonstrated a pattern of increasing bone strength in *G. g. gorilla* with no dramatic shifts in the strength ratio across ontogeny. These results matched previous studies assessing *Gorilla* ontogeny, with *G. g. gorilla* having relatively stable femoral to humeral strength across ontogeny (Ruff et al., 2018). Comparatively, within *G. b. beringei* there was a larger increase in femoral strength relative to humeral strength along ontogeny, coinciding with increased terrestrial behavior and larger body masses (Ruff et al., 2018). In a recent study, Sarringhaus et al. (2022) were able to distinguish between adult *G. g. gorilla* and *G. b. beringei*; consistent with reports that the former is more arboreal than the latter. These findings supported the prediction that arboreal vs. terrestrial locomotion can be detected in the skeleton at different points within ontogeny.

When comparing across species, in *Pan,* the femoral to humeral strength ratios are bracketed by *Pongo* and *Gorilla.* During infancy and juveniles with smaller *J* values (smaller cross sections, smaller body size) when forelimb dominated locomotion is at its highest, the ratios of femoral to humeral strength were similar to *Pongo.* As *J* values increased among juveniles, the strength ratios were similar to those of adult and adolescent *Pan,* and *Gorilla* at all ages. Skeletally, this matches predictions based on locomotion, though in some cases the trend is weakly significant or nonsignificant.

Cortical area was a strong predictor of *J* in all three species (Figure 3.5). However, there were no significant age effects in the relationship between *J* and CA. The ratio of *J*/CA did show age effects, with infant and juvenile *Pan* significantly differing from adults. Infants and juveniles both had lower ratios than adults in the femur and humerus. Overall, this indicated a step wise increase in the ratio of *J*/CA of *Pan* until adolescence when the adult phenotype is reached. Given the similarity in pattern of *J*/CA ratio increase in both the femur and humerus, the pattern was more likely due to increasing size rather than locomotion *per se* This potential mechanism had support in both *Pongo* and *Gorilla.* In *Pongo* there were no age differences, but rather a stepwise increase in the ratio until adulthood. In *Gorilla* adult males had a significantly higher ratio in the femur and humerus compared to infants, and juveniles, and significantly differed from the lower ratio of adult females in the femur. Adult male *Gorilla* had the highest ratios of *J*/CA across all species and ages.

3.5.2 Cortical Area vs. Total Area

There was a significantly positive relationship between CA and TA across all species and over ontogeny but within *Pongo* and *Gorilla* there was no effect of age. There was a significant effect of age in the femur of *Pan,* where infants and juveniles had significantly more cortical area given their total area than in adults or adolescents. Total area increased more than cortical area in adolescents and adults compared to infants. This pattern has been seen in human limb bone cross-sectional change, with a greater periosteal expansion and endosteal absorption through growth (Ruff et al., 1994; Goldman et al., 2009; Gosman et al., 2013). In the *Pan* femur, infants and juveniles had more cortical area relative to their total area which was likely not a function of locomotion but rather inherent development. This pattern was not likely to be locomotor, as there was no effect in the humerus. Given the increased forelimb dominated locomotion during those ages, it was expected that total area may increase relative to cortical area to facilitate increased bending strength (*J*). This is because as total area increases but cortical area remains more constant, it creates a larger medullary area (Bouxsein, 2005). This change creates a greater distance between the bone material and the neutral axis, resulting in increased torsional strength (Bouxsein, 2005). This pattern was seen in prepubertal tennis players in humans, where the dominant playing arm had a greater periosteal expansion in the humerus (increased total area) compared to cortical area and medullary area (Bass et al., 2009).

3.5.3 I_{max}/I_{min}

In all three species the femur increased in ellipticity across ontogeny, with limited differences in the humerus. In *Pan* there was no significant effect of ontogeny on humeral ellipticity. The femur showed the most change in response to locomotor variation, with infants and juveniles having rounder cross-sections compared to adults. Sarringhaus et al. (2016) also previously found that in *Pan* infant femora have rounder cross sections that become more elliptical in adulthood, while there was no age-effect in the shape of the humerus. In this study, the cross-sectional shape shifts proportionally across all age categories, moving from more round to more elliptical. Rounder cross-sections are thought to buffer against loading in multiple directions during arboreal locomotion, while elliptical cross-sections are thought to reflect increased stereotypical loading during terrestrial quadrupedalism (Sarringhaus et al., 2014; Sarringhaus et al., 2016). This study differs from Sarringhaus et al. (2016) in that young infants and old infants were combined into a single category to increase sample size and facilitate statistical robusticity.

Gorilla had a significant change in ellipticity from infancy compared to juveniles and adults, but all other ages did not significantly differ from one another. While skeletal data supports a shift from forelimb dominated loading to increased hindlimb dominance*,* there is no current locomotor data from *G. g. gorilla* to corroborate this change. This matches locomotor behavior in *G. b. beringei* where from infancy to juvenility there is a dramatic decrease in arboreal or forelimb dominant locomotion (Ruff et al., 2013). This change in locomotion is thought to be reflected in relative strength between the humerus and femur in both *G. b. beringei* and *G. g. gorilla,* with the transition from infancy to juvenility having a significant increase in femoral strength (Ruff et al., 2013; Ruff et al., 2018; Figure 3.6). *Gorilla* have relatively abducted hindlimbs, even given their large body size (Preuschof and Tardieu, 1996). Increased abduction and the predominant usage of less variable terrestrial locomotion supported the results found in the present study.

In *Pongo* the femur continually increases in ellipticity but the humerus decreases in ellipticity (becoming slightly rounder), though not significantly. In *Pongo*, abduction is also expected to increase across ontogeny, but for different reasons. As body size increases in Pongo, the lower limb increasingly is used in abducted postures to distribute body mass along branches. Increased abduction results in increased forces in the axial plane of the lower limb, resulting in increased ellipticity to compensate. As was detected within the skeleton, age had limited effect when studying the locomotion of Bornean *Pongo* (Manduell et al., 2011). Rather the major difference between adults and subadults was the position used during feeding vs. locomotion, with adults engaging in suspension predominantly for travel and subadults using suspension more for feeding (Manduell et al., 2011). Thorpe and Crompton (2005) found similar results in Sumatran *Pongo,* with substrate diameter rather than age-sex classes being a strong contributor locomotor behavior and thus limb bone ellipticity. Future studies assessing variation between *Pongo* populations could test if increased or decreased suspension within the groups is detectable in limb bone ellipticity.

Interestingly, all three species demonstrate differences in their patterns of change across ontogeny. Locomotion is linked with diaphyseal shape in *Pan*, with shape being more correlated with time spent in arboreal locomotion than with body mass (Carlson et al., 2006). However, the patterns of change across ontogeny cannot be solely attributed to locomotion, and body size may be a contributing factor. The present study considered taxa of highly variable size, both across genera and within genera, which could influence shape patterns. Terrestrial animals, including primates, show similar patterns of bone strength scaling between large and small body plans such that the peak strain is similar at large and small body masses (Steudel and Beattie, 1993; Biewener, 1989). This was achieved through changes in limb posture, with larger animals aligning their limbs in more columnar postures, limiting the ground reaction forces felt with larger masses (Biewener, 1989). This becomes more difficult to test in arboreal animals and it is not known if similar principles can be applied, particularly given the variable compliance of arboreal substrates. The cross-sectional properties will align themselves to protect against the greatest risk of fracture.

The pattern within the humerus is different. The limited change in *Pan* and *Pongo* could reflect the variability and the duration of locomotor postures used. For example, submodes of suspensory behavior may vary highly between individuals within age categories (Sarringhaus et al., 2014). Individuals experiencing more varied forces on the humerus would have rounder cross-sections compared to individuals experiencing more stereotypical forces. As stated above, all ages of *Pongo* are highly arboreal (Manduell et al., 2011). Rather than locomotor shifts, increasing size through ontogeny appears to be the major driver of strength differences. Within *Gorilla,* the shift to increased ellipticity from infancy to juvenility and beyond is the result of increased terrestrial behavior which loads the bone in a more stereotypical fashion. Alternatively, due to the expansion of the brachial flange, analyzing 40% bone length may be a better location to test humeral responses to locomotion (Sarringhaus et al., 2016).

3.5.4 Ellipticity Directionality

Results indicated that in all species the cross-sections became more elliptical and medio-laterally oriented across ontogeny, but there were no significant changes across age categories. The change in directionality in *Pan* and *Gorilla* corresponded with the shift to increased terrestrial locomotion, and matched strength changes reported in studies of *Gorilla* (Ruff et al., 2013; Ruff et al., 2018). The increased medio-lateral expansion of the femur in *Gorilla* is likely due to increased shear strain on the midshaft due to knuckle-walking, and a large body mass. *Gorilla* femora are positioned para-sagittally to the body with a large varus angle, and not directly under the center of mass. *Gorilla* have particularly large medial condyles, with enlarged lateral condyles, especially compared to *Homo* and even when compared to *Pan* (Tallman, 2016). As suggested by Ruff (1988) the expansion is likely due to weight distribution, particularly against medially concave bending moments (Preuschof and Tardieu, 1996). The large varus angle increases the necessity for buffering against these bending moments in the medio-lateral or frontal plane (Preuschof and Tardieu, 1996). The results of this study match what would be predicted in the directionality of ellipticity. Similarly, *Pongo* has increased medio-lateral elongation in the femoral cross-section. This corresponds with increased medio-laterally oriented and increased abduction dominated loading during suspension and quadrumanous locomotion with a larger body mass (Manduell et al., 2011; Thorpe and Crompton, 2005). *Pan* has a stepwise change, but remains rounder than *Pongo* or *Gorilla* though these results are not significantly different across age categories.

Gorilla and *Pongo* have increased medio-lateral expansion of their cross-sections relative to *Pan*, presumably due to increased stresses in the frontal plane or due to constraints imposed by anatomy, such as the wider distal femur. It is important to note that although there is a similar morphology in terms of medio-lateral expansion between adult *Pongo* and *Gorilla,* the cause of the expansion is different. Arboreal and terrestrial locomotion can cause similar impacts on the skeleton depending on body mass; the type of locomotion is also important to consider when using this data to reconstruct fossil taxa locomotor repertoires for example, if a taxon is large bodied (such as *Proconsul major*), an ML expanded elliptical midshaft could indicate either terrestrial locomotion with parasagittal leg placement, or it could indicate increased abduction.

The limitations of ellipticity and directionality of a cross-section were found in the humerus as well. In a study on humeral midshaft shape, suspensory species such as *Pan* and *Pongo* could not be discerned from one another in terms of their ellipticity (Patel et al., 2013). Further, while qualitatively it was noted that *Pan* and *Pongo* had rounder humerii than *G. g. gorilla* and *G. b. beringei,* the midshafts could not be statistically differentiated among any of the four taxa (Patel et al., 2013). It is necessary to pair the ellipticity and directionality of cross-sectional shape with other morphological variables. These could include bone length, articular surface shape and size, and critically whenever possible a comparison of fore to hindlimb strength. This will allow for the best reconstruction of locomotion using cortical cross-sections.

In addition to locomotion and body size, previous studies have shown some evidence for genetic canalization on midshaft shape (Morimoto et al., 2011; Canington et al., 2018; Cosneyfroy et al., 2022). In *Papio anubis,* there were no significant differences in Ix/Iy ellipticity corresponding to known shifts in locomotion and body mass across age stages (Cosnefroy et al., 2022). A study in *Pan* found similar results*,* with limited differences between captive and wild femoral ontogenetic I_x/I_y changes, even with locomotor restrictions in captivity vs. the wild (Morimoto et al., 2011). These findings showed that even with changes in locomotion and body mass between wild and captive, there was limited phenotypic plasticity in diaphyseal shape across ontogeny, suggesting a strong phylogenetic signal.

The role of genetics in influencing cross-sectional geometry in *Gorilla* is more equivocal. Captive infant *Gorilla gorilla gorilla* femoral cross sections more closely resembled those of wild *Gorilla gorilla gorilla,* while adult captive *G. g. gorilla* femora were similar to those of *G. b. beringei,* (Canington et al., 2018), who were more terrestrial than wild *G. g. gorilla.* If the shape of the cross-sections was more genetic, it would be expected that adult *G. g. gorilla* would look more similar to one another, regardless of the captive vs. wild status. These results support the prediction in this study that locomotion has an effect on adult skeletal morphology. A potential discrepancy between Canington et al., (2018) and Morimoto et al. (2011) is in the methodology used to measure cross sectional properties. Canington et al. (2018) used pQCT data, which were analyzed via the built in scanner software, while Morimoto et al. (2011) used novel morphometric mapping. The morphometric mapping creates a cylindrical coordinate system based on key features of the bone. This method does not use a traditional cross section to gather morphological data, but rather a combination of surface and internal cortical traits.

3.5.5 Femur vs. Humerus Patterns

The results of the present study indicated that the femur had a stronger locomotor signal than the humerus. The former showed more differences in diagnostic variables than the latter. This bias may be due to the increased hindlimb dominance of hominoids, even among more arboreal ones. Support for the role of hindlimb dominance in affecting cross-sectional geometry comes from studies of limb length and growth patterns. In hominoids, forelimb length scales slightly negatively relative to body mass, while hindlimb length scales more negatively than the forelimb (Jungers, 1996). *Pongo* females have longer forelimbs than expected given the quadrupedal primate baseline (+22.3 and +23.2 in *P. pygmaeus* and *P. abelli*) and both sexes of *Pongo* have longer forelimbs compared to other large bodied hominoids (+14.7 in male *P. pygmaeus* and +20.2 in female *P. abelli*) (*ibid.*). However, in all great apes, as body size increases, the forelimb length increases at a greater rate than the hindlimb (Jungers, 1996). The increase in forelimb length relative to hindlimb would facilitate reduced ground reaction forces in the humerus but increased ground reaction force in the hindlimb. Increased force could have been a contributing factor in the increased responsiveness of the femur relative to the humerus in age related locomotor change.

During *Pan* quadrupedal locomotion there is an increase in hindlimb weight support, allowing greater flexibility and reduced stress on the forelimb (Raichlen et al., 2009). The increased weight support of the hindlimb could plausibly have resulted in *Pan* and *Gorilla* having stronger femoral signals of locomotion compared to the humerus, especially given increased proportions of hindlimb dominated locomotion across ontogeny (Sarringhaus et al., 2016). This hypothesis was supported by limited humeral responses to locomotion relative to the femur in primates (Shaw and Ryan, 2012). The morphology of the humerus in hominoids results in difficulty reconciling locomotion depending on the location along the diaphysis that is tested. This is due to the deltoid tuberosity and medial distal flange morphology. The 50% midshaft site, used in this study, is less likely to demonstrate a locomotor signal compared to

40% midshaft used in others (Sarringhaus et al., 2016; Patel et al., 2013; Sarringhaus et al., 2022).

Ultimately while relative strength between the femur and humerus, ellipticity, and directionality were strong indicators of position behavior; the torsional strength of bone compared to cortical area and relative cortical area to total area were not. Rather, these variables may represent better signals of body size and limb posture. Further work is necessary to determine if posture during locomotion, particularly in male and female *Gorilla*, is related to bone strength. Overall, these results while not always significant supported the hypotheses and predictions; and demonstrated that ontogenetic shifts in locomotor positional behavior, or lack of change, can be detected across all three species.

3.6 Conclusion

This study set out to determine whether arboreal and terrestrial locomotor transitions across ontogeny would be reflected in the skeleton of *Pan troglodytes,* and if the changes matched the morphology of two bracketing species; one that used predominantly arboreal locomotion (*Pongo*) and another than used predominantly terrestrial locomotion (*Gorilla*). Results supported two out of three of the predictions: (1) *Pan* infants had femoral and humeral *J* ratios similar to those of *Pongo,* and *J* in both bones also increased across ontogeny as terrestriality increased. *Gorilla* had a steep increase in femoral *J* relative to humeral *J* from infancy to juvenility, while *Pongo* maintained relatively stronger humerii across ontogeny. This matched locomotor data, and predictions from this study. (3) *Pan* infants had more circular femora than adults, and the increase in ellipticity occurred in a stepwise pattern. Qualitatively the ellipticity of *Pan* infants and juveniles was more similar to *Pongo* than to *Gorilla,* whose infants most closely resembled *Pan* adults. These changes were only shown in the femur, which partially matched predictions from this study. The second prediction that *J*/CA will be higher in the humerus of *Pan* infants and juveniles, but become greater in the femur of adults and adolescents was not supported. As in previous studies, the femur demonstrates stronger locomotor signals compared to the humerus (Shaw and Ryan, 2012). Future studies should develop a study design that allows all of these factors to be considered together when assessing locomotor signal, particularly when attempting to predict how the proportion of arboreal and terrestrial may be reflected in the long bone shafts of fossil hominoids and hominins.

3.7 Appendix

Table 3.6: Statistical p-values from Kruskal-Wallis pairwise comparison of Femoral J/ Femoral CA across age in Pan and Gorilla. Significantly differences post-Bonferroni adjustment are italicized.

Pan	Adolescent	AF	AM	AU	Infant
AF	0.00778	-	-	-	-
AM	0.00778	0.73377	-	-	-
AU	0.83333	0.70467	0.70467	-	-
Infant	*0.00016*	*3.00E-06*	*2.20E-06*	0.00824	-
Juvenile	*0.00336*	*3.10E-07*	*1.60E-07*	0.00943	*1.70E-05*
Gorilla	Adolescent	AF	AM	Infant	
AF	0.3636	-	-	-	
AM	0.0794	*0.0017*	-	-	
Infant	0.2381	*0.0033*	0.0063	-	
Juvenile	0.4364	0.008	*0.0017*	0.0316	

Table 3.7: Statistical p-values from Kruskal-Wallis pairwise comparison of Humeral J/ Humeral CA across age in Pan and Gorilla. Significantly differences post-Bonferroni adjustment are italicized.

Pan	Adolescent	AF	AM	AU	Infant
AF	0.0279	-	-	-	-

AM	0.01058	0.40371	-	-	-
AU	0.40371	0.61224	0.7	-	-
Infant	*0.00016*	*3.00E-06*	*2.20E-06*	*0.03497*	-
Juvenile	*0.00089*	*1.10E-07*	*2.60E-08*	*0.01318*	*0.00016*
Gorilla	Adolescent	AF	AM	Infant	
AF	0.4444	-	-	-	
AM	0.102	0.0145	-	-	
Infant	0.2381	0.0059	0.0108	-	
Juvenile	0.641	0.0059	*0.0016*	0.0145	

3.8 References

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Chapter 4 Intraspecific Variation of Cross-Sectional Properties in *Pan* and *Gorilla*

4.1 Introduction

Reconstructing the behavior of fossil primates using fossilized remains is an important area of study for anthropologists. One of the challenges of studying fossil species, including hominoids, is the rarity of complete and well-preserved specimens. As a result, efforts to reconstruct locomotor behavior focus on understanding the form-function relationships between locomotion and suites of bones in modern taxa. However, such interspecific approaches to reconstructing locomotion typically rely on species means of skeletal properties, which masks any variation in form or function within species. As a result, anthropologists do not understand how much skeletal variation is present in modern species, let alone the degree to which genetic, behavioral, or environmental differences contribute to this variation. This makes it difficult to understand how much skeletal variation to expect in fossil species, and to tease apart the forces contributing to such variation. The problem is compounded in paleontological studies because it is challenging to know if the fossils found are representative of means or are outliers in a population. As a result, the ability to delineate species morphological bounds is limited, such as defining whether different phenotypes represent two species or one sexually dimorphic species. The first step to understanding the potential intraspecific variation present in fossil species is to quantify it in modern species with known locomotor repertoires and environment, which are constrained both in time and space. This allows for the comparison of behavioral and environmental impacts on skeletal variability. However, few studies have looked at patterns of intraspecific variation in locomotor elements, even though modern hominoids have a wide range of locomotor variation within species (Hunt et al., 1996); *Pan* in particular exhibits locomotor variation amongst adults (Doran and Hunt 1994; Pontzer and Wrangham, 2004).

Bone is a plastic tissue that responds to the loads placed upon it, with its size and shape reflecting a combination of genetic and external environmental influences. As a result, skeletal interspecific and intraspecific variation is the product of inherent genetic variation in a population as well as behavioral variation among individuals of a population/species. Traits such as articular surface shape are less plastic across ontogeny. Although these traits are useful for understanding interspecies differences, they may not strongly reflect behavioral variations among individuals but rather the range of potential behaviors those individuals were capable of (Ward, 2013). In contrast, cortical cross-sectional properties of long bones have greater plasticity over the life of an individual and are informative of individual behavior (For review see: Pearson and Lieberman, 2004; Ruff et al., 2006). These traits include the distribution of bone (cortical area and total area), the ellipticity or shape of the cross section (I_{max} and I_{min}, or I_x and I_y), and the resistance to bending and torsion, also known as strength (J).

There is some evidence for significant post-cranial intraspecific variation in primates. Buck et al. (2010) found that across catarrhines the forelimbs were significantly more variable than the hindlimbs, while the diaphyses were significantly more variable than the epiphyses. The bones in the forelimb and hindlimb were combined in this study, which prevents bone to bone comparison. There were no differences found between males and females (*ibid.*). In a study assessing intraspecific variation in the human pelvis, no significant differences between males and females were found (Kurki et al., 2013). Due to body size differences, many studies have assessed variation across individuals through use of coefficients of variation (CV), a size independent metric. CVs are quotients of the standard deviation and mean, which can be multiplied by 100 for comprehension (Buck et al., 2010; Stock, 2006; Plavcan, 2002).

A limitation of previous studies (e.g. Buck et al., 2010; Fulwood and Kramer, 2013; Kurki, 2013) is that they have focused on comparing linear measurements of character traits averaged across species, rather than comparing individual skeletal traits of specific taxa. In addition to using linear measurements, these studies used a measurement called V* which is a metric that uses a modified coefficient of variation to account for small sample sizes. Further, these studies used variable classes, or aggregations of multiple linear measurements (e.g., humeral length, diaphyseal diameter, humeral head diameter etc.) (Buck et al., 2010; Kurki, 2013). For each variable class, the mean of the V* was calculated across relevant measures in all species. Then the mean of all relevant variable class V*s was compiled and tested against other variable classes (e.g. all forelimb vs. all hindlimb measurements). Utilizing V* helps accommodate smaller sample sizes but does not allow direct comparison between variables, limiting its power to make specific inferences.

To address these gaps in knowledge, this study examines the levels of intraspecific skeletal variation in two species, *Pan troglodytes troglodytes* and *Gorilla gorilla gorilla*, and explore whether behavioral differences and size dimorphism impact intraspecific variation. Both the *Pan* and *Gorilla* skeletons were collected from the same two sites in Cameroon at around the same time, and present an interesting natural experiment because they are occasionally sympatric and can occupy similar environments (Tutin et al., 1993; Stanford and Nkurunungi, 2003; Head et al. 2012), yet differ in their proportions of positional behavioral submodes (Doran, 1997), body size and levels of sexual dimorphism (Jungers and Susman, 1984; Uehara and Nishida, 1987; Smith and Jungers, 1997). In addition, they are relatively closely related phylogenetically, sharing a common ancestor on the order of 10 million years ago (Langergraber et al., 2012). Studying two species of hominoids with known locomotor similarities and differences (but that live at essentially the same time and place) allows for a comparison of behavioral differences between sexes and across species, and for the study of how differing levels of sexual dimorphism impact skeletal variation. This will lead to a better foundation for studying variation in the fossil record, particularly amongst taxa purported to have varying degrees of sexual dimorphism and potentially sex-based differences in locomotion such as within the genus *Australopithecus* (Stern and Susman, 1983).

4.2 Questions

This study asks the question: Are there differences in intraspecific variation among the long bones of *Pan troglodytes troglodytes* and *Gorilla gorilla gorilla* by species, sex, and/or bone? To explore this question, the analysis had four main comparison groups:

Comparison 1: Are the CVs of males and females of a species the same or different for each parameter? **Comparison 2:** Are the CVs of males and females *across* species the same or different for each parameter? Within a given sex across species, do CVs differ by skeletal location? **Comparison 3:** Are the CVs across all four bones the same in each species and sex?

Comparison 4: Are there differences in the pattern of variation among cross-sectional traits within each species and sex? Does this differ between bones?

4.3 Methods

4.3.1 Samples

The sample in this study consisted of adult male and female *Pan troglodytes troglodytes* and *Gorilla gorilla gorilla* (Sample sizes are listed in Table 4.1). All bones are curated by the Cleveland Museum of Natural History (CMNH) in the Hamann-Todd Osteological Collection. Individuals were wild caught from two locations in Cameroon (called Ebolwa and Abong Mbong in the museum catalog), but more specific geographical data are unavailable. All individuals were aged as adult by degree of epiphyseal closure and had no observable pathologies or injuries on the sampled bones. Skeletal elements included the left femur, humerus, radius, and tibia. If the left side had post-mortem damage, the right was used in its place. While bilateral asymmetry is present in chimpanzees (Sarringhaus et al., 2005), the total number of right side bones used was minimal.

Table 4.1: Sample sizes per bone for each species and sex.

Species	Sex	Femur	Humerus	Tibia	Radius
Chimpanzee	Female	31	32	31	32
Chimpanzee	Male	16	14	15	15
Gorilla	Female	28	27	26	27
Gorilla	Male	30	30	27	29

4.3.2 Data Analysis

Skeletal trait quantification for each long bone was conducted using a peripheral quantitative computer tomography system (pQCT) (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). Each bone was scanned axially at 100 microns, using an in-plane pixel size of 0.10 mm x 0.10 mm. Calibration was performed daily using a standard phantom of known density to ensure image quality and consistency. Scans were taken at 50% (midshaft) of each bone according to functional bone length (as per Ruff, 2000) measured using an osteometric board. Bones were placed in custom holders that held them at the proximal and distal metaphyses (following Schlecht and Jepsen (2013)). The following traits were quantified: total cross-sectional area (TA, mm^2), cortical area (CA mm^2), second moments of area (I_{max} and I_{min}, mm^4), polar moment of inertia (J, mm^4), and cortical tissue mineral density (Ct.TMD, g/cm^3). For cross-sectional geometry, the image of each bone cross-section was input into Fiji, a distribution of ImageJ (Schindelin et al., 2012), and any bone fragments or tissue in the medullary cavity were removed using the fill tool in ImageJ. Following this, cross-sectional properties were obtained in Fiji using the MacroMoment plugin. Cortical tissue mineral density was estimated from mean grey values, using known densities from the standard phantom of the scanner (Schlecht and Jepsen, 2013).

4.3.3 Statistical Analysis

All statistical analyses were performed in R software (R Core Team, 2020). To account for the variation in body size in the sample, and the known effect of body size on skeletal tissue

(Buck et al., 2010) coefficients of variation (CVs) were used to provide a size-independent measure of variation, and thus allow for comparisons across groups with wide interspecific (e.g. male chimpanzee vs. male gorilla) or intraspecific (e.g. sexual dimorphism) disparities in body size. Statistical analysis used the package cvequality (Version 0.2.0; Marwick and Krishnamoorthy, 2019), which utilizes two different tests to assess statistical differences between CVs. The package includes a Modified Signed-Likelihood Rank test (MSLRT) and an Asymptotic test (AT). The MSLRT better controls for Type I error and allows for smaller sample sizes (Krishnamoorthy and Lee, 2014). The Asymptotic test is more widely recognized as an authoritative test for CVs but does not handle small sample sizes as well as the MSLRT (Feltz and Miller, 1996; Krishnamoorthy and Lee, 2014). Thus this study used both tests for increased statistical robustness and to leverage their complementary strengths, and report only results that reached statistical significance ($p< 0.05$) on both tests. Bonferroni corrections were applied to account for the multiple pairwise tests between each comparison.

To assess the effect of sex, pairwise comparisons within each species were made to assess if the traits for each bone differed between males and females. To test for interspecific differences, pairwise comparisons between species of males and of females were made. Finally, to assess intra-individual trait variation among bones, pairwise comparisons of a single trait between bones of each sex and species were made (e.g., variation in female chimpanzee cortical area between the femur and humerus).

Given the limited sample sizes, the data was resampled to create a distribution of CV values for each species and sex for each cross-sectional bone trait studied. For each species and sex class (e.g., female chimpanzee) and bone, the original data was resampled 1000 times to obtain a single CV, and then re-ran that process 1000 times. Then the original confidence interval for each bone parameter was compared to the distribution of 1000 resampled CVs (e.g., female chimpanzee radial J, see Figure 4.1). If the original CV was inside the 5%-95% confidence interval of the resampled data, then the value was considered to be a reasonable estimate of population variability. If the original CV was >95% or <5% of the distribution of resampled confidence intervals, it was concluded that the original data provided a poor estimate of population variability and discarded it. This was then repeated this procedure for the pairwise tests between sexes of the same species, and between sexes of different species (e.g., male and female chimpanzees, and female gorilla and female chimpanzee) from the original significant pairwise comparisons (Figure 4.1).

In the resampled data, of 112 individual resampled variables, only one mean value fell outside the range of the CI from the resampled data (Table 4.2): *Gorilla* femoral I_{min} was not significant in comparisons of male and female *Gorilla,* or female *Pan* and female *Gorilla*. This suggests that overall, our original means are adequately representing population variability. All figures for significant species to species and sex to sex comparison results are in the supplemental materials.

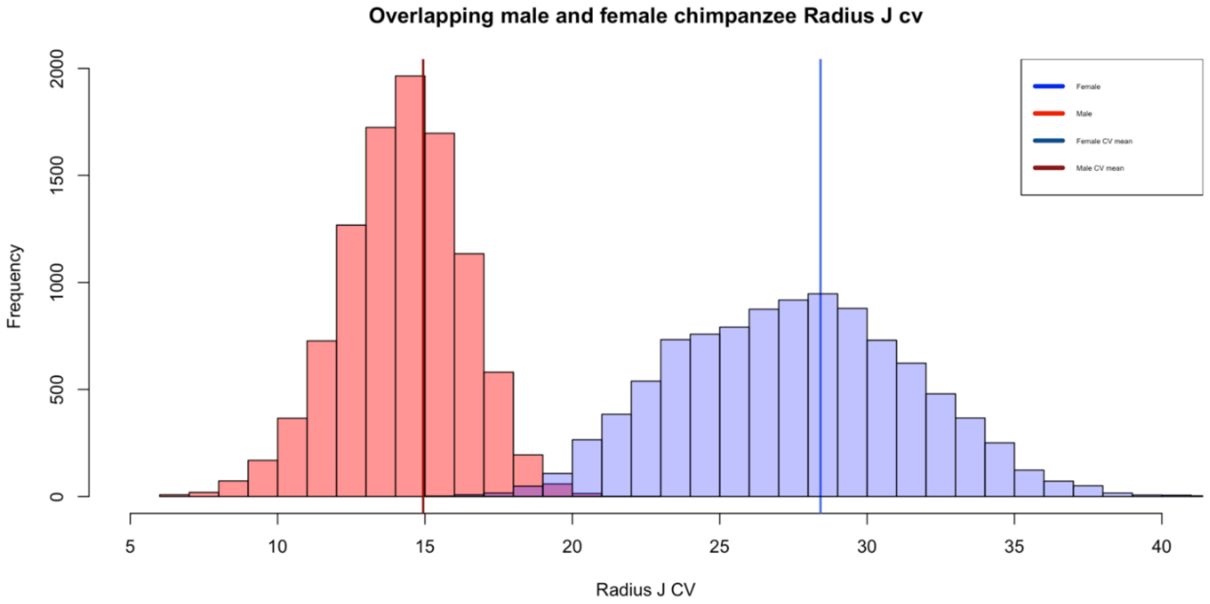

Figure 4.1: Example histogram showing distribution of resampled CV values for male and female chimpanzee radius J. The CV from the raw data is shown for males with the red line, and females with the blue line.

Table 4.2: Coefficient of Variation Values from Bootstrap for Male and Female Chimpanzees and Gorillas.

Bone	Trait	Chimp F Resampled mean	Chimp M Resampled mean	Gorilla F Resampled mean	Gorilla M Resampled mean
Femur	Bone Length	4.54	3.09	3.16	2.41
Humerus	Bone Length	4.49	4.04	4.36	3.11
Radius	Bone Length	5.18	7.08	4.58	3.38
Tibia	Bone Length	5.56	2.69	5.42	3.78
Femur	Ct.TMD	1.82	1.93	3.03	2.27
Humerus	Ct.TMD	2.84	2.05	2.54	1.8
Radius	Ct.TMD	2.95	2.67	2.4	1.53
Tibia	Ct.TMD	2.37	1.77	2.35	1.77
Femur	TA	11.9	10.4	18.3	14.1
Humerus	TA	15	8.35	15.9	12.5
Radius	TA	14.8	7.41	17.5	13.2
Tibia	TA	15.5	12.8	16.2	12.6
Femur	CA	20.5	13	14.1	9.88
Humerus	CA	15	11.3	103	7.23
Radius	CA	12.6	9.77	19.5	8.59
Tibia	CA	16.9	13.1	11.5	8.52
Femur	I_{max}	28.7	18.3	31.4	25.2
Humerus	I_{max}	24.3	18.5	24.7	20.2

Radius	I_{max}	26.9	14.3	30.6	23.2
Tibia	I_{max}	31.1	25.5	28	22.8
Femur	I_{min}	30.7	21.6	31	24.5
Humerus	I_{min}	25.6	20.5	23.5	18.2
Radius	I_{min}	26.8	15.8	32.5	25.7
Tibia	I_{min}	32.4	38	28.5	22.8
Femur	J	28.9	19.4	30.6	24.6
Humerus	J	24.5	18.7	24	19.3
Radius	J	27.5	14.2	30.3	23.7
Tibia	J	30.8	27.8	27.9	22.7

4.4 Results

The *Pan* and *Gorilla* specimens used in this study were from the same subspecies and were collected from two localities within Cameroon that were about 200km apart, so they were grouped together to increase sample size. When analyzed as separate localities, there were no differences in patterns of variation among *Pan* depending on site. In *Gorilla,* at Abong Mbong, males were consistently more variable than females, yet at Ebolwa, females were consistently more variable than males.

4.4.1 Intraspecific Variation in Chimpanzees

Sex: Overall, chimpanzee long bone traits tended to be more variable in females than in males in all four skeletal elements, but there was no consistent pattern in terms of which trait was more variable (Table 4.3). Traits that were significant prior to the Bonferroni adjustment are italicized in Table 4.3, and demonstrate that females were more variable than males more often than the reverse. However, when the Bonferroni adjustment was applied, only one variable, radius total area, was significantly more variable in females than in males (Radius: AT: 6.14 p=0.013; MSLRT: 6.99 p=0.008).

Bone: There were no significant differences in the CVs of traits across the bones in female chimpanzees. In male chimpanzees, the radius had more variable bone length when compared to the tibia (AT: 12.89 p=0.0003; MSLRT: 12.62 p=0.0004).

4.4.2 Intraspecific Variation in Gorillas

Sex: In *Gorilla*, there were no differences between the sexes in the variability of cross-sectional geometric traits (Table 4.4).

Bone: There were no statistically significant differences in either sex between bones.

Table 4.3: Coefficient of Variation Values for Male and Female Chimpanzees grouped by site; significant differences from both the Modified Signed-Likelihood Rank test (MSLRT) and Asymptotic test (AT) p<0.00714286, are bolded for the sex that was more variable. Nonsignificant differences with Bonferroni adjustment, but significant without the adjustment are italicized.

Bone	Trait	Chimp F	Chimp M
Femur	Bone Length	4.65	3.25
Humerus	Bone Length	4.59	4.26
Radius	Bone Length	5.29	7.70
Tibia	Bone Length	*5.69*	2.81
Femur	Ct.TMD	1.88	2.04
Humerus	Ct.TMD	2.94	2.16
Radius	Ct.TMD	3.02	2.85
Tibia	Ct.TMD	2.42	1.86
Femur	TA	12.30	10.90
Humerus	TA	*15.41*	8.83
Radius	TA	**15.08**	7.74
Tibia	TA	15.79	13.71
Femur	CA	*21.11*	13.56
Humerus	CA	15.34	11.81
Radius	CA	12.84	10.24
Tibia	CA	17.32	13.58
Femur	I_{max}	29.46	19.13
Humerus	I_{max}	24.79	19.44
Radius	I_{max}	*27.70*	15.06
Tibia	I_{max}	31.77	26.97

Femur	I_{min}	31.39	22.61
Humerus	I_{min}	26.15	21.71
Radius	I_{min}	*30.55*	16.58
Tibia	I_{min}	33.51	40.11
Femur	J	29.68	20.21
Humerus	J	25.05	19.69
Radius	J	*28.42*	14.94
Tibia	J	31.56	29.29

Table 4.4: Coefficient of Variation Values for Male and Female Gorillas grouped by site; significant differences from both the Modified Signed-Likelihood Rank test (MSLRT) and Asymptotic test (AT) p<0.007, are bolded for the sex that was more variable. Nonsignificant differences with Bonferroni adjustment, but significant without the adjustment, are italicized.

Bone	Trait	Gorilla F	Gorilla M
Femur	Bone Length	3.25	*5.36*
Humerus	Bone Length	4.48	4.76
Radius	Bone Length	4.70	5.09
Tibia	Bone Length	5.40	5.56
Femur	Ct.TMD	*3.10*	1.79
Humerus	Ct.TMD	2.62	2.29
Radius	Ct.TMD	2.27	2.49
Tibia	Ct.TMD	2.42	2.06
Femur	TA	18.83	14.82
Humerus	TA	16.28	13.34
Radius	TA	18.01	13.67
Tibia	TA	16.63	14.82
Femur	CA	14.63	16.47
Humerus	CA	10.60	12.26
Radius	CA	*20.27*	12.61
Tibia	CA	11.88	11.97
Femur	I_{max}	32.10	26.71
Humerus	I_{max}	25.27	24.12
Radius	I_{max}	31.47	28.54
Tibia	I_{max}	28.76	28.73
Femur	I_{min}	55.32	26.69
Humerus	I_{min}	24.13	23.24
Radius	I_{min}	33.40	23.66
Tibia	I_{min}	29.22	27.09
Femur	J	31.34	25.91
Humerus	J	24.57	23.09
Radius	J	31.04	24.76
Tibia	J	28.58	27.34

4.4.3 Interspecific Variation in Chimpanzee Males and Gorilla Males

In general, CVs were similar in *Pan* males and in *Gorilla* males. One parameter, tibia bone length, was significantly more variable (AT: 6.96 p=0.008; MSLRT: 7.88 p=0.005) in *Gorilla* males than in *Pan* males. This difference is driven by low variation in tibial bone length amongst male *Pan,* rather than by particularly high variation amongst male *Gorilla.*

4.4.4 Interspecific Variation in Chimpanzee Females and Gorilla Females

As in males, CVs did not differ between *Gorilla* females and *Pan* females for all but one comparison. In the femur, female *Gorilla* had significantly more variable cortical mineral density (AT: 7.05 p=0.008; MSLRT: 6.90 p=0.009). This difference is driven by relatively high variation in female *Gorilla* compared to all other groups for this trait.

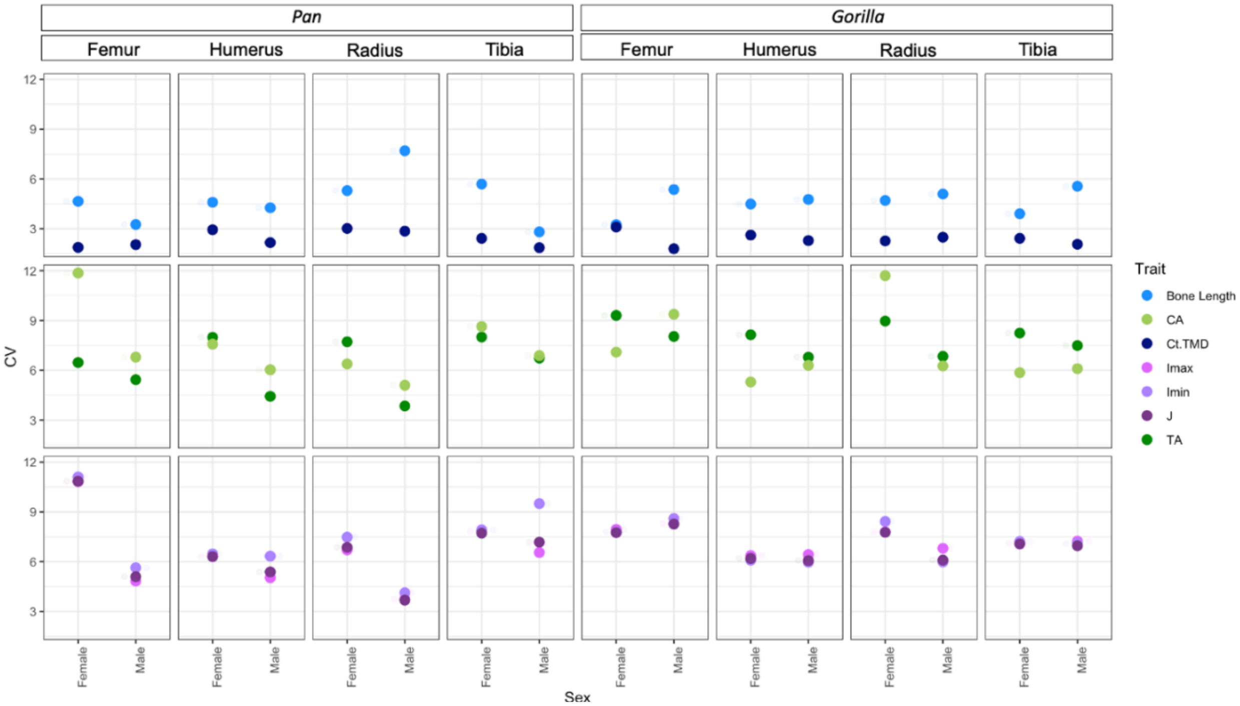

Figure 4.2: Distribution of trait coefficient of variation in both Chimpanzees and Gorillas. The bone and sex are along the X axis with the coefficient of variation on the Y axis for both species. Bone Length and Ct.TMD are in the top row, CA and TA in the middle, and I_{min}, I_{max}, and J in the last row.

4.4.5 Trait Patterns

The variability of different types of traits was assessed (bone length, cortical tissue mineral density, cross-sectional area (CA and TA), and cross-sectional geometry (I_{max}, I_{min}, and J)) across all four bones in each species. Overall, cortical tissue mineral density was less variable than all other traits (Figure 4.2). Cortical area, total area, I_{max}, I_{min}, and J had increased variation relative to bone length and to Ct.TMD (Figure 4.2).

4.5 Discussion

Understanding variation within species is important because natural selection occurs at the individual level, and variation can thus provide insights about the strength of selection on particular characters. Assessing intraspecific variation in modern populations is also critical because it provides key context for interpreting variation in the fossil record. This study quantified the level of intraspecific variation in long bone length, cortical mineral density, and cross-sectional structural properties in *Gorilla* and *Pan.* This study asked the question: *Are there differences in levels of intra and interspecific variation in these two taxa by species, bone, and sex?* Analysis revealed that while overall, differences in the extent of skeletal variation are subtle for wild African apes, some patterns emerge: (1) Within species, female *Pan* tended to be more variable than males, though these differences were limited in statistical significance. In *Gorilla,* the extent of variation was minimal and overall similar in males and females. (2) There were no clear statistical trends in intraspecific variation among bones either within or between species. (3) In interspecific comparisons, *Gorilla* long bone traits were sometimes significantly more variable than *Pan* long bone traits, while *Pan* long bone traits were never more variable than *Gorilla* traits. (4) There were some consistent trends in variability among the traits, with cortical mineral density and bone length being less variable than cross-sectional geometric traits. Given these findings, it is worthwhile to compare the behavior of male and female hominoids to investigate the cause of skeletal intraspecific variation. Studying modern species allows us to understand this variability in a single population occupying relatively similar time points and the same ecological niche. This is particularly important for fossil reconstructions, because males and females may engage in different behaviors. For example, as fossil sample sizes increase with continued collecting efforts, cross sectional geometric and linear measurements of individuals could be combined into proposed species-specific samples in order to determine whether CV's exceed, or fall within the range, of those from this study.

4.5.1 Sex Based Differences: Locomotor Variability

Although sex differences were limited, results indicated that long bone traits tended to be more variable in chimpanzee females than in males (i.e., females were more variable at $p \leq 0.05$ in 7 of 28 traits, but only 1 of 28 after Bonferroni correction, while males were more variable in 0 of 28 traits) (Table 4.3). Specifically, females had higher CV values in cortical area and total area as well as cortical tissue mineral density. Fewer traits had significantly different CV's between male and female gorillas; females were more variable in 2 of 28 traits, while males were more variable in 1 of 28 traits, and none of these differences remained significant after Bonferroni correction (Table 4.4).

One possible explanation for increased variability in long bone traits amongst female chimpanzees relative to males may be due to locomotor variability. Given that the magnitude, duration, and type of mechanical load influence the degree of osteogenic response (Turner, 1998), differences between males and females could be impacted by the *proportion* of arboreal and terrestrial behavior, as well as by *total locomotion* (day range). For example, primates who engage in proportionally more terrestrial locomotion have more elliptical long bone midshafts, while those that engage in more arboreal locomotion have rounder long bone midshafts to provide resistance to fracture in a variety of movement planes (e.g., Ruff, 2002; Pearson and Lieberman, 2004; Ruff et al., 2006, Carlson et al., 2008). Thus, if members individuals of one sex were more variable in their substrate use (e.g., some individuals within a sex are more terrestrial than others), they would be expected to display more variability in the midshaft cross-sectional properties of their bones. have more variability in its midshaft cross-sectional properties.

Among both *Pan* and *Gorilla*, habitat type (e.g., forest type and density) is strongly related to arboreal behavior (Doran, 1993; Remis, 1999), particularly for large-bodied primates. For example, Sarringhaus et al. (2014) found that *P. t. schweinfurthii* at Ngogo in Kibale National Park (Uganda) vertically climbed and descended trees at rates more similar to those of *P. t. verus* at Taï than to *P.t. schweinfurthii* at Gombe or Mahale. This most likely reflects habitat differences; Mahale and Gombe are more woodland and have little primary forest while Taï and Ngogo are both primary evergreen rainforests (Doran and Hunt, 1994; Struhsaker, 1997). Further, Taï chimpanzees spent more time at heights of 20 meters and over (50.7% for males and 52.3% for females), while at Gombe and Mahale, the chimpanzees did not go over 20 meters (Doran and Hunt, 1994). In addition to habitat differences, studies of chimpanzee positional behavior suggest sex differences in the proportions of total arboreal vs. terrestrial behavior time at some sites. Females are found in above ground arboreal substrates more than males are at Taï (Ivory Coast) (64.8% females vs. 48.9% males) and Mahale (Tanzania) (47.8% for females and 32.9% for males) (Doran and Hunt 1994), as well as at Gombe (Tanzania) (68.4% for females vs. 37.4% for males). Thus, females are spending more time above the ground than males are, although locomotor variability within the arboreal setting is unknown (Doran and Hunt, 1994).

At Taï and Mahale, male and female *Pan* spent similar amounts of time in different locomotor modes. By contrast, Gombe females spent more time in quadrumanous climbing than did males (Doran and Hunt 1994). It is also possible that because of the increased three-dimensionality and unpredictability of arboreal pathways, and the flexible behavioral responses they demand, arboreal substrates lead to more variable behaviors, and consequently, increased variability in the osteogenic response of bone. For example, female *Pan* were not only more arboreal than males (Doran and Hunt, 1994), they spent more time at greater heights and in branches away from the trunk (Doran, 1993). This would require increased usage of all four limbs along multiple branches to support the individual's weight. The increased diversity of limb postures as an individual moves further into terminal regions of the crown may result in increased skeletal variability through the differential usage of fore- and hindlimbs to support large bodies on small branches.

In addition to the proportion of arboreal and terrestrial behavior, there is some evidence for sex differences in daily range or daily path length in *Pan*, with males generally traveling longer and more consistent distances (terrestrially) than females (Chapman and Wrangham, 1993; Herbinger et al., 2001; Pontzer and Wrangham, 2004; Bates and Byrne, 2009). Among females, there are limited differences in daily travel between mothers and non-pregnant females at Kanyawara but more significant differences at Sonso (Uganda) with mothers travelling shorter distances (Pontzer and Wrangham, 2004; Bates and Byrne, 2009). Differences in female travel due to varying reproductive status may contribute to greater inter-individual variability in mechanical loading history and thus cross-sectional geometric parameters compared to males, although more work is needed to quantify these hypothesized differences.

In summary, while at some sites *Pan* female locomotor behavior may be more variable over the course of their life compared to that of males (Pontzer and Wrangham, 2004), patterns are inconsistent across sites. It is difficult to determine if locomotion is a cause of skeletal variability given the limited information currently available. Nevertheless, it is a plausible explanation given the strong relationship between form and function. These results are generally consistent with our findings that female *Pan* tended to be more variable than males in their skeletal traits (Table 4.3).

There were no differences in skeletal variability between male and female *Gorilla.* In *Gorilla gorilla gorilla,* there are few studies of sex differences in locomotion, and qualitative descriptions suggest no significant differences in arboreality between male and female lowland gorillas (Remis, 1999). Although time spent at different tree heights is significantly different between males and females, with females using smaller and less stable branches than males (Remis, 1995), this more varied use of limbs did not lead to higher skeletal variability in females.

Distance travelled also appears to be similar between male and female *Gorilla.* At Bai Hokou (Central African Republic), the overall day range including both terrestrial and arboreal locomotion was an average of 2.3 km (Remis, 1997). Males and females remain together while travelling, and even if a large group splits into smaller groups, there is always at least one silverback male with the females (Remis, 1999). This maintenance of group cohesiveness limits differences in distance travelled, and any potential influence on skeletal variation. The limited differences between male and female *G. g. gorilla* in locomotor variability and distance travelled thus match the finding of limited skeletal variability .

4.5.2 Sex Based Differences: Pregnancy and Lactation

A potential explanation for increased female skeletal variability in Ct.TMD is calcium mobilization for fetal bone growth during pregnancy and lactation. To accommodate the energetic stress of lactation and pregnancy, both humans and nonhuman apes increase energy intake and can decrease levels of physical activity (Dufour and Sauther, 2002). However, if pregnancy and lactation had strong effects on skeletal variability, it would be predicted that Ct.TMD would vary in all the bones of study in females in both species. Yet the results only indicate differences between male and female *Gorilla* in the femur, with the increased variability in female *Gorilla* driving the difference. Female and male *Pan,* and male *Gorilla* all have similar CV values in femoral Ct.TMD. Without any major differences in Ct.TMD across bones, species, or sex, it is unlikely that pregnancy or lactation are strong contributors to skeletal variability.

4.5.3 Sex Based Differences: Life History Traits

Another potential explanation for higher skeletal variability in female vs. male *Pan* could be sex differences in growth patterns and life history. Male and female primates, particularly amongst sexually dimorphic species, have different growth curves (Leigh, 1992; Leigh and Shea, 1995). Female growth in *Pan* does not reach the peak velocity seen in males, but duration of growth is more prolonged relative to males (Leigh and Shea, 1995). This combination in females of slower, more extended growth could contribute to increased skeletal variation (Leigh and Shea, 1995). Ecological factors such as food availability and seasonality (Altmann and Alberts, 1987; Altmann and Alberts, 2005) may also impact growth through limiting or increasing nutrients and stress. In addition, female *Pan* experience the stress of leaving their natal group on the cusp of adulthood at ~9-15 years (Stumpf et al., 2009; Wood et al., 2017), and travel an estimated 15 kilometers (n=2) during this emigration (McCarthy et al., 2018). Upon arrival, female immigrants are low-ranking and experience high physiological stress as assayed by urinary cortisol (Kahlenberg et al., 2008a). Both nutritional availability and stress are known to impact skeletal growth and maintenance (e.g., Bachrach et al., 1990; Misra et al. 2003; Devlin and Bouxsein, 2012), thus introducing skeletal variation into the population during periods of growth. It has also been noted that female *Pan* have smaller core areas than do males, who range more widely across a community's home range (Goodall, 1986). Since core areas for females can vary significantly in dietary quality with measurable impacts on reproductive success (Emery Thompson et al., 2007; Murray et al., 2007; Kahlenberg et al., 2008b), this could also contribute to greater skeletal variation in females than males.

4.5.4 Species Based Differences: Body Mass and Sexual Dimorphism

Although the differences were subtle, this study found slightly more skeletal variability in *Gorilla* than *Pan* in both males and females. Consistent with this finding, Plavcan (2012) found that the CV for femoral head diameter was 12.1 in *Gorilla* vs. 6.5 in *Pan*. However, body size is directly correlated with cross sectional geometric properties, and body size is less variable in *Gorilla gorilla gorilla* males than in either male or female *Pan troglodytes troglodytes* (Table 4.5), indicating that skeletal variability is not solely a product of body size. Although more data are needed, this study does not support a strong influence of body size variability or sexual dimorphism on postcranial cross-sectional skeletal variation.

While locomotion is a plausible explanation for the difference between male and female *Pan,* it has less explanatory power between species. Although *Gorilla gorilla gorilla* is reported with limited data as potentially spending as much as 20% of the time in trees, it is less arboreal than *Pan troglodytes* who occupy similar habitats (Remis, 1998). This is likely due to the large body size of *Gorilla* compared to *Pan.* There are no studies comparing the variability in locomotor behavior between *Gorilla* and *Pan*, so it is difficult to assess differences in locomotor variability. Further, the day ranges are similar between the two species. The day range of Gorilla at Bai Hokou is 1-3.25 kilometers (Remis, 1997), while for the Kanyawara chimpanzees, males range on average 2.4 kilometers and females 1.9 kilometers (Pontzer and Wrangham, 2006). However, this differs dramatically from Taï, where the day range varied by community (North males: 3.7 kilometers, North females: 3.2 kilometers; South males: 4.3 kilometers, South females: 4.1 kilometers; Middle males: 2.1 kilometers (Pontzer and Wrangham, 2004; Herbinger et al. 2001). Overall, these data fit our results indicating limited differences between *Gorilla* and *Pan* in populations occupying relatively similar habitats.

	Pan troglodytes Male	*Pan troglodytes* Female	*Gorilla gorilla* Male	*Gorilla gorilla* Female
Mean	40.9[1,3] kg	33.4[1,3] kg	169.5[2] kg	71.5[2] kg
Standard Deviation	4.6[1]	4.7[1]	25.8[2]	-
Coefficient of Variance	11.2[1]	14.1[1]	6.56 (Calculated from [2])	-
Range	39.2-45.1[3]	33.0-37.2[3]	132-218.2[2]	68.2-74.3[2]

Table 4.5: Body size comparison amongst Pan and Gorilla. ([1]Smith and Jungers, 1997; [2]Jungers and Susman, 1984; [3]Uehara and Nishida, 1987).

4.5.5 Bone Differences

In this study, results revealed few differences between the long bones in two hominoid species. Our results differ from Buck et al. (2010) who found that the forelimb was significantly more variable than the hindlimb in linear measurements of the diaphysis and epiphysis across a broader sample of twelve catarrhine species (five hominoids and seven old world monkeys). However, in addition to the broader range of species, Buck et al. (2010) combined the forelimb and hindlimb elements and did not separate them by bone as in this study, potentially masking some variability. Consistent with the findings of the present study, Fulwood and Kramer (2013) studied eight strepsirrhine species and did not find differences between forelimb and hindlimb diaphyseal or epiphyseal measurements.

4.5.6 Trait Differences

The overall pattern for the skeletal properties included in this study indicates that while overall variability in cortical cross-sectional geometry and strength, bone length, and bone mineral density traits is low, bone length and cortical bone mineral density tend to be the least variable. In the case of cortical bone mineral density, this fits with previous research that shows cortical mineral values are more heritable than cross-sectional values, and thus potentially more genetically controlled (Jepsen, 2009). Consistent with the findings, Tommasini et al. (2008) found that mean total area for 22 mouse strains had a CV of 13.3, mean cortical area had a CV of 11.1, and mean Ct.TMD had a CV of 4.8. This difference could potentially be due to differences in genetic pathways between longitudinal bone growth (length) and transverse bone growth (shape) (Li et al., 2002). Further, cortical area and total area are highly correlated with strength values such as *J* (Jepsen et al., 2007). Variability in cortical area and total area will impact variability in *J,* because the distribution and amount of bone is ultimately how *J* is derived. It is unsurprising that within a population, traits with higher powers, such as bone area (mm^2) and the polar moment of area (mm^4), have higher variation than traits such as bone length (mm). However, as demonstrated in our results, even when such higher power traits are converted into single power functions, variability remains higher amongst most cross-sectional geometric traits relative to that of cortical tissue mineral density and bone length.

4.6 Limitations and Future Directions

The study had some limitations, including difficulties aging adults in museum samples and the lack of specific locomotor data for the individuals from whom anatomical data were derived. Future studies analyzing intraspecific variation would also benefit from the inclusion of older adult specimens, to increase understanding of how aging in adults impacts skeletal variation. Many long-term field sites are now collecting individuals of known age who have died from natural causes. Studies of these individuals and populations with known ages, diet, ecology, and behavior will allow for more fine-grained analysis into how ecological factors influence skeletal variability within and between species. Finally, the addition of more species and samples will result in more robust analyses.

4.7 Conclusion

Accurately reconstructing the positional behavior of fossil taxa requires understanding the potential extent of intraspecific variation within the species. This study focused on cortical cross-sectional properties, and found limited overall differences between *Gorilla* and Pan. However, there were some notable differences in patterns of variation between males and females in each species. In terms of the fossil record, our finding that CV's differ minimally within hominoid species with different levels of dimorphism suggests that CVs should be relatively low, even in sexually dimorphic taxa such as *Australopithecus afarensis,* in which males and females have been reconstructed as having different forms and proportions of locomotion (Stern and Susman, 1983). More generally, studying extant populations provides the opportunity to assess skeletal parameters in species with known locomotor repertoire, ranging behavior and life history. Further, studying individuals from the same population, living in the same time and space, allows the identification of traits that are more genetically constrained vs. more plastic and thus indicative of phylogeny or behavior, respectively. In future studies, this approach can be applied to other skeletal traits such as trabecular bone microarchitecture, and to intraspecific ontogenetic changes in skeletal morphology.

4.8 Appendix

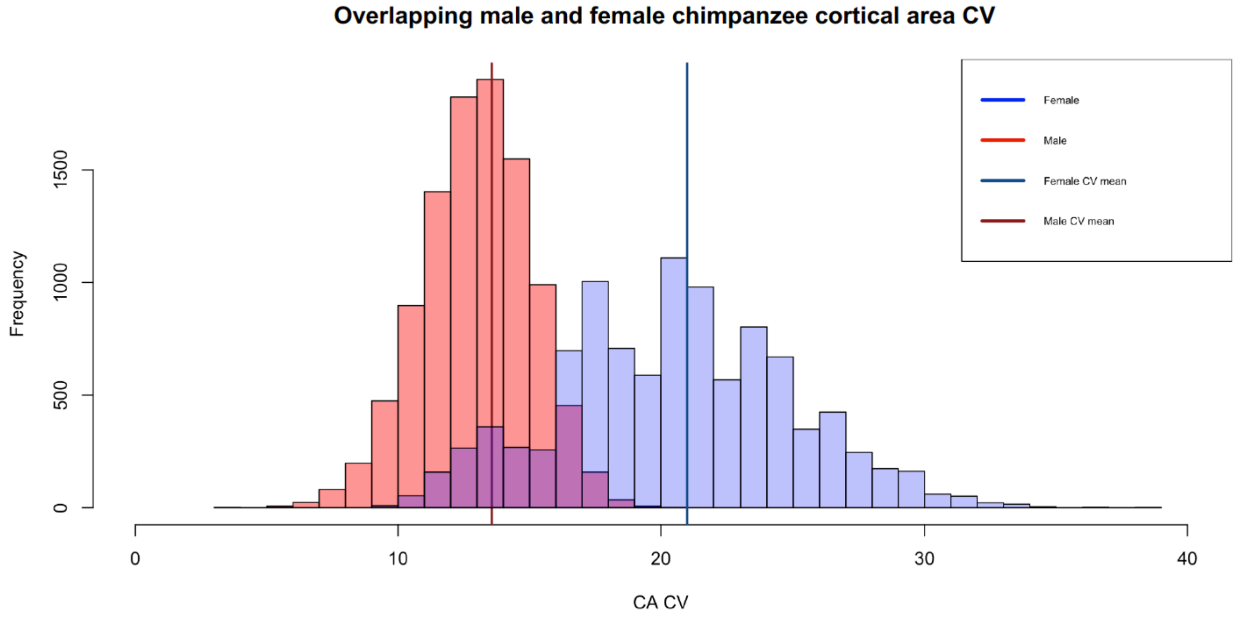

Figure 4.3: Histogram of bootstrap results from male and female chimpanzee femoral cortical area coefficients of variation. Red and blue lines denote the CV from original dataset.

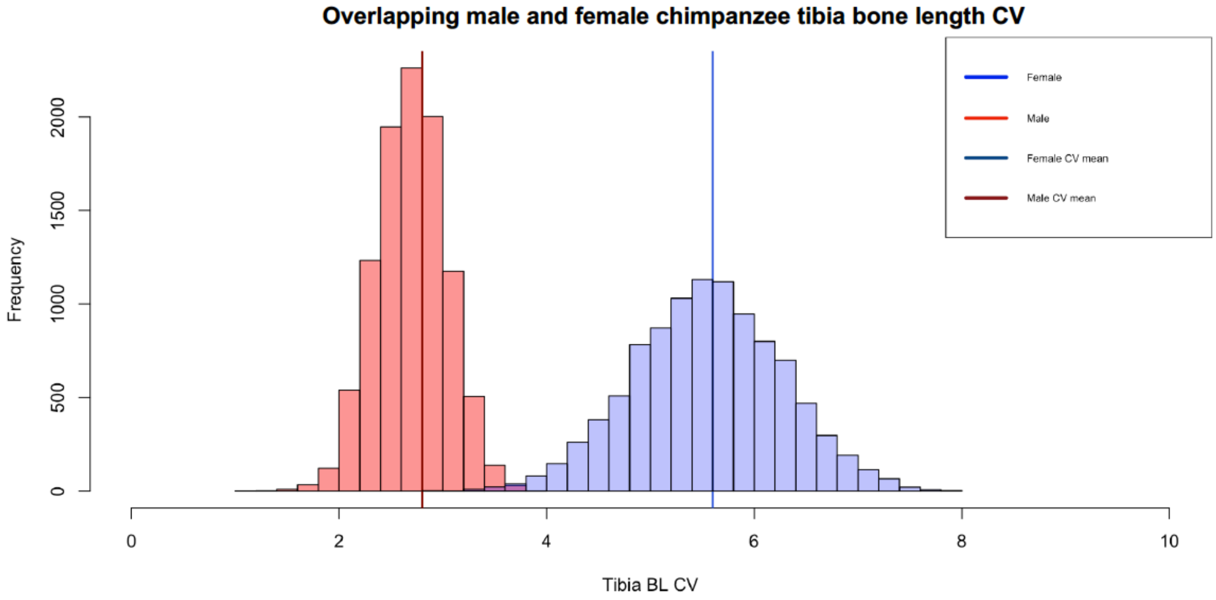

Figure 4.4: Histogram of bootstrap results from male and female chimpanzee tibial bone length coefficients of variation. Red and blue lines denote the CV from original dataset.

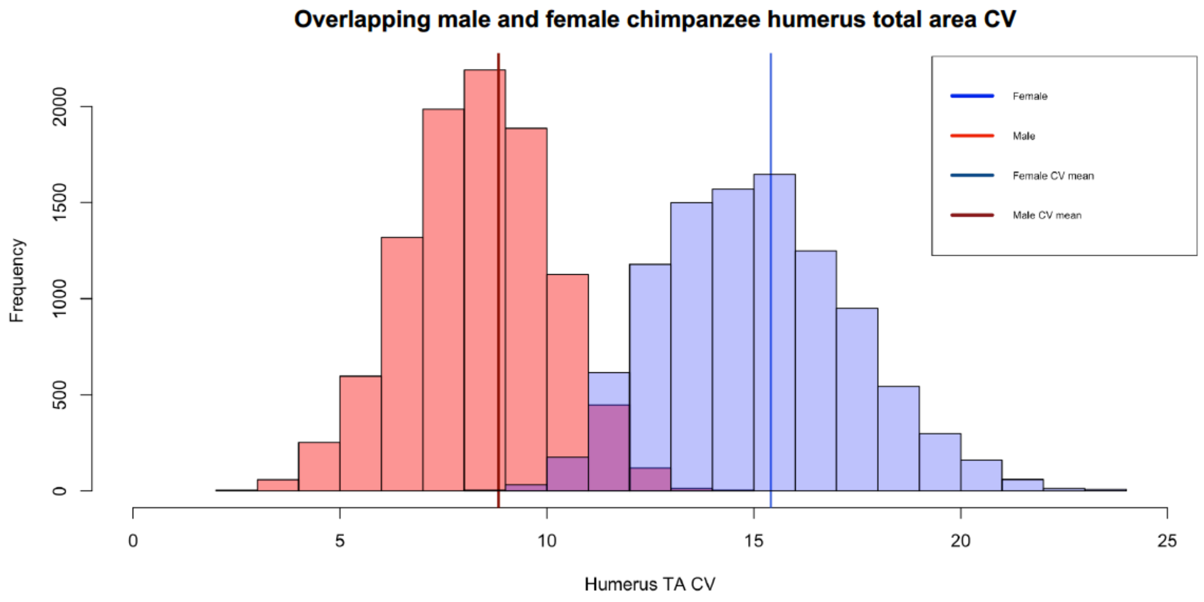

Figure 4.5: Histogram of bootstrap results from male and female chimpanzee humeral total area coefficients of variation. Red and blue lines denote the CV from original dataset.

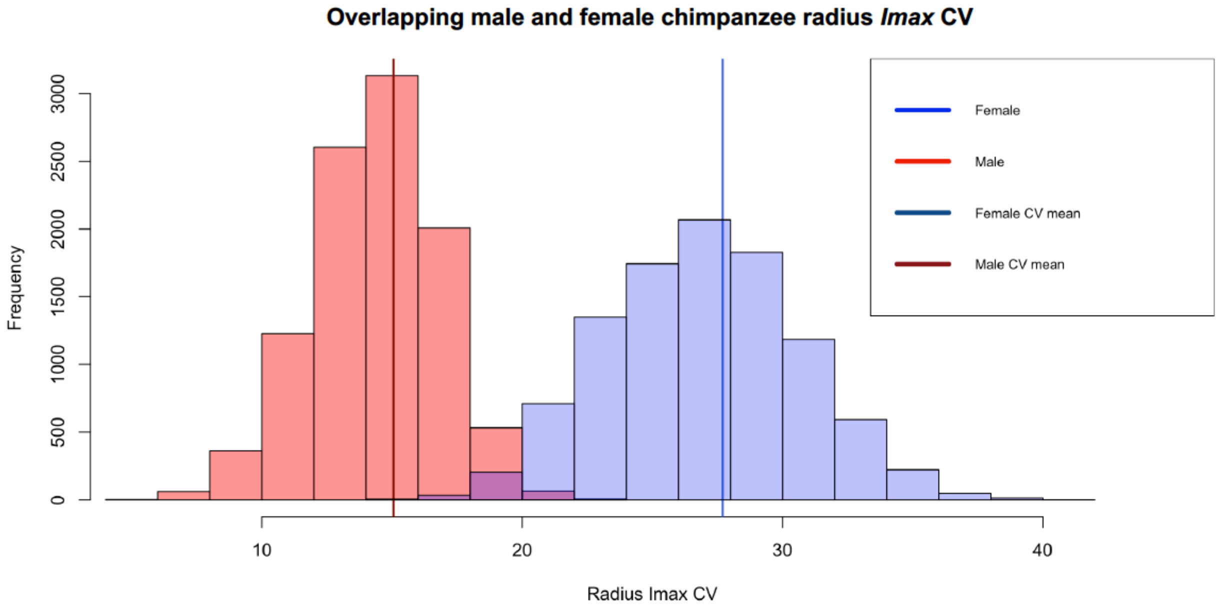

Figure 4.6: Histogram of bootstrap results from male and female chimpanzee radial Imax coefficients of variation. Red and blue lines denote the CV from original dataset.

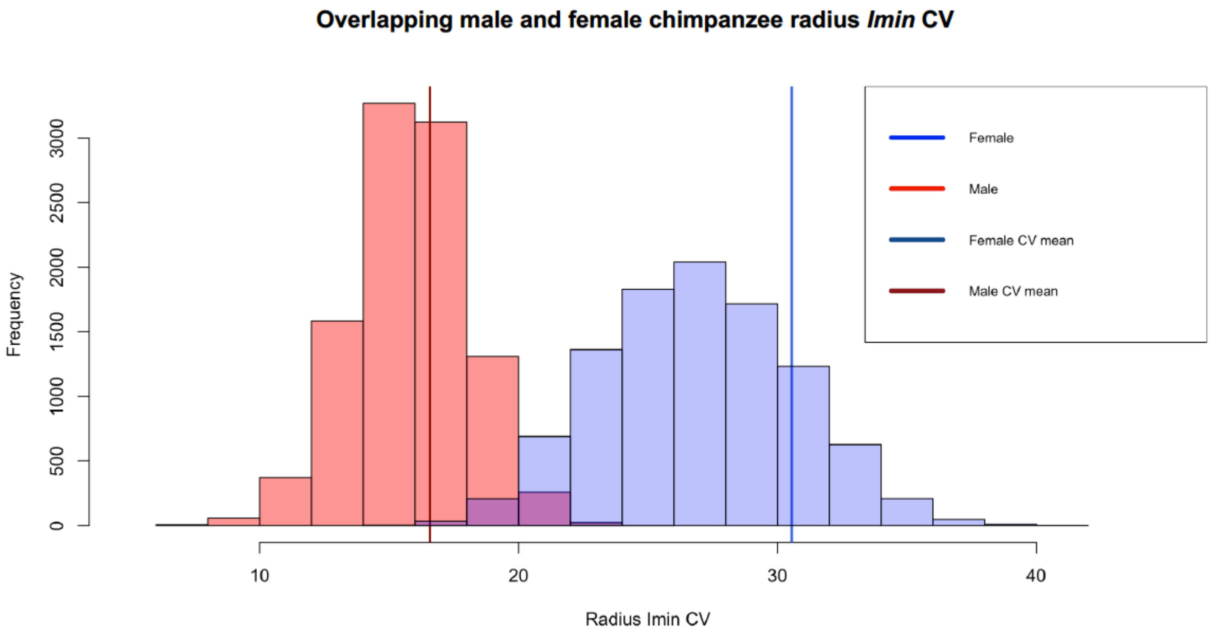

Figure 4.7: Histogram of bootstrap results from male and female chimpanzee radial Imin coefficients of variation. Red and blue lines denote the CV from original dataset.

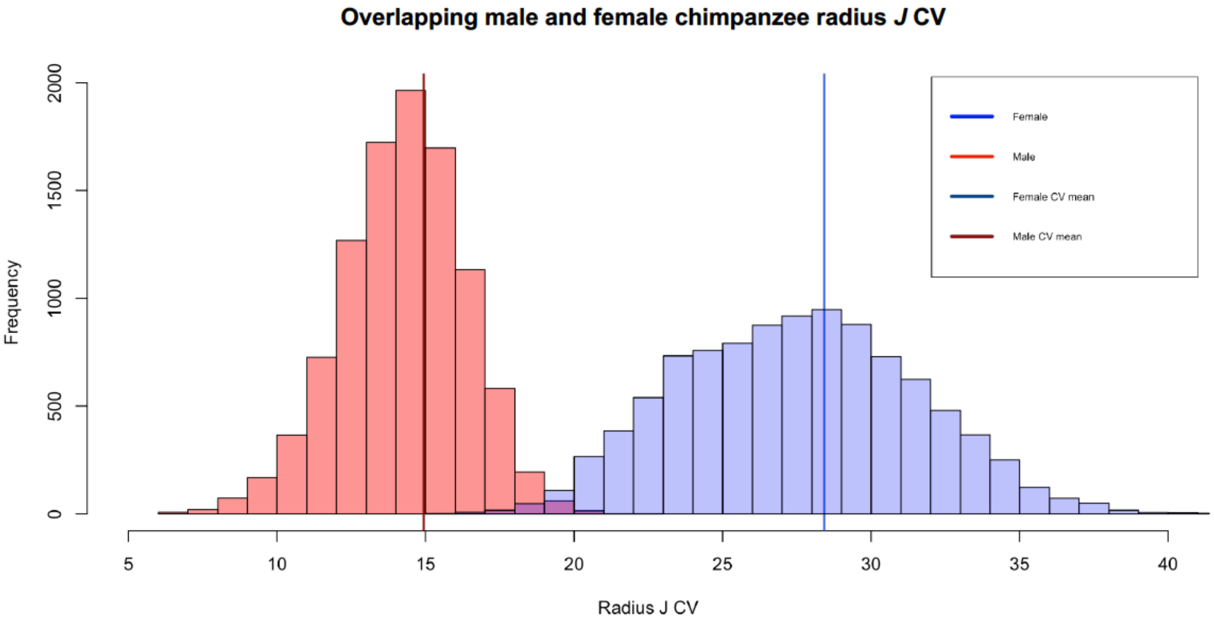

Figure 4.8: Histogram of bootstrap results from male and female chimpanzee radial J coefficients of variation. Red and blue lines denote the CV from original dataset.

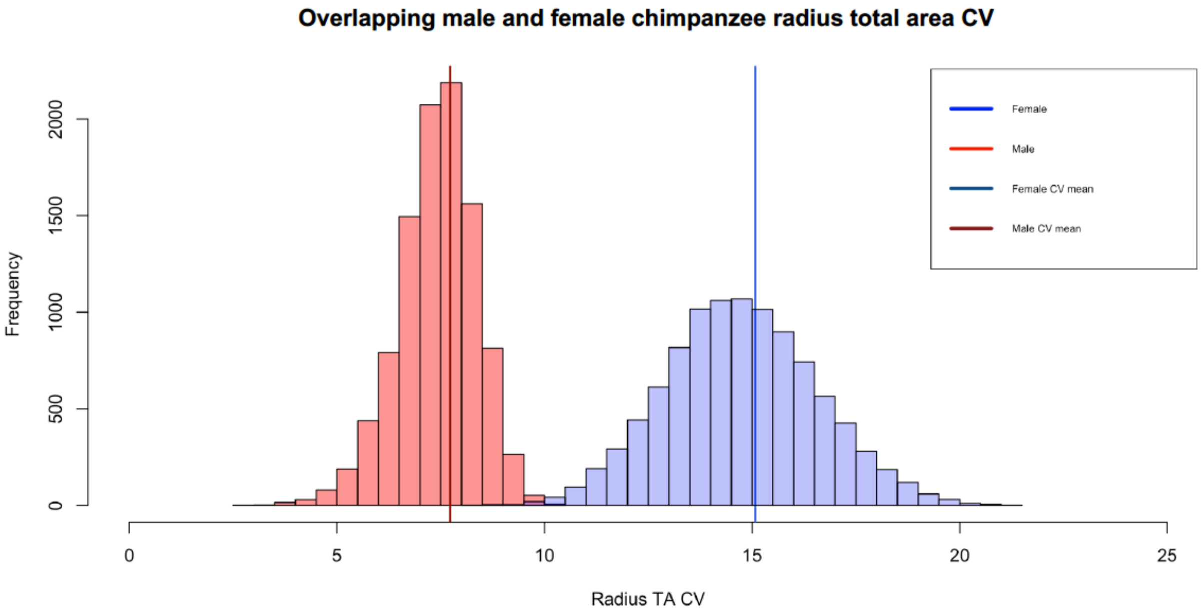

Figure 4.9: Histogram of bootstrap results from male and female chimpanzee radial total area coefficients of variation. Red and blue lines denote the CV from original dataset.

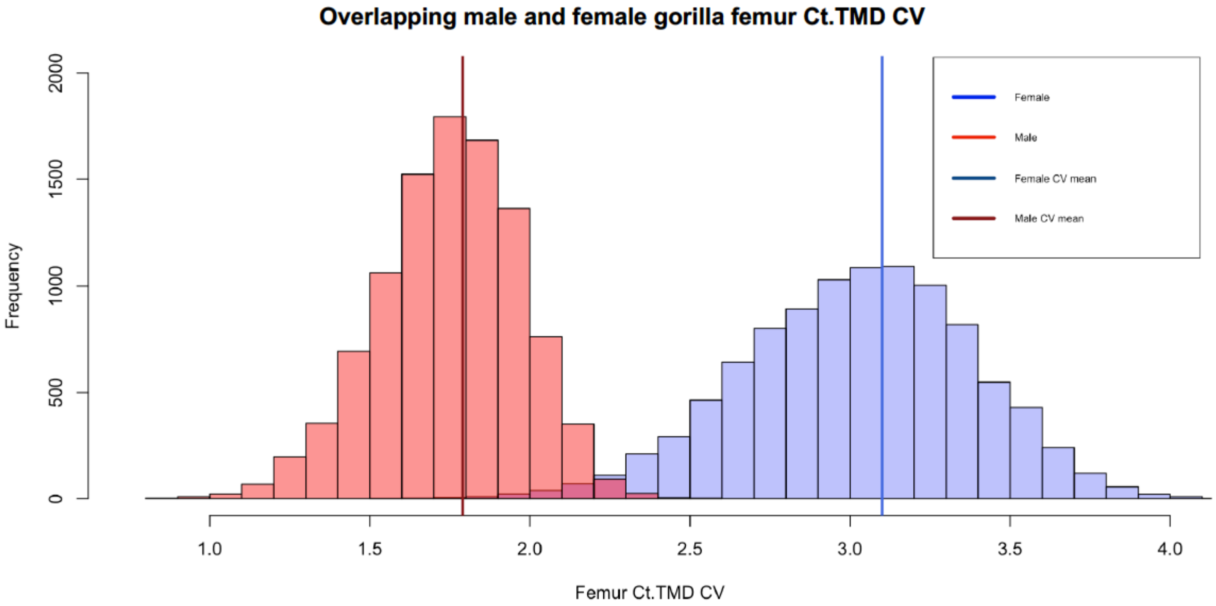

Figure 4.10: Histogram of bootstrap results from male and female gorilla femoral cortical tissue mineral density (Ct.TMD) coefficients of variation. Red and blue lines denote the CV from original dataset.

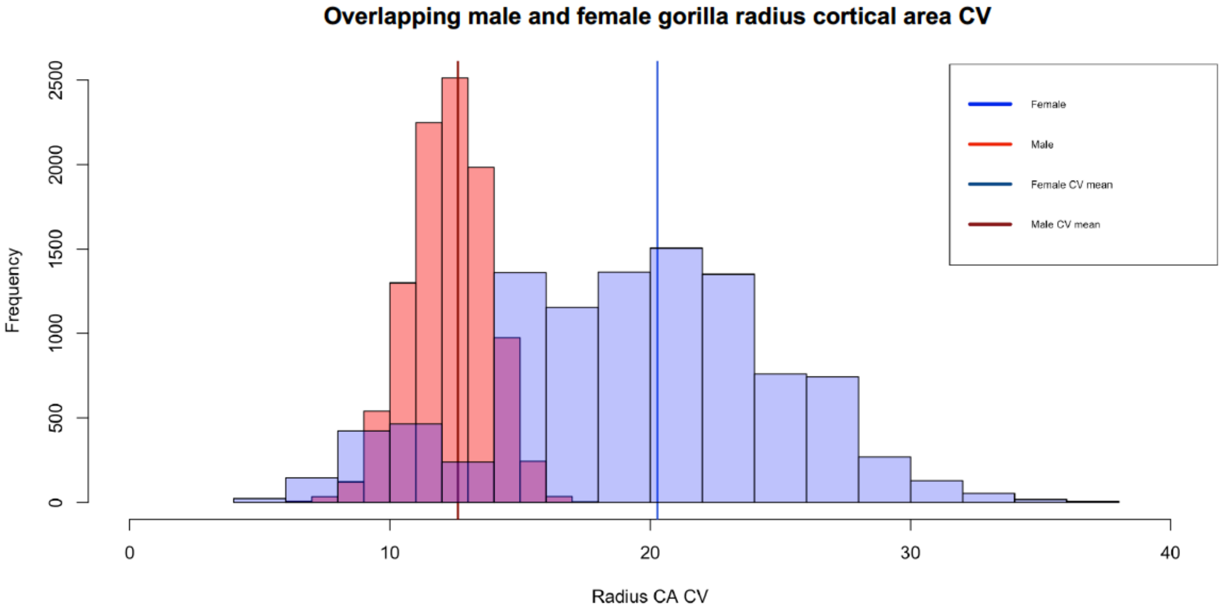

Figure 4.11: Histogram of bootstrap results from male and female gorilla radial cortical area coefficients of variation. Red and blue lines denote the CV from original dataset.

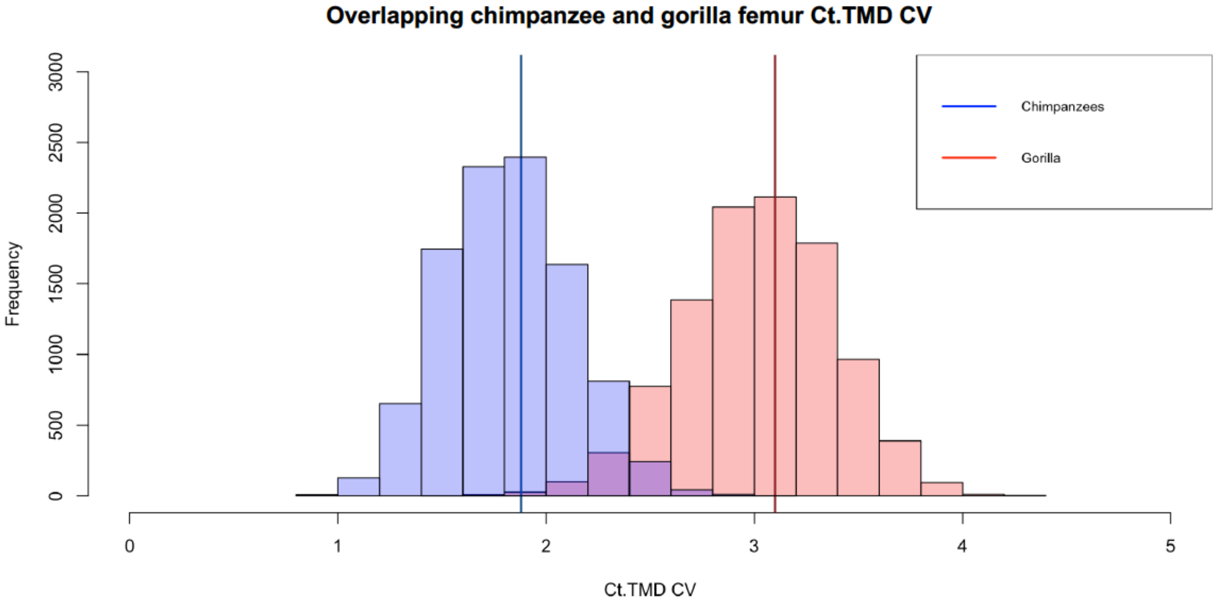

Figure 4.12: Histogram of bootstrap results from chimpanzees and gorilla female femoral cortical tissue mineral density coefficients of variation. Red and blue lines denote the CV from original dataset.

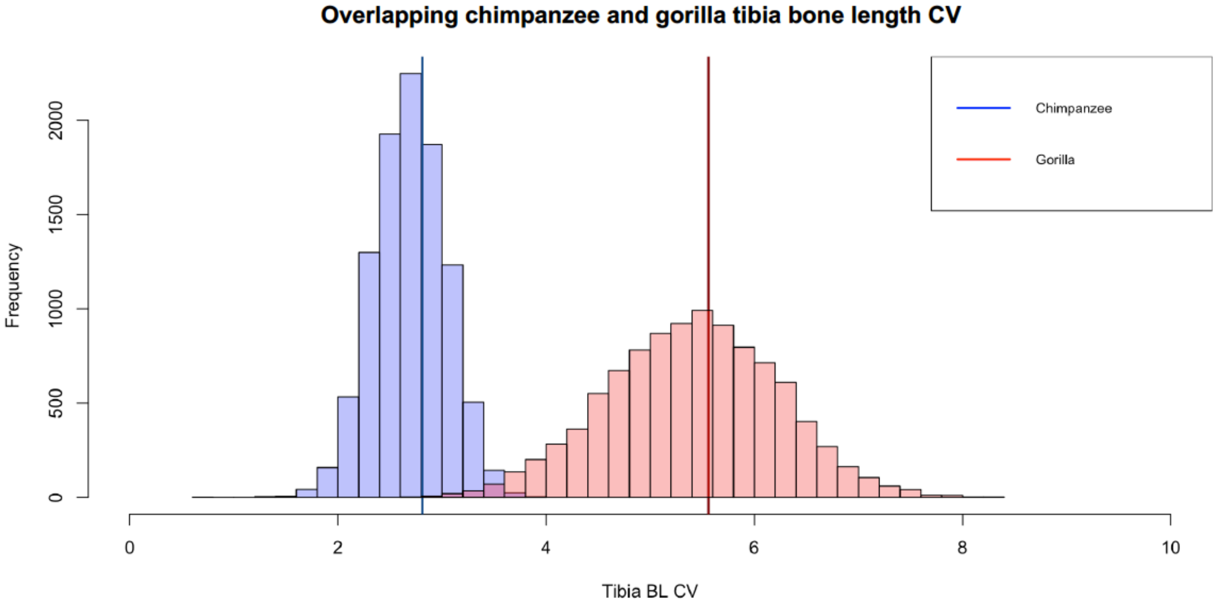

Figure 4.13: Histogram of bootstrap results from chimpanzees and gorilla male tibial bone length coefficients of variation. Red and blue lines denote the CV from original dataset.

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Chapter 5 Conclusion

This dissertation sought to understand how locomotor changes across ontogeny and across species impact skeletal morphology in great apes. In particular, this study examined how osteological responses to inferred differences in the loading environment on arboreal vs. terrestrial substrates are manifested phenotypically. Understanding the relationship between mechanical loading changes, such as shifts between arboreal and terrestrial locomotion, and bone will allow for better reconstruction of fossil hominoid locomotor behavior.

This project used microCT and pQCT data from *Pan troglodytes, Gorilla gorilla gorilla,* and *Pongo* from the femur and humerus. MicroCT of *Pan troglodytes* femora and humeri were collected from the proximal epiphysis and midshaft in both bones to determine how trabecular bone changed in response to changes in mechanical loading across ontogeny, and if these changes are correlated with cortical cross-sectional geometry. To determine how proportions of arboreal and terrestrial locomotion are reflected in the skeleton, microCT and pQCT of *Pan troglodytes, Gorilla gorilla gorilla,* and *Pongo* were collected, and cross-sectional geometric properties were compared across the three species and across age categories. Finally, given the differences in locomotion and body size between *Gorilla* and *Pan,* the levels of intraspecific variation were calculated. Factors such as body size, sexual dimorphism, and locomotor repertoire can all impact skeletal variation. Characterizing variation in two species that are closely related and occupy sympatric environments but have varying locomotion creates an ideal model to compare behavioral and environmental impacts on skeletal variability. In total, results demonstrate that while bone is a highly plastic tissue and responds to locomotor variation, there are differences between trabecular and cortical responses. Moreover, not all cortical factors match predicted phenotypes given locomotor behaviors. And finally, variation in skeletal morphology is not dictated by body size, sexual dimorphism, or locomotor behavior. While locomotion is a critical factor, responses were likely tempered by genetic and developmental constraints.

The second chapter investigated whether 1) trabecular bone responds to age related locomotor change in *Pan troglodytes* and 2) patterns of cortical and trabecular response to changes in locomotor behavior are correlated. Results demonstrated that trabecular bone volume fraction ratios in the femur and humerus (BV/TV) tracked with locomotor change across ontogeny, as predicted. The femoral BV/TV in infants and juveniles was significantly lower compared to adults and adolescents, but in the humerus, only infants had significantly lower BV/TV from all other age categories. The degree of anisotropy (DA) had an opposite trend, with increasing isotropy in the femur and the humerus across ontogeny. However, the humerus maintained more consistency across ontogeny. Infants, juveniles, and adolescents had more anisotropic orientations compared to adults. Given locomotor variability, it would be expected that adults would have more anisotropic femora with increasing terrestrial quadrupedalism. Contrary to predictions, there were no correlations between trabecular and cortical bone across ontogeny.

The third chapter tested whether changes in the proportion of arboreal and terrestrial locomotion in the skeleton of *Pan troglodytes* are reflected in humeral and femoral cross-sectional morphology. *Gorilla* and *Pongo* were used as bracketing taxa to determine if the morphology of younger and more arboreal (e.g., infant and juvenile) *Pan* trended toward *Pongo*'s morphology, and if the older, less arboreal ontogenetic stages trended toward *Gorilla*'s morphology. Results demonstrated that in cortical cross-sectional geometry, femoral and humeral strength (*J*) ratios can accurately discern between proportions of arboreal and terrestrial locomotion both across ontogeny and between species. The ellipticity of the cross-section was also able to accurately reflect locomotor change across ontogeny, with all species moving from rounder cross-sections to more elliptical ones in the femur. A limitation of ellipticity as a metric of locomotor behavior was the finding that both *Pongo* and *Gorilla* had medio-laterally expanded cross sections. Given their pronounced differences in positional behavior, it seems reasonable to suggest that the cause of the expansion may differ between the two taxa. More details on locomotor behavior, particularly kinematic data, may help to resolve this ambiguity. For now, it is hypothesized that the varus position of the femur during terrestrial locomotion in *Gorilla* may generate medio-lateral (ML) bending loads, whereas in *Pongo*, high ML bending loads may be attributed to more abducted femoral postures during versatile climbing.

The fourth chapter asked if there were differences in intraspecific variation among the long bones of *Pan troglodytes troglodytes* and *Gorilla gorilla gorilla* by species, sex, and/or bone. Understanding how differences in locomotor behavior and body size impact skeletal variation is critical for reconstructing locomotion in the fossil record. Results indicated that there were limited differences in variation between the two species. Intraspecifically, females tended to be more variable than males in both *Pan* and *Gorilla*. Interspecifically, *Gorilla* tended to be more variable than *Pan.* These findings demonstrate the complicated relationships among locomotor behavior, phylogeny, and body size.

The findings of this dissertation indicated that the relationship between skeletal morphology and mechanical loading is complex, and trends in one bone compartment cannot necessarily be applied to another. Although trabecular and cortical bone morphology have been found to be linked in studies searching for interspecific correlates of locomotion, in this study, changes in the two bone types were not strongly correlated across ontogeny. However, both trabecular bone (chapter two) and cortical bone (chapter three) had some features that followed predicted patterns given locomotor behavior in *Pan troglodytes, Gorilla gorilla gorilla,* and *Pongo.* When comparing changes in proportions of behaviors across ontogeny, cortical bone may be a stronger indicator of these changes compared to trabecular bone. To address the lack of trabecular signal to locomotor behavior in this study, it is necessary to test the levels of variation in trabecular and cortical bone. If trabecular bone has a greater range of variation than cortical bone, it is plausible that any locomotor signal is being masked due to inherent variation within a population. Until baseline levels of variation are reported for trabecular bone, it will be difficult to test the difference in magnitude of responsiveness and subsequent variable response levels to mechanical loading between cortical and trabecular bone.

Variation among adult *Pan* and *Gorilla* cross-sectional properties was found to be limited, and future studies should (1) test variability across ontogeny and (2) compare variability in trabecular bone and cortical bone. If locomotor variation has an effect on skeletal morphological variation, species with limited locomotor change across ontogeny (similar to *Gorilla* studied here) should maintain similar levels of variation, while species with greater locomotor variation (such as *Pan troglodytes* studied here) should have ontogenetic patterns in the level of variation between the femur and humerus that match locomotor trends.

In conclusion, cortical and trabecular bone are extremely useful in gaining detailed insights into the relationship between locomotion and skeletal morphology in extant taxa. This relationship appears to differ between cortical and trabecular bone, which is important when considering applications to the fossil record. Cortical cross-sectional geometry demonstrates clear ontogenetic signals, but is best used with multiple bones for comparison (e.g. femur and humerus). This is rarely possible with fossils, due to the fragmentary nature of fossil preservation. Trabecular bone is less informative of ontogenetic changes in locomotion, but with further study the relationship between locomotion and trabecular morphology across ontogeny will be more clear.