

ONTOGENETIC VARIATION IN LONG BONE MICROSTRUCTURE IN
CATARRHINES AND ITS SIGNIFICANCE FOR LIFE HISTORY RESEARCH

by

SHANNON CHRISTA MCFARLIN

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ABSTRACT**ONTOGENETIC VARIATION IN LONG BONE MICROSTRUCTURE IN
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by

Shannon C. McFarlin

Advisor: Professor Timothy G. Bromage

This research was motivated by an interest in the significance of bone histology for primate life history investigations. Its purpose was to provide a baseline understanding of ontogenetic, regional and taxonomic variability in bone microstructure at the midshaft femur and humerus of *Chlorocebus aethiops*, *Hylobates lar* and *Pan troglodytes*, and to consider the manner in which it reflects species growth patterns. Histological thin sections were imaged in LM and CPL microscopy for assessment of variability in primary bone tissue types, which has been suggested to reflect variability in bone depositional rates across growth stages and among organisms characterized by different overall rates of body growth. Because bone also undergoes architectural modifications during growth, this variability was examined within the context of variation in mechanically-relevant features of bone, including cross-sectional geometry and secondary intracortical remodeling.

Results demonstrated significant variability in primary tissue type proportions across biological age classes. Younger individuals were characterized by higher proportions of faster-forming (e.g., fibrolamellar) tissues, while older individuals were

characterized by higher proportions of slower-forming (e.g., lamellar) bone.

Interestingly, taxa differed in the timing of this transition, and the particular tissue types observed among individuals of similar biological age. When interpreted within the context of local growth and remodeling history, intra- and interspecific patterns of microstructural development revealed several consistencies with body mass growth patterns reported previously. In some cases, however, results were not in agreement with expectations. With respect to this latter point, this study identified several patterns warranting further investigation, including age and sex variability in vascular patterns, the occurrence of growth marks, and variability in preferred collagen fiber orientation within more broadly-defined tissue ‘types’, all of which may reflect subtle variations in bone depositional rates during periods of relatively fast or slow overall growth. Results also suggest that teasing apart developmental versus mechanical factors influencing taxonomic and intraskeletal distributions of secondary remodeling should be an important component of future research. Finally, this study provides an important foundation for future studies, and it demonstrates the exciting potential of bone microstructural analyses as an independent line of inquiry into primate life history in paleontological contexts.

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LIST OF ABBREVIATIONS

Chapter 3: Materials and methods

CPL	Circularly-polarized light microscopy
DECID	Age class, defined on the basis of emergence of the deciduous dentition
EPIPH	Age class, defined on the basis of complete long bone epiphyseal union and dental eruption
LM	Conventional (i.e., unpolarized) transmitted light microscopy
MCZ	Museum of Comparative Zoology, Harvard University, Boston
MNH	Museum für Naturkunde der Humboldt Universität, Berlin
M1	Age class, defined on the basis of emergence of the 1 st permanent molars
M2	Age class, defined on the basis of emergence of the 2 nd permanent molars
M3	Age class, defined on the basis of emergence of the 3 rd permanent molars
SLW	Sherwood L. Washburn skeletal collection, University of California at Santa Cruz

Chapter 4: Cross-sectional geometry variables

CA	Cortical area (mm ²)
I _{max}	Maximum second moment of area (mm ⁴)
I _{min}	Minimum second moment of area (mm ⁴)
J	Polar second moment of area (mm ⁴)
MA	Medullary canal area (mm ²)
TA	Total subperiosteal area (mm ²)
Z _x	Section modulus about the x axis (mm ³)
Z _y	Section modulus about the y axis (mm ³)
Z _p	Polar section modulus (mm ³)
Ø	Angle theta, the orientation of the plane of greatest bending rigidity (I _{max})

Chapters 5 and 6: Tissue types

ECCC	Compacted coarse cancellous, of endosteal origin
ELAM	Lamellar, of endosteal origin
END	Tissue of endosteal origin
EPF	Parallel-fibered, of endosteal origin
EPF-LAM	Parallel-fibered to lamellar (transition tissue type), of endosteal origin
FLC	Fibro-lamellar complex, of periosteal origin
FLC-LZPO	Fibro-lamellar complex to lamellar-zonal with primary osteons (transition tissue type), of periosteal origin
HAV	Secondary osteonal (i.e., Haversian) bone
INT	Interstitial bone
LAM	Lamellar, of periosteal origin
PER	Tissue of periosteal origin
PF	Parallel-fibered, of periosteal origin
PF-LAM	Parallel-fibered to lamellar (transition tissue type), of periosteal origin
SF	Sharpey's fiber bone, of periosteal origin
WOV	Woven, of periosteal origin

CHAPTER 1.

INTRODUCTION

The study of life history is concerned with those events and developmental processes that occur during the life cycle, and which determine the schedule by which individuals grow, mature, reproduce and ultimately die. Life histories are traditionally characterized in terms of a suite of somatic and reproductive traits, which describe morphologic attributes and the timing of key life cycle events (e.g., neonatal and adult body and brain size, gestation length, age at weaning, age at first reproduction, interbirth interval, and longevity) (e.g., Harvey and Clutton-Brock, 1985; Harvey et al., 1987; Promislow and Harvey, 1990; Read and Harvey, 1989). Many of these traits are highly inter-correlated across species, and natural selection is proposed to act on them to produce strategies that maximize fitness over the lifetime. Life history strategies exhibit a great deal of variation, both across mammals and within the order Primates, and much attention has focused on identifying the factors (e.g., age-specific mortality and metabolic risks) which explain this variation (recently reviewed by Kappeler and Pereira, 2003; and Leigh and Blomquist, 2007).

Primate ontogeny has figured prominently in recent approaches to the study of life history (e.g., Bolter and Zihlman, 2007; Charnov and Berrigan, 1993; works in DeRousseau, 1990b; Godfrey et al., 2001; Leigh and Blomquist, 2007; works in Pereira and Fairbanks, 1993). Primates are unusual among most mammals of similar body size in their relatively slower growth rates, later ages at maturity, low reproductive rates, and longer life spans (Case, 1978; Charnov and Berrigan, 1993; Ross and Lee, 1999).

However, they also exhibit considerable diversity in patterns of growth and development, and similar adult phenotypes may arise through different developmental mechanisms. For instance, body mass variation may arise from differences in growth rate, growth duration, or both (Leigh, 1992; Leigh and Terranova, 1998; Shea, 1986). Early versus later stages of ontogeny may be subject to independent selection, as may different attributes of ontogenetic trajectories (e.g., growth rates and timing) (Leigh, 2001; Leigh and Park, 1998; Pereira and Leigh, 2003). Further, the developmental timing of different anatomical organs/systems (e.g., brain, body, dentition, and reproductive) may be dissociated from one another (Dirks, 2003; Godfrey et al., 2004; Pereira and Leigh, 2003; Watts, 1990). These diverse ‘modes’ of development are suggested to underlie important variations in life history (Leigh and Blomquist, 2007; Pereira and Leigh, 2003).

In order to place studies of primate ontogeny and life history more firmly within an evolutionary framework, a major focus has been the establishment of methods for extending this research to paleontological contexts. Since paleoanthropologists have only the fossilized remains of once-living primates available to them for study, this research has naturally focused on the surviving bones and teeth (e.g., Smith, 1992; Smith and Tompkins, 1995). In recent decades, there has been widespread recognition that examinations of developmental processes recorded in mineralized tissues, particularly those in teeth, can provide an important longitudinal perspective in this endeavor (Dean, 2000; DeRousseau, 1990a; Macho and Wood, 1995). Histological investigations of crown and root development (particularly focusing on evidence of enamel and dentin formation) have been especially important in elucidating dental development patterns in extant primates (e.g., Beynon et al., 1991; Dirks, 2003; Reid et al., 1998), and have

yielded considerable insight into the developmental schedules of Plio-Pleistocene hominins (e.g., Bromage and Dean, 1985; Dean et al., 2001; Guatelli-Steinberg et al., 2005) and other extinct primate taxa (e.g., Beynon et al., 1998; Kelley, 1997; Kelley and Smith, 2003; Schwartz et al., 2005).

The microscopic organization of bone also records a history of its growth and development, and thus, represents a potentially rich source of information concerning the various ontogenetic and local factors which influence its formation (Enlow, 1963, 1966). Among anthropologists, this has been most widely recognized in studies of the developmental processes underlying variation in craniofacial morphology (e.g., Bromage, 1989; Duterloo and Enlow, 1970; Mowbray, 2005). Among investigators of other vertebrate groups, however, there is a tradition of examining bone microstructure (particularly in limb elements) as a means of deciphering basic characteristics of life history, including growth rates, maturational timing, and longevity (see reviews by Castanet et al., 1993; Chinsamy-Turan, 2005; Erickson, 2005; Klevezal, 1996; Ricqlès, 1993; Ricqlès et al., 1991). This research has focused largely on the microscopic organization of primary bone tissues (i.e., those tissues deposited during the process of growth). This approach has figured prominently in paleobiological studies of dinosaurs and extinct avians, where it has yielded important insight into such issues as the developmental basis for major changes in body size throughout their evolutionary history and the degree to which their physiology and growth may have been influenced by environmental factors (Chinsamy-Turan, 2005; Erickson, 2005).

Despite the promising results of this research, the significance of bone microstructure for deciphering aspects of life history in primates has been relatively

unexplored. Primates have been included in a number of broadly comparative studies of vertebrate bone histology (Amprino and Godina, 1947; Demeter and Mátyás, 1928; Enlow and Brown, 1958; Foote, 1916; Quekett, 1855; Singh et al., 1974), which have demonstrated considerable variation among taxa and among age groups, in both the microscopic organization of primary bone tissues and in the extent and distribution of secondary remodeling. While these studies have led to an appreciation of general trends and the range of variation observed among primates, they have tended to utilize bone samples collected opportunistically from a variety of anatomical locations, from animals frequently of unknown sex and age, and/or covering a broad range of vertebrate taxa (for a discussion of these and other limitations, see Enlow, 1966; and Ricqlès, 1993).

More recent systematic examinations of long bone microstructure in primates have tended to focus on its biomechanical significance (e.g., Kalmey and Lovejoy, 2002; Paine, 1994; Paine and Godfrey, 1997; Schaffler and Burr, 1984), often without attention to developmental history, and/or the age-related dynamics of secondary remodeling (e.g., Burr, 1992; Havill, 2004; Mulhern and Ubelaker, 2003; Robling and Stout, 2000). Only a few studies (Castanet et al., 2004; Enlow, 1962; Herrmann and Danielmeyer, 1994; Newell-Morris and Sirianni, 1982) have examined the significance of ontogenetic variation in primary bone microstructure for interpreting aspects of growth and development in primates. The current study begins to address this shortcoming. If the potential of this approach for anthropological investigations can be demonstrated, it may open new avenues of research and an independent line of inquiry into primate life history in paleontological contexts.

Interpreting growth and developmental history from bone

A major focus of research on other vertebrate groups has been the microscopic organization of primary bone tissues (i.e., those tissues deposited during the growth process), and their significance for discriminating variation in bone depositional rates. The significance of bone microstructure for such studies was first recognized by Amprino (1947), based on observations that different primary bone tissue types are deposited at different stages during ontogeny, and distinguish taxa characterized by different overall rates of body growth. ‘Amprino’s rule’ has since been substantiated in both comparative and experimental studies across a range of extant vertebrate groups (e.g., Castanet et al., 1996; Castanet et al., 2000; Margerie et al., 2002; Margerie et al., 2004; Newell-Morris and Sirianni, 1982; Ricqlès et al., 1991). Bone growth rate variability is also expressed in other histological features of primary bone, such as ‘lines of arrested growth’, which themselves have been the focus of considerable attention (Castanet et al., 1993; Klevezal, 1996; Peabody, 1961).

Unlike other mineralized tissues, however, bone undergoes substantial architectural modifications during growth, and these may continue into adult life. Enlow (Enlow, 1962, 1963, 1982) clarified the manner in which adjustments in whole bone architecture are accomplished by tissue-level mechanisms of bone growth remodeling at endosteal and periosteal surfaces, which may involve differential rates of bone deposition across cortical regions and local resorption of bone tissues deposited during earlier growth stages. Concurrently, adjustments in bone material properties (i.e., compositional and microstructural organization) may be accomplished through a process of secondary remodeling, involving the resorption of older cortical regions and their sequential

replacement with bone of more recent origin (Martin et al., 1998).

The mechanical significance of bone growth and secondary remodeling processes has received a great deal of attention (Currey, 2002; Frost, 1990a, 1990b; and Martin et al., 1998), and their examination can yield important insight into aspects of functional biology. These processes have important consequences for the microscopic organization and distribution of bone tissues within cortices, producing a heterogeneous and highly stratified cortex when viewed in cross-section (Enlow, 1962, 1963, 1982). Thus, if features of primary bone microstructure are to be properly interpreted within the context of life history, the influence of local (e.g., mechanically-regulated) dynamics of bone growth and development must also be considered (see Enlow, 1966; Ricqlès, 1993).

RATIONALE AND SCOPE OF THE DISSERTATION

The current study was motivated by an interest in the significance of bone microstructure for studies of primate life history in both modern and paleontological contexts. In ontogenetic series comprised exclusively of wild-collected individuals, the purpose of this study was to provide a foundation of understanding for ontogenetic and regional variation in bone microstructural organization at the midshaft femur and humerus of *Chlorocebus aethiops* and *Hylobates lar* and the midshaft femur of *Pan troglodytes*, and to consider the manner in which this variation reflects 1) aspects of life history, namely taxon-specific growth patterns, and 2) local mechanically-influenced dynamics of bone growth and development. This study examined variation in two microstructural features (primary bone tissue ‘types’, secondary intracortical remodeling) and one macrostructural feature (cross-sectional geometry):

Primary bone tissue types. Variability in a number of features of primary bone microstructure (e.g., primary bone tissue types and their microstructural characteristics, lines of arrested growth) has been suggested to reflect variability in bone growth rates. The current study examined variation in the proportion and distribution of primary bone tissue types within cortices. Researchers have historically relied on typological classifications to characterize diversity in primary bone microstructural organization to facilitate comparisons among groups. Current classifications (e.g., Francillon-Vieillot et al., 1990) integrate multiple criteria, each having relevance for interpreting variability in bone depositional rates (i.e., developmental origin, collagen fiber organization, and vascularization), into a single biologically-meaningful framework. Although these criteria have been examined independently in a number of recent studies, the approach taken here provides important baseline information regarding patterns of primary bone microstructural organization within entire cross-sections, both across individuals of different ages within taxa, and among taxa.

Cross-sectional geometry and secondary intracortical remodeling. Ontogenetic variation in primary bone microstructure was considered within the context of mechanically-relevant changes in bone macro- and microstructure. First, analysis of cross-sectional geometry provided a means of quantifying changes in whole bone architecture (i.e., size and shape) at midshaft, which occur as a consequence of growth and local circumstances of remodeling. Secondly, ontogenetic variation in the proportion and distribution of secondary intracortical remodeling was also analyzed.

Due to the nature of the sampling protocol to prepare histological thin sections, specimen availability was limited in both the taxonomic scope and element representation

in the current study sample. Nevertheless, this study represents the first systematic investigation of ontogenetic and regional variability in several features of bone macro- and microscopic structure across a range of catarrhine taxa characterized by varying life history. This study forms the groundwork for future intra- and interspecific comparisons with other extant primates, and it contributes to the current foundation for extending bone microstructural analyses to life history investigations in paleontological contexts.

ORGANIZATION OF THIS THESIS

Chapter 2 of this dissertation provides a general review of bone biology, followed by a discussion of determinants of bone macro- and microstructure. This discussion focuses particularly on the manner in which bone geometry and microstructure reflects the influence of 1) individual growth patterns, and 2) mechanical loading history.

Chapter 3 provides a general overview of the sample chosen for study, and a discussion of the criteria that were prioritized for the selection of appropriate specimens for examination. This is followed by a detailed description of the specimen preparation methodology, imaging methods, and a brief overview of those image processing techniques common to the separate analyses performed as part of this dissertation.

The main body of this dissertation research encompasses three separate analyses. While this introduces some redundancy in the thesis, the goals, background research, methods, results and discussion relevant to each of these analyses are presented separately in Chapters 4, 5 and 6, respectively. *Chapter 4* presents results of analyses of cross-sectional geometry. *Chapter 5* presents results of analyses of primary bone microstructure. *Chapter 6* presents results of analyses of secondary intracortical

remodeling.

Finally, *Chapter 7* summarizes the main results of this research and their significance. This is followed by a discussion of the broader implications of this research for studies of primate life history, and avenues for future investigation.

CHAPTER 2.

BACKGROUND: BONE BIOLOGY AND STRUCTURE

Bone is a dynamic tissue, which exhibits considerable variation in its macroscopic and microscopic organization. This diversity can be largely understood as a reflection of ontogenetic, local (e.g., functional), environmental, and phylogenetic factors (Castanet et al., 2001; Enlow, 1963; Martin et al., 1998; Ricqlès, 1993), the first two of which are the focus of the current thesis. This chapter reviews the basic composition and structure of bone, principles of bone growth and remodeling, and determinants of bone macro- and micro- structural organization. This is followed by a brief summary of research on nonhuman primate bone structural and microstructural characteristics undertaken to date. The latter studies will be reviewed in Chapters 4 – 6 of this thesis.

BONE COMPOSITION AND MICROSCOPIC STRUCTURE

Bone serves a number of supportive and metabolic functions (Currey, 2002; Martin and Burr, 1989; Martin et al., 1998; Parfitt, 2001). A primary function of the skeleton is support; bones withstand loads engendered by the growing and adult organism, and they serve as levers to permit movement resulting from the action of muscles. The skeleton also protects soft tissues of the body. The marrow cavities of bones also provide an environment for hematopoiesis, particularly in regions of the axial skeleton, pelvis and proximal femur which are composed primarily of cancellous bone. Finally, bones serve as a storage site for calcium, phosphorous and other minerals, and they serve an important role in mineral metabolism. All of these functions that bones

must serve have an important influence on their growth, development and maintenance in the adult skeleton. The following reviews aspects of bone composition and structure, which are relevant for an understanding of bone biology and function.

Cells of bone

Four types of bone cells are responsible for the formation, maintenance, and resorption of bone tissues: osteoblasts, osteocytes, bone lining cells, and osteoclasts. The first three cell types represent stages in the maturation of a single cell lineage of mesenchymal origin, while osteoclasts have a distinct origin. The differentiation and activities of these cells are regulated by a number of different hormones, transcription factors and growth factors, which have been the focus of many recent reviews (e.g., Boyle et al., 2003; Harada and Rodan, 2003; Jee, 2001; Lian and Stein, 1999; Lian et al., 2003; Majeska, 2001).

Cells of the osteoblast lineage: osteoblasts, osteocytes, and bone lining cells. Cells of the osteoblast lineage arise from a population of pluripotent stem cells of mesenchymal origin. The marrow stroma is thought to be an important source for progenitor cells committed to the osteoblast lineage (Jee, 2001; Lian and Stein, 1999; Lian et al., 2003; Majeska, 2001). Endothelial cells and pericytes (in perivascular regions) may serve as another source (Parfitt, 2000). The first cell type committed to the osteoblast lineage is the *osteoprogenitor cell*. These cells retain the capacity to proliferate, and they are present at sites near the bone surface (i.e., in the periosteum, endosteum, or marrow stroma).

Osteoblasts: bone forming cells. The *osteoblast* is the first mature cell type in the osteoblast lineage, and it functions primarily in bone formation. Osteoblasts can be recognized by their cuboidal shape, and by ultrastructural characteristics of cells that are actively engaged in synthesis and secretion of proteins. Osteoblasts are located at the bone surface, where they exhibit polarity; collagen and a variety of other bone matrix proteins are secreted (in the form of unmineralized *osteoid*) on the forming front of growing bone. As there is a lag between matrix deposition and its subsequent mineralization, a thin layer of osteoid separates osteoblasts from the mineralized matrix of bone. Osteoblasts exert a regulatory influence over matrix mineralization through the bone matrix proteins they secrete. Osteoblasts (and other osteoblast-lineage cells, see below) also participate in the regulation of osteoclast differentiation and activity (Jee, 2001; Lian and Stein, 1999; Lian et al., 2003; Mackie, 2003; Majeska, 2001; Rodan and Martin, 1981).

Osteocytes: mechanosensory cells. Some osteoblasts become embedded within the matrix of forming bone, enclosed within spaces called *lacunae*. These cells are then referred to as *osteocytes*, which represent the final stage of differentiation in the osteoblast lineage. Osteocytes are stellate-shaped cells, which have lost most of the cytoplasmic organelles associated with matrix synthesis and secretion. They are separated from the lacunar wall by a thin layer of unmineralized matrix, referred to as *interstitial fluid*. They develop elongated cytoplasmic processes, which course through small canals termed *canaliculi* to maintain connections with adjacent osteocytes and cells at the bone surface. Cytoplasmic processes of adjacent cells communicate through transmembrane channels called gap junctions, which permit exchange of small molecules

(e.g., calcium and other ions, signaling molecules) between cells. Osteocytes may participate locally in the passage of minerals into and out of bone. They are thought to serve a primary role in sensing and responding to mechanical strain (Burger and Klein-Nulend, 1999; Cowin, 2002; Cowin et al., 1991; Doty, 1981; Jee, 2001; Majeska, 2001; Nijweide et al., 1996).

Bone lining cells. On quiescent bone surfaces, resting osteoblasts take on a flattened shape and are referred to as *bone lining cells*. Bone lining cells form a barrier between the bone fluid compartment and interstitial fluids. They communicate with osteocytes and adjacent bone lining cells via cytoplasmic extensions and their gap junctions. They are capable of becoming active osteoblasts, and may secrete factors which promote osteoblast differentiation. Bone-lining cells also participate in the initiation of bone resorption during the process of remodeling, in part by undergoing changes in their disposition on the bone surface to provide access by osteoclasts (Cowin et al., 1991; Jee, 2001; Majeska, 2001; Miller and Jee, 1987; Parfitt, 1994).

Osteoclasts: bone resorbing cells. *Osteoclasts* are large (~ 100 μm) multinucleated cells, whose primary function is the resorption of bone. They are derived from fusion of mononuclear cells of the monocyte/macrophage hematopoietic lineage, and their differentiation is mediated by osteoblasts. Osteoclasts form depressions on the bone surface called *Howship's lacunae* and, like osteoblasts, active cells exhibit a distinct polarity in their ultrastructure and function. The cell surface in contact with bone is characterized by numerous cytoplasmic infoldings, forming what has been called a “ruffled border”, surrounded by a zone of adhesion. Osteoclasts secrete hydrogen ions

into this space, thereby lowering the pH and creating a microenvironment conducive to the break down of bone mineral. They also secrete a variety of proteolytic enzymes, which digest organic components of the matrix (Boyle et al., 2003; Jee, 2001; Junquiera and Carniero, 2005; Lian and Stein, 1999; Mackie, 2003; Majeska, 2001; Rodan and Martin, 1981).

Extracellular matrix of bone

Bone, like other connective tissues, contains populations of cells embedded in a rich extracellular matrix (ECM). The ECM of bone is first deposited as an organic *osteoid* substance, which is later mineralized. Mammalian bone is made up of approximately 50-70% mineral (Robey and Boskey, 2003). The major organic constituents of the ECM of bone are collagen, water and ground substance. This composite nature of bone is critical to its ability to function mechanically (Currey, 2002; Martin et al., 1998). For instance, mineralization is a major determinant of bone stiffness (Currey, 1984), while collagen is thought to contribute to the compliance and fracture toughness of bone (Burr, 2002).

Organic matrix components. The major organic components of the ECM of bone include collagen, non-collagenous proteins, and water. The term *ground substance* is commonly used to refer to non-collagenous organic components of the ECM (e.g., Junquiera and Carniero, 2005).

Collagen comprises approximately 90% of the organic component of bone. It exists predominantly in the form of type I collagen, a triple helical molecule composed of

two $\alpha 1(I)$ chains and one $\alpha 2(I)$ chain (Boskey, 1999; Robey and Boskey, 2003; Rossert and Crombrughe, 1996). Type I collagen is synthesized and secreted by osteoblasts into the ECM in the form of procollagen. Following further modification, tropocollagen molecules aggregate to form fibrils. Within fibrils, tropocollagen molecules lie end to end, with each molecule separated from the next by a 40 nm hole. Parallel arrangements of tropocollagen molecules overlap in a staggered manner, with a characteristic 67 nm repeat between adjacent hole + overlap regions (Currey, 2002; Rossert and Crombrughe, 1996). Collagen fibrils themselves aggregate to form branching fiber bundles, visible with the aid of transmitted light microscopy (Boyde, 1972).

Non-collagenous proteins comprise approximately 10-15 % of the overall protein content of bone. The functions of many proteins are not well understood, but they include: participation in cell-matrix and matrix-matrix interactions; regulation of cell proliferation, differentiation and function; binding of growth factors and other matrix proteins; participation in collagen fibril formation and matrix mineralization. Among the most abundant non-collagenous proteins in bone are osteocalcin, osteopontin, osteonectin, and bone sialoprotein. These proteins are present in bone undergoing active growth and remodeling, where they participate in regulation of bone formation and resorption, and matrix mineralization (Alford and Hankenson, 2006; Boskey, 2001; Hall, 2005; Robey and Boskey, 2003). The ECM of bone also contains proteoglycan aggregates, such as decorin and biglycan, which are important for matrix integrity (Robey and Boskey, 2003).

Water is also a significant component of the organic matrix of bone. It is present in vacancies within collagen fibrils and mineral crystallites, and as a thin layer on the

surface of mineral crystallites where it may mediate interactions between mineral and organic matrix constituents (Boyde and Jones, 1998; Wilson et al., 2006). It also exists freely and bound to components (e.g., proteoglycans) of the ground substance (Junquiera and Carniero, 2005; Martin et al., 1998). Much of this water is replaced by mineral as mineralization proceeds, while the remaining exists as structural water binding mineral to its associated organic matrix (Boyde and Jones, 1998).

Mineral. Bone mineral consists mainly of calcium and phosphate in an apatite form similar to hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$. It differs from hydroxyapatite in the incorporation of impurities into its lattice structure, the most abundant of which is carbonate (Boskey, 1999; Boyde and Jones, 1998). While the stages of mineral deposition in the ECM are not completely understood, the majority of mineral in bone is associated with collagen fibrils. Mineralization of bone is thought to be first initiated in the hole zones within collagen fibrils, and it progresses to overlap regions within collagen fibrils and along the fibril surfaces. Mineral crystals are plate-like in shape, and are oriented with their long axes approximately parallel to the long axes of the collagen (see Boskey, 2001 for a review; Glimcher, 1984; Landis et al., 1996; Landis et al., 1993; Su et al., 2003; Traub et al., 1989; Weiner and Traub, 1986; Weiner and Traub, 1992).

This initial phase of mineralization associated with collagen fibrils proceeds rapidly, over the course of a few days to a week after bone deposition, during which time the majority (approximately 70 %) of the final mineralization density is achieved (Akkus et al., 2003; Boyde and Jones, 1998). Mineral continues to accumulate in the remaining spaces of the ECM over a longer period of time, which may last as long as two decades in

human bone (Akkus et al., 2003). This period of secondary mineralization, or *maturation*, is characterized by an increase in the size of mineral crystals and by changes in their chemical properties (Akkus et al., 2004; Akkus et al., 2003).

Bone Tissue Types

The cellular and extracellular constituents of bone may combine to produce a diversity of patterns of organization, which may be observed at macroscopic and microscopic levels. To characterize this diversity, particularly at the microscopic level, researchers have typically classified patterns of organization into a number of distinct “tissue types”.

When bones are observed macroscopically, two types of bone can be recognized on the basis of porosity. *Cancellous* (also called *spongy* or *trabecular*) bone is very porous, with 75-95% of its volume being comprised of marrow-filled space (Martin *et al.*, 1998). The bone tissue matrix forms interconnecting slips of bone, called trabeculae. Cancellous bone forms the majority of irregular-shaped bones (*e.g.*, vertebrae), flat bones, and the ends of long bones. *Compact* or *cortical bone* comprises the dense outer shell of irregular and flat bones, the ends of long bones, and it forms the majority of long bone diaphyses. Cortical bone is less porous than trabecular bone, with vascular canals and resorption spaces generally comprising 5-10% of its volume (Martin *et al.*, 1998).

If cancellous and cortical bone is examined more closely, one can appreciate that the microscopic organization of bone exhibits considerable diversity both within and among vertebrate skeletons. A number of different bone tissue types have been defined on the basis of criteria such as developmental origin (*e.g.*, endosteal versus periosteal),

organization of the collagen fiber matrix, and vascular patterns (Enlow, 1962, 1982; Enlow and Brown, 1956; Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991). Some of the more commonly recognized tissue types among vertebrates are briefly described here. Bone tissue types will be considered in greater detail in Chapters 5 and 6 of this thesis.

Tissue types recognized on the basis of collagen fiber organization. The collagen fiber matrix of bone is organized along a continuum, from being randomly organized to having a highly-ordered arrangement. Three fundamental patterns can be recognized.

1) *Woven bone* [also referred to as “fibrous” bone by some authors (Boyde and Jones, 1998; Enlow, 1963; Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991; Smith, 1961)] is a rapidly formed tissue, which constitutes the fetal skeleton, regions of the young postnatal skeleton, and areas of callus formation. It is characterized by loosely-packed collagen fibers ranging in diameter from 0.1 – 3 μm (Boyde and Jones, 1998) and coursing in all directions in a more or less random fashion. Osteoblasts are incorporated into the forming matrix of woven bone as plump and irregularly-shaped osteocytes, which are randomly distributed and more numerous compared to other bone tissue types. This tissue type is frequently richly vascularized.

2) *Parallel-fibered bone* (Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991) is intermediate in organization between woven bone and lamellar bone (discussed below). It is characterized by closely-packed collagen fibers having a single preferred orientation, running approximately parallel to one another. Osteocyte lacunae are slightly flattened in shape, and tend to have a more regular arrangement than observed in woven bone (Jones and Boyde, 1976).

3) *Lamellar bone* (Boyde and Jones, 1998; Enlow, 1963, 1982; Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991) is characterized by a highly-ordered collagen fiber arrangement, and is comprised of layers of bone, called lamellae, which are frequently 2 -6 microns in width (Currey, 2002). Osteoblasts become incorporated into the forming matrix as spindle-shaped osteocytes, oriented with their long axes parallel to the underlying collagen fiber matrix (Jones and Boyde, 1976). They tend to follow a linear course along lamellae. Osteocyte lacunae are less densely distributed than in other tissue types described above (Boyde and Jones, 1998). The majority of current investigators agree that the layered appearance of lamellar bone is the result of a regular change (whether continuous or discontinuous) in the orientation of collagen fiber bundles between successive lamellae, which results in their optical distinction when viewed under polarized light [(Ascenzi and Benvenuti, 1986; Giraud-Guille, 1988; Weiner et al., 1997; Ziv et al., 1996), but see (Marotti, 1993; Marotti and Muglia, 1988)].

Bone tissues belonging to the above tissue types are frequently grouped into two broad categories (Francillon-Vieillot et al., 1990b): 1) *lamellar-zonal bone*, which includes parallel-fibered and lamellar tissue types, and 2) *fibro-lamellar bone*, which is defined as a composite of woven bone and lamellar bone. The latter tissue type (also referred to as compacted fine cancellous bone by Enlow, 1962, 1963, 1982) is deposited as an initial framework of woven bone organized into fine cancellous trabeculae, characterized by numerous spaces containing connective tissue cells and blood vessels. This framework is later in-filled by lamellar (or parallel-fibered) bone, which is deposited along the cancellous surfaces, gradually “compacting” the cancellous spaces and resulting in the formation of primary osteons where blood vessels exist. Fibro-lamellar

bone is densely vascularized, with vascular canals incorporated into the forming matrix in a variety of orientations. Several fibro-lamellar tissue types are recognized on the basis of vascular patterns (e.g., reticular, plexiform, laminar, radial), discussed further in Chapter 5 of this thesis.

Endosteal versus periosteal origin. Bone tissues formed during growth may be deposited at the periosteal surface or at the endosteal surface. Thus, woven, parallel-fibered, and lamellar bone can be classified accordingly, as either endosteal or periosteal in origin. However, one tissue type not yet discussed originates only at the endosteal surface: *compacted coarse cancellous bone* (Enlow, 1962, 1963, 1982). This tissue type is formed when endosteal growth takes place within a region originally comprised of coarse cancellous bone (usually near the metaphysis), which is “compacted” as a result of lamellar bone deposition in the available spaces between cancellous trabeculae. During compaction, lamellar tissue is deposited along the irregular contours of coarse cancellous trabeculae, giving this tissue type a distinctively convoluted appearance.

Primary versus secondary origin. All bone tissues may be classified as being either primary or secondary in origin (Castanet and Ricqlès, 1986-7; Currey, 2002; Enlow, 1963, 1982; Francillon-Vieillot et al., 1990b; Martin et al., 1998). *Primary bone tissues* are those deposited during growth at the periosteal or endosteal surface, where bone tissue did not previously exist. Vascular canals in primary tissues are embedded within the forming front of growing bone. *Secondary bone tissues* are those formed during the process of secondary remodeling, which may take place at endosteal, periosteal, and

intracortical surfaces within bone. Secondary bone is deposited following the resorption of an existing bone volume, and through the sequential action of osteoclasts and osteoblasts at single bone sites. If this occurs at intracortical surfaces within bone, the resulting structure is a *secondary osteon* or *Haversian system*, delimited from surrounding tissue by the presence of a cement (reversal) line. The formation of secondary osteons is discussed further below.

BASIC PRINCIPLES OF LONG BONE GROWTH

Bone is a mesenchymal tissue, originating from neural crest and mesoderm in the developing embryo. Elements that will form part of the limb skeleton, in particular, are derived from the parietal layer of the lateral plate mesoderm (Gilbert, 2003). Bone formation takes place via two mechanisms: 1) *endochondral ossification*, in which bone deposition occurs within a cartilaginous model, and 2) *intramembranous ossification*, in which bone deposition takes place directly within condensations of mesenchyme without a cartilage intermediate (see recent reviews by Scheuer and Black, 2000; Young and Heath, 2000).

Cartilage is an avascular connective tissue having a firm yet unmineralized ECM. As such, it is capable of undergoing both *interstitial* (i.e., by cell proliferation and matrix production *within* the cartilage mass) and *appositional* (i.e., by cell proliferation and addition of new matrix at its surface) growth (Enlow, 1982; Enlow and Hans, 1996). Interstitial and appositional growth of hyaline cartilage at the epiphyseal growth plate, and its subsequent replacement by bone during endochondral ossification, provides a mechanism for linear growth of long bones. As a mineralized tissue, bone is only

capable of growing appositionally. Thus, as bones grow in linear and cross-sectional dimensions, they must also be continuously re-shaped or remodeled. Endochondral and intramembranous ossification are considered in further detail below. Bone remodeling during growth will be considered in a following section.

Endochondral ossification

Endochondral ossification most notably takes place in long bones, vertebrae, sternum, the pelvis, bones of the skull base, and carpals and tarsals (Scheuer and Black, 2000). This mechanism of bone formation involves the formation of a hyaline cartilage model, which is subsequently replaced by bone. Thus, ossification is preceded by a period of chondrogenesis, or cartilage formation. Endochondral bone formation in the limb skeleton is regulated by a variety of systemic and local factors that have been the subject of recent reviews (e.g., Colnot, 2005; Hall, 2005; Horton, 2003; Kronenberg, 2003; Olsen et al., 2000).

The sites of future long bones in the developing limb bud first are first evidenced by the local proliferation and condensation of mesenchymal cells. These cells begin expressing matrix proteins (mainly collagen Type II) that are specific to cartilage, resulting in the formation of a hyaline cartilage mass. This mass of hyaline cartilage will eventually serve as a template for future bone development. *Interstitial growth* of this cartilage occurs from within, as a result of cell proliferation and secretion of extracellular matrix by individual cartilage cells (now termed chondrocytes). Mesenchymal cells at the periphery of this cartilage model will form the perichondrium, the inner layer of which is a source of differentiating cartilage cells for *appositional growth* at the surface

(Scheuer and Black, 2000; Young and Heath, 2000).

With further maturation, chondrocytes at the center of the hyaline cartilage mass cease proliferation and undergo a sequence of degenerative changes beginning with chondrocyte hypertrophy. Hypertrophic chondrocytes secrete an extracellular matrix rich in collagen Type X and other constituents not characteristic of proliferating chondrocytes, and they are thought to have an important regulatory influence over later stages of cartilage maturation and bone formation. Under their influence, cells of the adjacent perichondrium differentiate into osteoblasts, and form a collar of bone around the periphery of the developing midshaft region via intramembranous ossification (discussed further below). Hypertrophic chondrocytes also secrete factors that promote invasion by blood vessels, which is necessary for endochondral ossification and development of the marrow cavity. The cartilage matrix becomes calcified, and is partially resorbed by chondroclasts brought to the region by invading blood vessels (Colnot, 2005; Gilbert, 2003; Kronenberg, 2003; Olsen et al., 2000).

Hypertrophic chondrocytes eventually undergo apoptosis, and the remaining calcified matrix becomes the scaffolding on which bone matrix is initially deposited by osteoblasts. Once bone formation begins, this site is referred to as the *primary ossification center*. Chondrocytes of the primary ossification center show a clear directionality in their maturation. The cartilage model continues to grow lengthwise by proliferation of chondrocytes towards the epiphysis, while older cartilage matrix is replaced by bone towards the developing diaphysis. As the bone develops, hyaline cartilage of the future epiphysis undergoes a sequence of changes similar to that described above, forming a *secondary ossification center* (Scheuer and Black, 2000;

Young and Heath, 2000).

Linear growth of long bones results from continued proliferation and hypertrophy of chondrocytes located between the primary and secondary ossification centers, in a zone of rapid interstitial growth referred to as the *growth plate*. Linear bone growth stops when chondrocytes at the growth plate(s) stop proliferating (Scheuer and Black, 2000; Young and Heath, 2000). Chondrocyte proliferation slows and eventually ceases during puberty under the influence of systemic hormones, mainly estrogen (Frank, 2003). This may also reflect some intrinsic (i.e., genetically-determined) capacity for chondrocyte proliferation in the growth plate itself (Nilsson and Baron, 2004; Weise et al., 2001). Cessation of chondrocyte proliferation is accompanied by a decline in the thickness of the growth plate and, when the ossification fronts of the epiphysis and diaphysis meet, by the onset of epiphyseal union (Nilsson and Baron, 2004; Parfitt, 2002; Weise et al., 2001). Long bone epiphyseal union occurs at varying times across skeletal sites. The distal humerus is among the first to fuse and the proximal humerus usually among the last to fuse in primates (Cheverud, 1981; Kimura and Hamada, 1990; Scheuer and Black, 2000; Shigehara, 1980; Washburn, 1943; Wintheiser et al., 1977).

Intramembranous ossification

Intramembranous bone formation takes place within local condensations of mesenchymal cells, which form what is often termed a connective tissue “membrane”. These mesenchymal cells differentiate into osteoprogenitor cells, and then osteoblasts, as they begin secreting extracellular matrix specific to bone. This matrix subsequently becomes mineralized. Bone matrix is initially deposited in the form of isolated patches,

or “spicules”, which are scattered among differentiating mesenchymal cells. These spicules expand with continued deposition of bone along their surfaces, and they eventually fuse to form larger trabeculae. Mesenchymal tissue around the periphery differentiates to form the periosteum, which retains its osteogenic potential into adulthood (Scheuer and Black, 2000; Young and Heath, 2000).

Intramembranous bone formation takes place within all skeletal elements, but it is the primary mechanism of bone formation in the clavicle, the flat bones of the skull, bones making up the face, and parts of the mandible (Scheuer and Black, 2000). Although long bones are often cited as a textbook example of endochondral ossification, bone is first deposited intramembranously as a periosteal bone collar (see above). Further, the majority of bone tissue comprising the mature diaphyseal cortex is formed by intramembranous ossification, as is the outer cortical shell of other long bone regions. The periosteum and endosteum retain their osteogenic potential into adulthood (Scheuer and Black, 2000).

MECHANISMS OF BONE REMODELING

The term *remodeling* has historically been used to refer to a continuum of biological processes involving the coordinated activities of osteoclasts and osteoblasts on bone surfaces, which take place during growth and in the adult skeleton (for a review of this concept, see Enlow, 1963; Enlow, 1976). Remodeling may occur at different times and locations to fulfill a number of different functions, and on this basis Enlow (Enlow, 1976, 1982; Enlow and Hans, 1996) distinguished four different varieties: 1) ‘biochemical remodeling’, which is involved in mineral homeostasis; 2) remodeling

following pathology or trauma; 3) ‘growth remodeling’, which constitutes a basic component of the bone growth process; and 4) ‘secondary (or Haversian) remodeling’, which is a replacement process involving the sequential resorption and formation of bone at single bone sites. The latter two varieties of bone remodeling are relevant to the current study.

Growth remodeling entails coordinated patterns of bone resorption and deposition occurring simultaneously at different endosteal (including cancellous) and periosteal surfaces of bone (Enlow, 1962, 1963, 1976, 1982; Enlow and Hans, 1996). Growth remodeling accomplishes the sequential re-shaping of bones as they grow in linear and cross-sectional dimensions during development, although it may also continue throughout adult life. *Secondary remodeling* refers to the sequential action of osteoclasts and osteoblasts at single bone sites, most commonly on endosteal (including cancellous) and intracortical surfaces of bone. In secondary remodeling, bone resorption always precedes a period of bone formation, resulting in the replacement of the original bone volume with bone of more recent origin (Martin et al., 1998). During both processes, coordinated activities of bone cells effect appropriate adjustments in bone structure in response to local (e.g., mechanical) influences. Growth remodeling and secondary remodeling are considered in further detail below.¹

¹ The current study follows terminology clarified and discussed by Enlow (Enlow, 1976, 1982; Enlow and Hans, 1996). However, it is also noted that Frost (Frost, 1986, 1990a, 1990b, 2003) proposed that growth remodeling and secondary remodeling are operationally different processes, initiated under different mechanical strain conditions (e.g., disuse and overload) and in an opposing manner. These processes also differ in their sensitivity to mechanical loading conditions with age. On this basis, Frost proposed a terminological distinction between *modeling* (synonymous with the term ‘growth remodeling’ proposed by Enlow) and *secondary remodeling*. (Thus, according to Frost,

Growth remodeling

Enlow (Enlow, 1962, 1963, 1976, 1982; Enlow and Hans, 1996; Hoyte and Enlow, 1966) characterized the basic tissue-level mechanisms involved in the bone growth process, and which provide a means by which bones acquire and maintain their characteristic shapes during development. *Growth remodeling* involves temporally- and spatially - coordinated patterns of bone deposition and resorption at endosteal (including cancellous) and periosteal surfaces. It results in the sequential relocation of bone regions into new locations during growth, serving either to maintain or to make local adjustments in bone shape with increases in overall bone size.

Mechanisms of growth remodeling may entail local destruction of tissues deposited during earlier growth stages, and differential rates of osteogenesis across cortical regions. Their result is a stratified and heterogeneous appearance of cortical bone in cross-section, where each stratum represents a cycle of bone deposition of tissue that

the term *remodeling* refers only to the secondary remodeling process.) According to this view, secondary remodeling is also distinguished on the basis of its “coupled” action of osteoclasts and osteoblasts, while ‘modeling’ is said to involve the “independent” actions of these cell types (as defined by Martin et al., 1998, p. 60-61.)

In the years since Frost’s proposal, the terminology used to describe remodeling processes has varied among investigators and subfields. For instance, investigators with a primary research interest in bone biomechanics have tended to follow his distinction between modeling and remodeling (e.g., Drapeau and Streeter, 2006; Lieberman et al., 2003; Martin et al., 1998). However, investigators with a primary interest in the surface activities of bone cells during processes of bone growth (e.g., Bromage, 1989; McCollum, 1999; Mowbray, 2005) have continued to use the term ‘growth remodeling’ as clarified by Enlow. That these two perspectives have yet to be fully reconciled likely reflects an understanding of basic mechanisms of bone growth and ‘remodeling’ (*sensu* Enlow) which is still emerging, and this issue may not be fully resolved until the various factors controlling the temporal and spatial coordination of bone cells during both growth and secondary remodeling are better understood.

may vary in its microstructural organization, or a reversal in growth direction, from adjacent strata. Microstructural characteristics of bone growth remodeling observed in transmitted light microscopy of histological thin-sections have been clarified and discussed at length by Enlow (1962; 1963; 1976; 1982), and their consistency with features associated with formative and resorptive activity on the bone surface observed in scanning electron microscopy has been demonstrated (Saunders, 1985). Several principles of growth remodeling fundamental to an understanding of long bone growth and development are considered further below.

Cortical drift, metaphyseal remodeling, and relocation. 1) *Cortical drift* (Enlow, 1962, 1963; Enlow and Hans, 1996; Smith, 1961). Cortical drift is accomplished by coordinated patterns of bone deposition and resorption on complementary endosteal and periosteal bone surfaces, resulting in the directional movement or “drift” of the bone cortex in morphological space. Bone is deposited on surfaces facing towards the direction of drift, while bone is removed from surfaces facing away from the direction of drift. For example, cortical drift of a whole bone (cross-section) in an anteromedial direction is accomplished: 1) in the anteromedial cortex, by bone deposition on the periosteal surface and bone resorption on the endosteal surface; and 2) in the posterolateral cortex, by bone resorption on the periosteal surface and bone deposition on the endosteal surface. (Figure 2-1). In a bone undergoing cortical drift, depositional rates in the direction of drift may be substantially faster than in other regions of the cortex (e.g., Newell-Morris and Sirianni, 1982). Cortical drift is an important process involved in the development of long bone diaphyseal curvature.

2) *The “V” principle and metaphyseal remodeling* (Enlow, 1962; 1963; Leblond et al., 1950). The “V” principle refers to a special circumstance of cortical drift, which occurs in skeletal elements having funnel - shaped proportions. In this case, cortical drift must operate both to relocate cortical regions through space and to maintain their characteristic shapes. The long bone metaphysis provides an example of the “V” principle. As the metaphysis keeps pace with the proximal or distal advance of the growth plate during linear bone growth, its proportions are maintained as a result of a coordinated pattern of bone deposition on the endosteal surface and bone resorption on the periosteal surface. (Figure 2-2 a). As the metaphysis is sequentially relocated towards the end of the long bone, its dimensions are enlarged towards the wide end of the “V” as it advances with the growth plate. At the same time, the diameter of older metaphyseal tissue is reduced shaft-ward (i.e., at the closed end of the “V”) as it is sequentially incorporated into the elongating diaphysis.

3) *Relocation* (Enlow, 1963, 1982; Enlow and Hans, 1996). Relocation is an important component of the bone growth remodeling process. As a result of formative and resorptive activities on bone surfaces, cortical regions are sequentially relocated in space relative to other regions of the bone as growth proceeds. For instance, bone deposited at the endosteal surface of a developing long bone cross-section may be sequentially relocated towards the periosteal surface under conditions of marked cortical drift, as a result of addition of new bone layers at the endosteal surface and removal of bone at the periosteal surface. (Such a pattern may be expected in the posterolateral cortex of the bone depicted in Figure 2-1 a.) Also, as shown in Figure 2-2 b, cortical levels along the length of a growing long bone may be sequentially relocated relative to

the position of the metaphysis as linear bone growth proceeds. As a result of linear bone growth and metaphyseal remodeling, levels located in the region of the metaphysis during early development are sequentially relocated to become part of the diaphysis during later stages of development.

Concurrently with these remodeling processes, radial growth of the long bone diaphysis is accomplished by intramembranous bone formation at the periosteal surface. Bone deposited endochondrally at the growth plate undergoes subsequent growth remodeling, either being resorbed during the process of medullary expansion or relocated to different cortical regions as a result of cortical drift. Long bone regions that have undergone metaphyseal remodeling during growth, particularly in the proximal and distal thirds, are typically characterized by high proportions of compacted coarse cancellous bone (in areas previously containing endosteal coarse cancellous bone) or other tissues of endosteal origin. These tissues may be removed as a result of medullary expansion later in life.

Cement lines: spatial and temporal discontinuities in bone deposition. Growth remodeling processes described above may entail both spatial and temporal discontinuities between cycles of bone deposition, represented by the formation of cement lines. Cement lines can be readily identified in transmitted light microscopy where they appear as thin (frequently 1-2 microns in width; Frasca, 1981) translucent bands, in polarized light where they generally appear brighter than surrounding tissue, and with the aid of histological staining techniques (Castanet, 1981; Castanet et al., 1993; Klevezal, 1996). They are generally thought to be collagen deficient and rich in ground

substance, and most investigators (e.g., Boyde and Jones, 1998; Castanet, 1981; Frasca, 1981; Skedros et al., 2005) consider them more highly mineralized relative to adjacent bone tissues (but see Burr et al., 1988a; Schaffler et al., 1987).

Two types of cement lines are recognized. *Reversal lines* indicate a reversal in growth direction (e.g., from periosteal to endosteal deposition) and/or a spatial discontinuity between cycles of bone deposition. Reversal lines are formed following a cycle of bone resorption, and they are typically distinguished from other cement lines by their scalloped contour following the outline of resorption (Howship's) lacunae (Enlow, 1963; Ricqlès et al., 1991). *Resting lines*, in contrast, represent temporal discontinuities (i.e., temporary cessations) between cycles of bone deposition. Their formation does *not* involve an intervening period of bone resorption (Castanet, 1981; Castanet et al., 1993; Klevezal, 1996).

Secondary remodeling

Like growth remodeling, secondary remodeling also occurs at a reduced rate following skeletal maturity. However, it may also be prevalent in the adult skeleton (Martin et al., 1998). Secondary remodeling results in the resorption of an existing bone volume and its immediate replacement by bone of more recent origin (see recent reviews by Currey, 2002; Dempster, 1999; Martin et al., 1998; Parfitt, 1994). Together, groups of osteoclasts and osteoblasts involved in a local secondary remodeling event comprise a *basic multicellular unit (BMU)*. Three sequential stages in the activity of a BMU are recognized (Frost, 1986, 1990b): 1) *activation* in response to a hormonal or mechanical stimulus, involving changes in bone lining cells and local differentiation and recruitment

of osteoclasts to the bone surface; 2) *resorption* of a bone volume by osteoclasts, creating a resorption cavity; and 3) *formation* of a new volume of bone at the same bone site by recently-differentiated osteoblasts. This process takes approximately 4 months in humans, with the majority of this time (~3 months) corresponding to the formation stage. The mechanism responsible for “coupling” of osteoclasts and osteoblasts at single bone sites, during the reversal period between resorption and formation, is not entirely understood. However, it has been suggested to involve growth factors released from the bone matrix following resorption, mechanical stimuli, and signals emanating from vasculature associated with the BMU (Martin and Rodan, 2001; Parfitt, 2000).

The sequential activities of a given BMU are similar whether secondary remodeling occurs at endosteal (including cancellous), periosteal or intracortical surfaces of bone. However, secondary remodeling at intracortical surfaces results in the formation of a cylindrical structure, termed a *secondary osteon* or *Haversian system* (Currey, 2002; Dempster, 1999; Martin et al., 1998; Parfitt, 1994). (Figure 2-3). Newly-differentiated osteoclasts form a cutting cone (rather than a trench-like depression, as on other bone surfaces) approximately 200 μm in diameter, which tunnels through existing bone in a predominantly longitudinal direction. As osteoclasts resorb older bone tissue, a resorption space is left behind. The BMU brings with it a growing capillary loop, which provides nourishment and progenitor cells for osteoclasts and osteoblasts. The reversal from osteoclastic resorption to osteoblastic formation occurs over a period of several days, and results in the formation of a cement line. The formation phase begins with the appearance of osteoblasts along the surfaces of the resorption space. Inward concentric deposition of lamellar bone follows at an average rate of 1-2 $\mu\text{m}/\text{day}$ (Martin et al.,

1998), refilling the space up to the blood vessel wall. The entire bone volume is not replaced, but a canal is left in the center for passage of blood vessels and nerves, termed the *Haversian canal*. This vascular supply provides nourishment for adjacent osteocytes and bone lining cells, and a passageway for exchange of minerals between the skeleton and bloodstream (Martin et al., 1998; Parfitt, 1994).

FACTORS INFLUENCING BONE STRUCTURE

Bones play a primary role in structural support of the body and, in doing so, must bear the mechanical loads engendered by weight-bearing and muscle action during locomotion and other activities. It has long been appreciated that bones within and among skeletons exhibit a range of structural and material characteristics, which appear to be appropriately and efficiently suited to their mechanical loading environments. This has led to the idea that bone structure reflects, at least in part, its mechanical loading history (Currey, 2002; Dibbets, 1992; Martin et al., 1998; Roesler, 1987; Ruff et al., 2006).

In addition to their obvious role in structural support, bones are also subject to a variety of systemic and environmental influences, which may at times impose conflicting demands on bone growth and maintenance. For example, adequate levels of calcium and other nutrients (e.g., vitamin D, protein) are vital for bone health, particularly during postnatal growth when the large majority of bone mass is acquired (Davies et al., 2005; Marcus, 1996; Mühlbauer and Li, 1999; Root, 1990). Hormones are also important factors influencing bone. Bone mass acquisition during postnatal growth and its maintenance in the adult skeleton is under the influence of the growth hormone / IGF-I

axis and sex steroids (i.e., estrogen and androgen) (Compston, 2001; Davies et al., 2005; Marcus, 1996; Olney, 2003; Vanderschueren et al., 2004). Increased levels of glucocorticoids (e.g., cortisol), however, have a negative influence on bone balance (Canalis and Delany, 2002; Rehman and Lane, 2003). Further, as the skeleton serves as a reservoir for calcium and other minerals, bone cell activity is influenced by hormones involved in calcium homeostasis (e.g., parathyroid hormone, calcitonin, vitamin D) (Parfitt, 1993, 2001; Rizzoli and Bonjour, 1999).

Evidence indicates that hormones (e.g., estrogen and parathyroid hormone) may also influence the osteogenic response of bone to mechanical loading (Lanyon et al., 2004; Lanyon, 1996; Ryder and Duncan, 2000). Aside from their direct influence on bone cell activity, hormones may also act indirectly to promote bone growth via their effects on growth in body size and muscle mass (thereby increasing mechanical loads; Marcus, 1996; Vanderschueren et al., 2004), which themselves are thought to be important determinants of diaphyseal geometry (Moro et al., 1996; Ruff, 2003; Schoenau, 1998; Schoenau et al., 2000; van der Meulen et al., 1996).

Teasing apart the influence of genetic and other systemic factors versus local mechanical factors on bone structure is difficult (for a recent review, see Ruff et al., 2006). As much as 60-70 % or more of the variance observed in peak bone mass is genetically determined (Davies et al., 2005; Prentice, 2001). Genetic background partly determines variation in bone geometry, material properties, and patterns of growth remodeling at periosteal and endosteal surfaces (Price et al., 2005; Volkman et al., 2004; Volkman et al., 2003). The responsiveness of bones to mechanical stimuli is also determined in part by genetic factors (Robling and Turner, 2002). Further, Lovejoy and

colleagues (Lovejoy et al., 2003; Lovejoy et al., 2002) have recently argued that strains arising from external loads have a relatively minor influence on bone morphology; rather, adult bone size and shape is largely the outcome of genetically-determined pattern formation and assignment of positional information at various stages of bone morphogenesis.

However, evidence also indicates that “...regardless of genetic background, appropriate mechanical loading is necessary to develop normal adult form” (Ruff et al., 2006: 491). While limb elements relieved of normal mechanical loading continue to grow in linear and cross-sectional dimensions, they also exhibit diminished bone mass and fail to develop their characteristic cross-sectional shapes and diaphyseal curvatures (Biewener and Bertram, 1994; Lanyon, 1980). Further, experimental studies have demonstrated that bone is capable of responding to changes in loading conditions by making mechanically-appropriate adjustments in macro- and micro- structure (discussed below; see reviews by Martin et al., 1998; Ruff et al., 2006). As proposed by Carter and colleagues (Carter and Beaupré, 2001; Carter et al., 1996; van der Meulen and Carter, 1995; van der Meulen et al., 1993), both intrinsic (the “biological” component) and mechanical (the “mechanobiological” component) influences are important for the normal growth and development of long bone diaphyses. Mechanobiologic influences on bone development include intermittent strains generated by functional loading and growth in body mass, which are proposed to exert an increasingly important influence on bone geometry as development continues.

Thus, genetics, nutrition, hormones, and mechanical loading all have a significant and interactive influence on the growth, development and maintenance of bone, although

the relative importance of these factors is not yet fully understood. The current project focuses specifically on ontogenetic variation in several features of bone structure which have been shown to relate to growth – related aspects of life history (primary bone microstructure) and skeletal function (cross-sectional geometry and secondary intracortical remodeling). These features and their significance are briefly discussed below, and will be considered in further detail in subsequent chapters.

The influence of life history: primary bone microstructure

There is a long tradition of examining variation in primary bone microstructure as a source of information about aspects of life history, particularly relating to organismal growth patterns (e.g., Amprino, 1947; Castanet et al., 1993; Klevezal, 1996; Peabody, 1961; Ricqlès, 1993; Ricqlès et al., 1991). This approach has figured prominently in palaeobiological studies of dinosaurs and extinct avians, where it has yielded important insight into such issues as their growth rates and durations, the developmental basis for major changes in body size throughout their evolutionary history, and the degree to which their growth patterns may have been influenced by environmental factors (recently reviewed by Chinsamy-Turan, 2005; and Erickson, 2005). This research relies on an empirical relationship between the microscopic organization of primary bone tissues and the rates at which they are deposited. First, variation in the microscopic organization of primary bone tissue types has been shown to reflect variation in bone depositional rates, from fast to slow. Second, temporary disturbances in bone growth are also recorded in features of bone microstructure, such as annuli and resting lines.

Bone growth patterns and primary bone tissue types. Amprino (1947) was the first to propose a relationship between the microstructural organization of primary (particularly periosteal) bone tissues and bone growth rates, with more loosely-organized woven bone tissues being characterized by faster depositional rates than more highly-ordered lamellar bone tissues. Thus, variation in bone microstructural organization was suggested to reflect variability in bone depositional rates. This hypothesis, often referred to as ‘Amprino’s rule’, has since been confirmed by both comparative (Amprino and Godina, 1947; Enlow and Brown, 1956, 1957, 1958) and experimental studies (see reviews by Castanet et al., 1993; Ricqlès et al., 1991) of bone histology across vertebrates. This relationship between primary bone microstructure and bone growth rate is reflected in (1) the organization of the collagen fiber matrix and (2) vascular patterns of bone.

With respect to the former, Amprino (1947) observed a transition in the organization of the collagen fiber matrix of bone across different growth stages within taxa. He noted that deposition of loosely-organized woven-fibered bone coincides with periods of rapid growth during early ontogeny, in which large quantities of bone tissue are required to build the skeleton. This is followed by a transition to deposition of parallel-fibered bone, and then more highly-organized lamellar bone, as rates of body growth decrease. Further, he observed that animals characterized by different patterns of body growth (e.g., antelopes vs. dogs, and among the latter, small breeds vs. large breeds) are characterized by differences in the pattern and timing of this succession of primary bone tissue types.

Recent efforts have employed fluorescent vital-labeling techniques to obtain quantitative measurements of bone depositional rates which can be related to patterns of

bone microstructural organization (Castanet et al., 1996; 2000; Margerie et al., 2002; 2004; Newell-Morris and Sirianni, 1982; and earlier studies reviewed by Ricqlès et al., 1991). Systematic studies have focused on relatively few vertebrate species to date, and have been particularly scarce in mammals. However, they have provided experimental support for the ideas originally proposed by Amprino. According to a review (Ricqlès et al., 1991), depositional rates of $0.26 - 5 \mu\text{m} / \text{day}$ have been observed for avascular woven-fibered bone. Depositional rates for avascular periosteal parallel-fibered bone are reportedly in the general range of $0.10 - 0.5 \mu\text{m} / \text{day}$, and avascular periosteal lamellar bone depositional rates are $< 0.05 - 0.3 \mu\text{m} / \text{day}$ (Ricqlès et al., 1991; see Table 1, p. 32-33, and references therein).

More subtle variability in bone depositional rates may also be discriminated within more broadly defined bone tissue ‘types’. For instance, in a vital labeling study of bone growth in laboratory rats during space flight, Bromage and colleagues (Bromage et al., 2002; Bromage et al., 1998) demonstrated a circadian periodicity in the formation of individual bone layers in lamellar bone. They also identified variability in the widths of individual lamellae coincident with variation in bone depositional rates, in association with changes in gravitational conditions.

Evidence also indicates that variation in the vascularity of primary cortical bone tissues reflects variation in bone growth rates, with more highly vascularized tissues being deposited at faster rates compared to less vascularized tissues (also reviewed by Ricqlès et al., 1991). This has been most clearly demonstrated recently in a series of systematic studies of extant avians by Castanet and colleagues (Castanet et al., 1996; 2000; Margerie et al., 2002; 2004). According to these studies, vascular fibro-lamellar

tissues are the most rapidly growing primary bone tissues, generally deposited at rates greater than 5 – 10 μm / day, compared to less vascular and lamellar-zonal tissues.

Depositional rates as high as 171 μm / day have been recorded for a type of fibro-lamellar bone observed in king penguin chicks (Margerie et al., 2004). Features of the vascular canal network which have been studied in relation to bone depositional rates include vascular canal density, size, and orientation (Castanet et al., 1996; 2000; Margerie et al., 2002; 2004; Starck and Chinsamy, 2002). This is discussed in greater detail in Chapter 5 of this thesis.

Growth marks in bone: annuli and lines of arrested growth. In accordance with this relationship between bone depositional rate and bone microstructural organization, regular variations in bone growth rates (e.g., owing to seasonal growth rhythms) are also recorded in microscopic features of bone. Peabody (1961) published an extensive comparative examination of what he referred to as “annual growth zones” in the bones of living and extinct vertebrates, which were attributed to patterns of cyclical growth. As a result of his work and research by others, including experimental approaches, three types of growth marks are recognized histologically in the bone tissues of vertebrates (for recent reviews, see Castanet et al., 1993; Francillon-Vieillot et al., 1990b; Klevezal, 1996). (1) *Zones* represent periods of rapid growth, and they are typically comprised of more vascular and / or less ordered primary bone tissue types (e.g., woven-fibered or parallel-fibered bone). (2) *Annuli* represent periods of slow growth, most commonly being comprised of less vascular lamellar bone. (3) *Resting lines* (also commonly referred to as *lines of arrested growth*) are cement lines, which represent temporary

cessations of bone growth.

Annuli and resting lines have been observed in the bone cortices of a variety of vertebrates, where they are usually attributed to environmentally-mediated growth rhythms (for reviews, see Castanet et al., 1993; Chinsamy-Turan, 2005; Klevezal, 1996). Their annual occurrence in many organisms has been established experimentally through the implementation of mark-release-recapture studies, fluorescent vital labeling and known-aged reference samples. In cases where the periodicity of these marks is known, information about other aspects of life history can also be discriminated from their occurrence, such as age at death, growth duration, and periods of physiologic stress (e.g., Castanet et al., 2004; Castanet et al., 1993; Francillon-Vieillot et al., 1990a; Klevezal, 1996; Klevezal and Fedyk, 1978).

Mechanically-adaptive changes in bone structure: growth remodeling and secondary intracortical remodeling.

While intrinsic growth patterns are primary determinants of the rates of bone growth, mechanical factors are also important determinants of the manner in which the appropriate form of a given bone is acquired. As discussed above, bone remodeling during growth may entail differential rates of bone deposition across different regions of the same bone, as well as local destruction of tissues deposited during earlier growth stages. These local variations in bone growth and remodeling processes are thought to produce changes in whole bone structure which are mechanically appropriate (e.g., Mosley and Lanyon, 1998; Mosley et al., 1997).

Considerable attention has focused on mechanical adaptation as an important

determinant of bone structure. This is considered below, focusing on the role of growth remodeling and secondary intracortical remodeling as primary mechanisms by which adaptive changes in bone structure are achieved. This discussion relies on a foundational understanding of the mechanical properties of bone, which are reviewed in Appendix A of this thesis.

Mechanical adaptation. The concept of functional or mechanical adaptation is based on several principles that originated in the nineteenth century (following reviews by Dibbets, 1992; Martin et al., 1998; Roesler, 1987; and Ruff et al., 2006). (1) The idea that bone structure is appropriately suited to its functional loading environment is attributed to Julius Wolff (1892; translated by Maquet and Furlong, 1986), who proposed that there is a mathematical correspondence between the orientation of cancellous trabeculae and principle stress trajectories in long bones. (2) Wilhelm Roux formulated the idea that bone adaptation results from a dynamic interaction between the functional loading environment and bone structure, regulated by bone cells which themselves are sensitive to local functional stimuli and have the ability to effect changes in bone structure. (3) A third idea is that of optimization, namely that bone structure reflects a compromise between the conflicting demands of tissue economy (i.e., the requirement to minimize bone mass for metabolic reasons) and bone strength (i.e., the requirement to minimize risk of fracture).

In recent decades, a variety of experimental approaches and animal models have been used to characterize mechanically-adaptive changes in bone (for recent reviews and/or critical evaluations of these studies, see the following: Bertram and Swartz, 1991;

Burr et al., 2002; Forwood and Turner, 1995; Martin et al., 1998; Robling et al., 2001; and Ruff et al., 2006). Studies have examined not only the manner in which bone structure is altered in response to changes in mechanical loading conditions, but also its ability to repair damage induced by fatigue (for the latter, see review by Martin et al., 1998). These studies have identified *strain* as the primary mechanical stimulus evoking adaptive changes in bone. It is clear that strains engendered during cyclic or intermittent loading are more important than those engendered during static loading. Further, in addition to peak strain magnitude and unusual strain distributions, other aspects of the strain stimulus are also important; the osteogenic response to increased strains is enhanced by loading at higher strain rates, strain frequencies, and by introducing recovery periods between loading bouts. These observations suggest that dynamic behaviors are likely to be particularly important in eliciting adaptive alterations in bone structure.

The manner in which bone cells sense and respond to strain-related stimuli has received a great deal of attention (see recent reviews by Burger and Klein-Nulend, 1999; Cowin, 2002; Cowin et al., 1991; Duncan and Turner, 1995; Ehrlich and Lanyon, 2002; Lanyon, 1993; and Pearson and Lieberman, 2004). The location of osteocytes within the matrix of bone, and their ability to communicate with each other and with cells at the bone surface, make them ideally suited for sensing mechanical stimuli and conveying that information to effector cells (i.e., osteoblasts and osteoclasts). Other cells of the osteoblast lineage, particularly bone-lining cells, also appear to be capable of sensing and responding to mechanical strain. Mechanical loading produces a heterogeneous distribution of strains throughout bones, and evidence suggests that local strain gradients

influence the flow of interstitial fluid through the osteocyte lacunar – canalicular network. This, in turn, creates stimuli for mechano-sensation by osteocytes, such as electrical streaming potentials and shear stress in the osteocyte cell membrane. However, the exact mechanisms by which these mechanical signals may be detected by osteocytes (e.g., stretch-activated ion channels, changes in the cytoskeleton at cell-matrix attachment sites) are still being elucidated.

Studies have also focused on the manner in which mechanical stimulation results in a coordinated response by bone cells. Following mechanical loading, osteocytes (and bone lining cells) are known to exhibit changes in energy metabolism and production of factors which promote the recruitment, differentiation and functioning of osteoblasts (Burger and Klein-Nulend, 1999; Duncan and Turner, 1995; Ehrlich and Lanyon, 2002; Pearson and Lieberman, 2004). Regulation of bone resorption in response to mechanical stimuli is not entirely understood, although osteoblast-lineage cells are known to play an important role (Boyle et al., 2003; Mackie, 2003; Majeska, 2001; Rodan and Martin, 1981). It has been proposed that osteocytes secrete factors which inhibit initiation of secondary remodeling by bone lining cells (Martin, 2000), and that osteoblast-lineage cells inhibit bone resorption by osteoclasts (Rodan and Martin, 1981). Accordingly, loss of osteocyte viability and its inhibitory signaling may provide a mechanism for the initiation of secondary remodeling in conditions of disuse (if mechanical strain promotes the flow of nutrients and waste products in the interstitial fluid), and for the targeting of osteoclasts to areas of accumulated fatigue damage (Burger and Klein-Nulend, 1999; Lanyon, 1993; Martin, 2000; Pearson and Lieberman, 2004). In support of this, studies have shown that fatigue damage is associated with localized osteocyte apoptosis,

disruption of the lacunar-canalicular network, and changes in interstitial fluid flow (Bentolila et al., 1998; Chamay, 1970; Schaffler et al., 1996; Tami et al., 2002; Verborgt et al., 2000). Localized patterns of osteocyte apoptosis observed in actively growing bone suggest that such a mechanism may also be involved in regulation of bone cell activities during growth remodeling processes as well (Noble et al., 1997).

Predictive models proposed to characterize the adaptive response of bone have been largely based on the premise that *in vivo* strains serve not only as a *stimulus*, but also as a primary *objective* of adaptive bone remodeling (see Lanyon, 1987). According to *dynamic strain similarity* and similar models (Biewener, 1991; Biewener and Taylor, 1986; Lanyon, 1987; Rubin and Lanyon, 1982; Rubin and Lanyon, 1984), changes in bone architecture occur as part of a larger adaptive process (i.e., accompanied by changes in limb posture and diaphyseal curvature), which has the objective of maintaining peak bone strains and safety factors within an acceptable range appropriate for normal mechanical usage. (What is deemed “appropriate” is likely genetically-determined.) This can be most optimally achieved if the manner of loading is also similar throughout an animal’s range of locomotor behaviors. This model is supported by experimental observations indicating that peak bone strains (or stresses) experienced at functionally-equivalent sites during locomotor behaviors are similar across vertebrates of widely varying body size (Biewener and Taylor, 1986; Biewener et al., 1988; Rubin and Lanyon, 1982; Rubin and Lanyon, 1984), with peak compressive strains ranging from 2,100 – 3,200 $\mu\epsilon$ in most animals studied (Rubin and Lanyon, 1984). Dynamic strain similarity is also an objective of alterations in bone structure during growth (Biewener et al., 1986), although recent studies have shown that bones may operate at higher safety factors (and

thus exhibit lower peak strain magnitudes) during early ontogeny to compensate for the less developed locomotor skills of young juveniles (Main and Biewener, 2004, 2006).

According to the dynamic strain similarity model, loading-induced changes in strain distribution and local strain magnitudes (resulting in local peak strains that fall above or below this acceptable range) are suggested to be particularly important stimuli for bone adaptation. Growth remodeling at periosteal and endosteal surfaces is thought to be the primary mechanism by which bone adapts to such changes, as it serves to locally adjust bone quantity and distribution to restore optimum strain conditions. However, secondary intracortical remodeling may also be necessary to repair accumulated fatigue damage and to maintain an acceptable fatigue life (Lanyon, 1984; Lanyon, 1987; Lanyon and Baggott, 1976; Rubin and Lanyon, 1985).

The *mechanostat* model proposed by Frost (Frost, 1986, 1990a, 1990b, 1992, 2001, 2003) is also based on the premise that adaptive changes in bone serve to maintain *in vivo* strain magnitudes within threshold limits optimized for normal mechanical loading. However, this model makes explicit predictions concerning the manner in which *both* growth remodeling (at periosteal, endosteal and cancellous surfaces; termed ‘modeling’ by Frost) and secondary remodeling (at periosteal, endosteal, cancellous and intracortical surfaces) act in concert to maintain equilibrium strain conditions. Initiation of secondary remodeling is predicted to increase under two mechanical usage conditions, defined by minimum effective strain thresholds: (1) *disuse* (*in vivo* strains $< \sim 50\text{-}100\ \mu\epsilon$, possibly as high as $400\ \mu\epsilon$), where it serves to decrease bone mass and increase strains to optimum levels; and (2) *pathologic overload* (*in vivo* strains $> \sim 3000\ \mu\epsilon$), where it is a microdamage-initiated response and serves to prolong fatigue life. Growth remodeling is

predicted to be initiated (and secondary remodeling decreased) under (3) *mild overload* conditions ($1,000-1,500 \mu\epsilon < \text{in vivo strains} < \sim 3,000 \mu\epsilon$), where it serves to increase bone strength and reduce peak strains to acceptable levels. Bone strains are said to fall within the (4) *adapted* mechanical usage window between 100 and $1,500 \mu\epsilon$, where growth remodeling is not initiated and secondary remodeling proceeds at baseline rates (determined by systemic factors).

The implication of the mechanostat model is that growth remodeling at endosteal and periosteal surfaces is the primary adaptive mechanism for increases in mechanical loading, while secondary remodeling serves two roles; it is the primary adaptive mechanism for decreases in mechanical loading, and for prolonging fatigue life in regions of abnormally high strains. Experimental studies have provided support for many predictions of the mechanostat model (e.g., reviewed by Forwood and Turner, 1995; Martin et al., 1998). However, inconsistencies include observations that secondary remodeling is not limited to its mechanical thresholds, namely that it may also be initiated in response to high strain magnitudes during normal functional activities (Bouvier and Hylander, 1996; Lanyon and Baggott, 1976; Lanyon et al., 1979; Lieberman et al., 2003; Martin et al., 1998).

Characterization of the adaptive response to changes in mechanical loading is complicated by several factors. For instance, site-specific differences in strain thresholds and in the growth and secondary remodeling response to alterations in mechanical loading suggest that genetic factors may play a role in determining appropriate strain levels at a local level. Further, adaptive responses may be coordinated across skeletal sites to achieve more global adjustments in skeletal (or whole bone) architecture

(Biewener et al., 1986; Hsieh et al., 2001; Lieberman et al., 2003; Mosley and Lanyon, 1998; Mosley et al., 1997; Rubin and Lanyon, 1987). In addition, the adaptive response of bone varies with age, with changes in mechanical loading thought to have a greater influence on bone structure during growth compared to later in life (Bertram and Swartz, 1991; Forwood and Burr, 1993; Lieberman et al., 2003). Some of these issues have been addressed recently in a model proposed by Lieberman and colleagues (Lieberman et al., 2003; Pearson and Lieberman, 2004).

Growth remodeling and cross-sectional geometry. Bone growth remodeling is thought to be the primary mechanism by which bones adapt to increases in mechanical loading, although its responsiveness declines after skeletal maturity is reached (Frost, 1990a; Martin et al., 1998; Pearson and Lieberman, 2004). Remodeling responses at different bone envelopes (i.e., endosteal and periosteal) also appear to vary in their sensitivity to changes in mechanical loading with age. The periosteal surface is suggested to be more responsive during childhood and early adolescence, while the endosteal surface is more responsive during later adolescence and adulthood in humans (Lieberman et al., 2003; Pearson and Lieberman, 2004; Ruff et al., 1994; but see Woo et al., 1981).

The study of cross-sectional geometry provides a useful means of quantifying the consequences of remodeling at periosteal and endosteal surfaces during growth for attributes of bone shape and size having mechanical relevance (Huiskes, 1982). Based on principles of engineering beam theory, several cross-sectional geometric properties (characterizing the amount and distribution of bone around a given cross-section) are proportional to the resistance of long bones to different kinds of loading (Biewener, 1992;

Ruff, 1989; Ruff and Hayes, 1983; Wainwright et al., 1976). These properties are discussed in detail in Chapter 4 of this thesis. To briefly summarize, bone strength (i.e., rigidity) can be improved by making several adjustments in bone structural properties: (1) by increasing cortical area, thereby improving resistance to axial loads, and (2) by increasing subperiosteal dimensions, particularly in the axis subjected to the greatest bending strains, thereby increasing resistance to bending and torsional loads (Biewener, 1992; Ruff and Hayes, 1983; Wainwright et al., 1976).

Secondary intracortical remodeling. Secondary intracortical remodeling is less well understood than growth remodeling (and its consequences for cross-sectional geometry) in terms of its influence on bone strength, although it is thought to be the primary mechanism by which adaptive changes in bone material properties are achieved.

Secondary osteonal bone is characterized by reduced material strength under monotonic loading compared to primary bone (Currey, 2002; Martin et al., 1998). This difference may be attributed in part to its consequences for increased porosity, decreased mineralization relative to adjacent older tissue, and the presence of a compliant cement line interface (Burr et al., 1988b; Currey, 1959, 1975; Martin and Boardman, 1993; Martin et al., 1998; Martin and Ishida, 1989; Schaffler and Burr, 1988; Schaffler et al., 1987). However, intracortical remodeling may also result in local improvements in bone material properties by altering the preferred collagen fiber orientation, which has consequences for bone strength under different modes of loading (Bromage and Boyde, n.d.; Martin et al., 1996; Mason et al., 1995; McMahon et al., 1995; Riggs et al., 1993a;

Riggs et al., 1993b). ²

Examinations of the material properties of secondary osteonal bone under cyclic loading have reached varying conclusions (Carter and Hayes, 1976; Carter et al., 1976; Evans and Riolo, 1970; Gibson et al., 2006; Schaffler et al., 1990), although the prevailing view is that it improves the resistance of bone to fatigue failure. It is thought to do this in two main ways (reviewed by Martin et al., 1998). First, cement lines and inter-lamellar boundaries serves as microstructural interfaces, which are thought to be particularly well suited for slowing or preventing the spread of fatigue-induced microcracks. Second, intracortical remodeling is suggested to function in repair of cortical regions with accumulated microdamage, thereby extending fatigue life. Secondary intracortical remodeling is discussed in greater detail in Chapter 6 of this thesis.

BRIEF SUMMARY OF NONHUMAN PRIMATE STUDIES

Structural properties of long bone diaphyses have been examined in a variety of nonhuman primate taxa, in an attempt to assess the influence of body size and skeletal function on diaphyseal geometry. Previous studies by other researchers have shown that a number of cross-sectional geometric properties vary with respect to positional behavior across a broad range of adult primates. However, postnatal development of diaphyseal cross-sectional geometry has received relatively little attention. The few studies that have been undertaken thus far indicate that ontogenetic variation in diaphyseal geometry

² The influences of mineralization, porosity, collagen fiber orientation and other variables on bone material strength have been discussed in several recent reviews (Bromage et al., 2003; Currey, 2002; Martin et al., 1998).

reflects the influence of both growth-related increases in body size and behavioral changes as immatures acquire adult postural and locomotor skills. These studies are reviewed in Chapter 4 of this thesis.

Cortical bone microstructure has been comparatively unexplored in long bone diaphyses of nonhuman primates. Primates were included in a number of broadly comparative investigations of vertebrate bone histology conducted in the nineteenth and first half of the twentieth century. Although many of these studies suffered from a number of limitations, they nevertheless demonstrated that vertebrates in general, and primates specifically, exhibit substantial variation in secondary remodeling and the microscopic organization of their primary bone tissues. However, the few systematic studies that have been undertaken in recent decades have focused to a large extent on secondary intracortical remodeling, or other aspects of cortical bone microstructure and composition (not explicitly considered in the current thesis) of interest for their mechanical significance (e.g., studies of bone mineral density, reflecting tissue mineralization and porosity, and studies of collagen fiber orientation: Bromage et al., 2003; Burr, 1979; Carlson, 2002; Jowsey, 1966; Kikuchi et al., 2003; Sumner et al., 1989; Warshaw et al., 2002). Ontogenetic and taxonomic variation in primary bone microstructure and its significance for life history has been largely unexplored, with a few notable exceptions. Previous studies of primary bone microstructure and secondary intracortical remodeling in nonhuman primates are reviewed in Chapters 5 and 6 of this thesis.

SUMMARY

This chapter presented a general background on bone biology. This was followed by a discussion of determinants of bone macro- and micro-scopic structure, focusing particularly on the manner in which aspects of bone structure reflect the influence of 1) individual growth patterns (namely, primary bone tissue types), and 2) mechanical loading history (namely, cross-sectional geometry and secondary intracortical remodeling). A brief summary was presented of prior research on the structural and microstructural characteristics of nonhuman primate diaphyseal bone. This research is reviewed in more detail in following chapters.

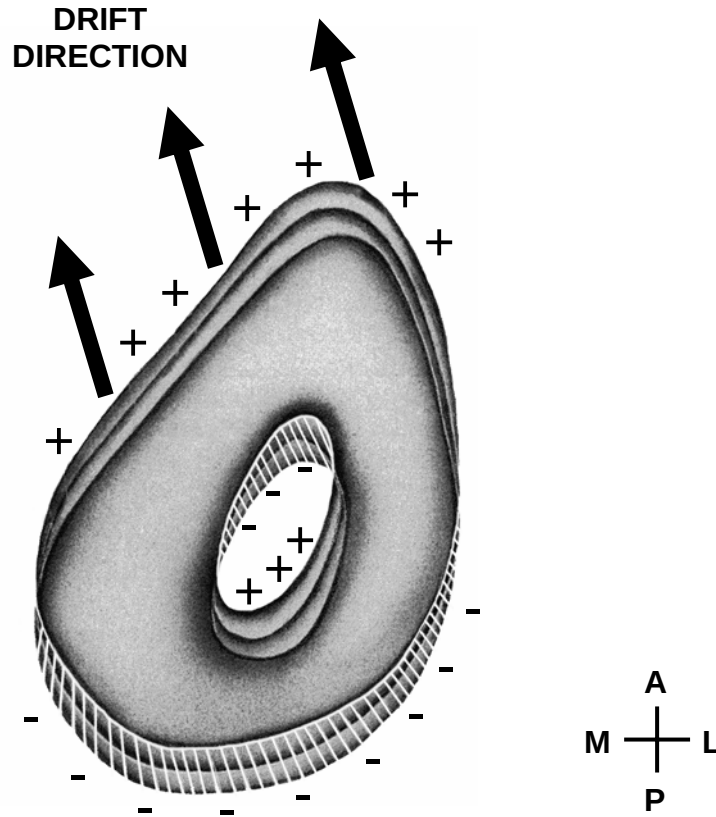


FIGURE 2-1. Cortical drift. In this generalized representation, bone area is shaded gray, while previously-resorbed bone regions are represented by hatched lines. Cortical drift in the direction indicated by the arrows is accomplished by a coordinated pattern of bone deposition (+++) and bone resorption (---) on complementary bone surfaces. Bone deposition occurs on surfaces facing the direction of cortical drift, i.e. on the periosteal surface of the anteromedial cortex and on the endosteal surface of the posterolateral cortex. Concurrently, bone is resorbed on surfaces facing away from the direction of cortical drift.

Modified from Enlow, DH (1963) *Principles of Bone Remodeling: An Account of Postnatal Growth and Remodeling Processes in Long Bones and the Mandible*. Springfield: Charles C. Thomas. Pp. 131.

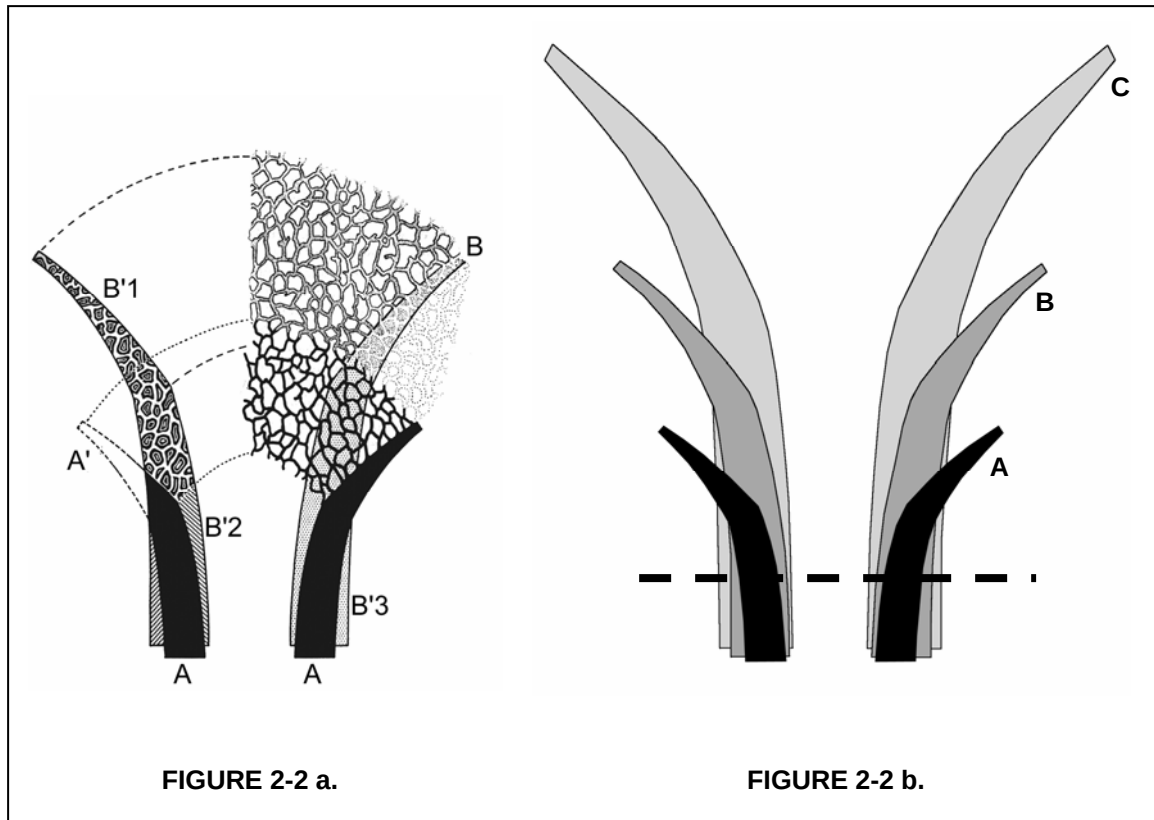


FIGURE 2-2. Metaphyseal remodeling and relocation.

FIGURE 2-2 a. Metaphyseal remodeling. Original bone areas are represented in solid black (A), while new bone areas are stippled or hatched (B). Metaphyseal remodeling is accomplished by resorption at the periosteal surface (A') and deposition at the endosteal surface (B). As a result of this process, the diameter of older metaphyseal tissue is reduced so that it can be incorporated into the elongating diaphysis. Deposition of bone at the endosteal surface results in formation of compacted coarse cancellous bone where deposition occurs in regions previously occupied by coarse cancellous trabeculae (B'1). If cancellous trabeculae are not present, deposition at the endosteal surface may form endosteal circumferential bone (B'2). During later growth stages as diaphyseal dimensions enlarge, bone may be deposited at the periosteal surface (B'3). Bone in endosteal regions may also be subject to continued remodeling.

FIGURE 2-2 b. Relocation of cortical levels. With continued linear bone growth and remodeling, cortical levels (- - -) that were once part of the metaphysis during earlier stages of development (A) are sequentially relocated to become part of the elongating diaphysis during later stages of development (B and C).

Modified from Enlow, DH (1963) *Principles of Bone Remodeling: An Account of Postnatal Growth and Remodeling Processes in Long Bones and the Mandible*. Springfield: Charles C. Thomas. Pp. 131.

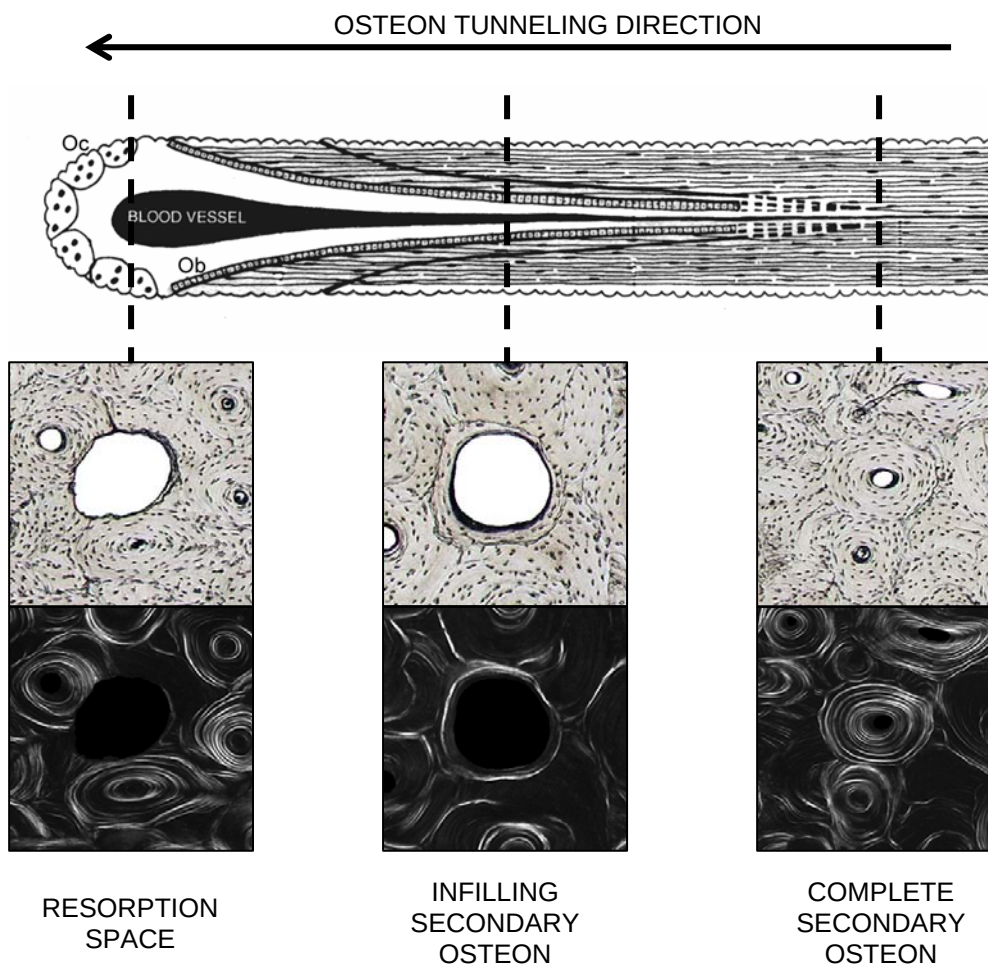


FIGURE 2-3. Secondary osteon formation. Secondary remodeling at intracortical surfaces results in the formation of a cylindrical structure, termed a *secondary osteon* or *Haversian system*, as depicted here. LM and CPL images of different stages in this process are also provided.

Left: Newly-differentiated osteoclasts (Oc) of the BMU tunnel through existing bone in a predominantly longitudinal direction, creating a resorption space. The BMU brings with it a growing capillary loop. *Middle:* The formation phase begins with the appearance of differentiated osteoblasts (Ob) around the surfaces of the resorption space. Inward concentric deposition of lamellar bone begins, infilling the space up to the blood vessel wall. *Right:* A Haversian canal is left in the center of the completed secondary osteon, for passage of blood vessels and nerves.

Modified from from Roberts WE, Turley PK, Brezniak N, and Fielder PJ. (1987) Bone physiology and adaptation. *Calif. Dent. Assoc. J.* 15(10):54-61. Reprinted in Enlow DH and Hans MG. (1996) *Essentials of Facial Growth*. Philadelphia: WB Saunders Company. Pp. 303.

CHAPTER 3.

MATERIALS AND METHODS

This chapter describes the sample utilized in the current study and the criteria that were prioritized in the selection of appropriate specimens for study. This is followed by a detailed description of the specimen preparation methodology, including sample acquisition, preparation and embedding, and histological thin-section preparation. Finally, the imaging methods are described, and a brief overview is presented of those image processing techniques common to the separate analyses performed as part of this dissertation.

DESCRIPTION OF THE SAMPLE

This project utilized midshaft bone samples collected from the femur and humerus of *Chlorocebus aethiops* (vervet monkeys, $N=32$) and *Hylobates lar* (white-handed gibbons, $N=28$) and the midshaft femur of *Pan troglodytes* (common chimpanzees, $N=12$). These taxa were selected to provide a focused comparative context for the investigation of ontogenetic variability in bone microstructural features of catarrhine primates and its relationship to aspects of life history, namely taxon-specific growth patterns. Due to the nature of the sampling protocol to prepare histological thin sections, specimen availability limited the taxonomic scope and element representation of the current study sample. Nevertheless, this study represents the first systematic investigation of ontogenetic and regional variability in several features of bone macro- and microscopic structure across a range of catarrhine primates characterized by varying

life histories. Table 3-1 a summarizes characteristics of growth and life history in the study taxa, which are most relevant to the current study. Table 3-1 b summarizes the most common locomotor behaviors characterizing adult members of each taxon, in order of their relative frequencies (reflected in % of total locomotor time spent in each behavior).

Priorities in the selection of appropriate specimens for study

Several criteria were prioritized in the selection of appropriate collections from which specimens would be obtained for study.

1) Collections comprising wild-caught individuals from restricted geographic areas were prioritized. Development in some wild primate populations has been shown to be delayed relative to captive conspecifics (e.g., Altmann and Alberts, 1987; Hamada et al., 1996; Phillips-Conroy and Jolly, 1988; Zihlman et al., 2004). Variability in growth strategies has also been documented among wild populations (e.g., Strum, 1991; Turner et al., 1997) and among artificially provisioned groups (e.g., DeRousseau, 1990; DeRousseau and Reichs, 1987; Fauchaux et al., 1978; Hamada, 1994; Hamada et al., 1986; Marzke et al., 1996) living under different environmental conditions. The current study reduced these potential sources of variability in life history and growth strategies by focusing on wild-caught individuals from restricted geographic areas within each species sample. Further, with the inclusion of additional samples in future studies, inter-population comparisons among wild-caught animals can also be undertaken to examine variability among individuals living under different ecological conditions.

2) Collections comprising individuals with reasonably complete skeletons and

representing all ages were prioritized, so that bone microstructural variables suggested to reflect skeletal growth rate variability could be examined within the context of more traditional measures of postnatal growth. For each species sample, individuals were chosen to meet several criteria as closely as possible: A) presence of relatively complete dental and postcranial remains, making possible the estimation of biological age (from alveolar dental emergence and epiphyseal union) and the collection of linear skeletal measurements; B) equal distribution of individuals among different age classes throughout the postnatal growth period (discussed below); C) equal distribution of males and females; and D) associated body mass.

3) Since the goal of this study was to examine variability in bone microstructure related to growth and development, only immature individuals and non-geriatric adults were included. Geriatric adults were excluded on the basis of extreme tooth wear and evidence of osteoarthritis. Individuals showing obvious evidence of skeletal fractures and pathologies (as described by Schwartz, 1995; White, 1991) were also avoided.

Where possible, long bones with broken proximal or distal ends were chosen for study in an effort to minimize the impact of this research on valuable skeletal collections. In these situations, bone length and sampling location (midshaft) were estimated based on the presence of a complete contra-lateral element. Despite such efforts, however, both the invasive nature of the sampling protocol required for histological investigation and the importance of the above selection criteria meant that both the taxonomic range and appropriate samples available for this study were limited. While it was generally possible to meet these criteria for the *Chlorocebus aethiops* and *Hylobates lar* samples, many criteria were difficult to meet when selecting appropriate material to comprise the *Pan*

troglydytes sample. Further, as fewer *P. troglodytes* humeri were available for sampling, only the femur was examined in the current study. However, the sample available for this study still provides the first such data on ontogenetic variability in bone microstructure and its relationship to life history in this species. Details of samples utilized in this study are described further below.

Classification of biological age.

Establishment of a chronological framework is critical to the investigation of ontogenetic variability in bone microstructure and its relationship to life history. In the absence of chronological age information for individuals examined in the current study, however, it was necessary to rely on other criteria to estimate biological age. This was important both in the selection of materials for study, to ensure that the study samples were broadly representative of the entire postnatal growth period, and for the interpretation of patterns of bone microstructural development both within and between species.

Dental development as a proxy for biological age. Dental alveolar emergence was chosen as the most appropriate criterion for estimating biological age in the current study for several reasons. While dental development is accelerated in some primates living in captivity relative their wild conspecifics (Phillips-Conroy and Jolly, 1988; Zihlman et al., 2004), it is considered to be highly heritable and less resistant to nutritional and environmental influence than other aspects of somatic (i.e., skeletal development and body mass growth) and reproductive maturation (Garn et al., 1965a; Garn et al., 1965b;

Lewis and Garn, 1960; Marzke et al., 1996; Murchison et al., 1988). Additionally, aspects of dental development have been shown to correlate with other timed life history events (e.g., age at weaning, age at first reproduction, longevity), as well as size-related markers of physical maturation (e.g., brain size), across primates (Macho, 2001; Smith, 1989, 1991, 1992). Age at emergence of the first permanent molar, in particular, is highly correlated with life history variables and scales isochronically with age at weaning. Finally, dental eruption can be readily observed and is frequently preserved in the fossil record. In the absence of chronological age information, dental development therefore serves as a good proxy for establishing a timescale for somatic growth and life history in the study taxa, and it extends the applicability of this research to paleontological contexts.

In this study, individuals were grouped into five biological age classes based on alveolar dental emergence and long bone epiphyseal union. (Table 3-2). Individuals having emergent deciduous teeth and lacking molars in the process of eruption were assigned to the ‘Infant’ (Deciduous) biological age class. For older age classes, it is noted that the age at eruption of second and third permanent molars scale isochronically with age at eruption of the first permanent molar (an important life history attribute), while replacement teeth show differences in their eruption timing relative to the molar teeth among primate taxa (Smith, 1989, 1992). Thus, individuals were assigned to ‘Juvenile’ (M1, M2, and M3, respectively) biological age classes on the basis of alveolar emergence of the permanent molars. Individuals continue skeletal development after full eruption of the dentition (e.g. personal observations, and Bolter and Zihlman, 2003; Watts, 1990). Thus, individuals were only considered ‘Adult’ after complete fusion of all

long bone epiphyses. (Methods for assessment of alveolar dental emergence and long bone epiphyseal union are described in further detail below.)

At times, it was desirable to further refine this biological age classification, for the purpose of establishing the relative ages of individuals within biological age classes (particularly within age classes based on molar emergence) as defined above and for visualizing growth-related increases in variables over biological time *within* taxa. In these instances, individuals were further grouped into eight biological age classes according to the classification provided in Bolter and Zihlman (2003) for *C. aethiops*. (Table 3-3). Use of this biological age classification system also allowed comparison between the current study and previous studies of growth and development undertaken on other wild *C. aethiops* samples (Bolter and Zihlman, 2003; Turner et al., 1997). As *P. troglodytes* also shares the same sequence of dental eruption and the dental chronology is known (Schultz, 1935; Smith et al., 1994), this biological age classification was also applied to this taxon.

Assessment of dental eruption and epiphyseal union. Alveolar dental eruption and long bone epiphyseal union status were assessed according to the methods of Wintheiser et al. (1977) and Bolter and Zihlman (2003), which are appropriate for skeletal samples without soft tissues preserved. Each representative (right, left, maxillary, mandibular) of a given tooth type and both epiphyses (right, left) were initially assigned a score of 1, 2, or 3 to indicate the stage of eruption or union:

Dental eruption:

- 1 = tooth not visible above the level of the alveolar margin (unerupted);
- 2 = emergence of any part of a tooth cusp or crown above the level of the alveolar margin, but not yet reaching the occlusal plane (emergence or partial eruption);
- 3 = tooth at the occlusal plane (completely erupted).

Epiphyseal union:

- 1 = epiphysis completely separate (unfused);
- 2 = from the formation of the first visible bone spicules connecting the epiphysis to the metaphysis, and including a state of fusion where a small opening (with no visible bone spicules) is still present at the site of union (partial fusion);
- 3 = no visible opening at site of union, including the presence of an epiphyseal union scar (completely fused).

In many cases, adhering soft tissue from the joint capsule made it difficult to determine whether an epiphysis was completely unfused or had begun the process of fusion. Thus, epiphyseal fusion scores were converted to a two-stage classification, similar to that of Washburn (1943) and Cheverud (1981).

- 1 = epiphysis completely unfused, or in the process of fusion (corresponding to a score of '1' or '2' above)
- 2 = epiphysis completely fused (corresponding to a score of '3' above).

Assignment of individuals to biological age classes. Specifically, individuals in the current study were assigned to a biological age class on the basis of the most advanced (in terms of its stage of eruption) representative of each tooth type (e.g., Schultz, 1935). For example, those individuals with at least one deciduous tooth emergent / in the process of eruption (i.e., having a score of '2' or '3', as described above) and lacking emergent first permanent molars (M1; all four representatives not yet emerged and having a score

of '1') were assigned to the 'DECID' biological age class. Similarly, individuals with at least one emergent M1 were assigned to the 'M1' biological age class. Individuals with at least one emergent M2 were assigned to the 'M2' biological age class. Individuals with at least one emergent M3 were assigned to the 'M3' biological age class.

Individuals were only assigned to the 'EPIPH' age class and considered 'adult' on the basis of complete eruption of the permanent dentition (all teeth having a score of '3') and complete fusion of all long bone epiphyses (all epiphyses having a score of '3')¹.

Among those 'Adult' individuals with fully erupted dentitions and fully fused long bones, geriatric adult specimens were excluded on the basis of tooth wear. Adult individuals were chosen that exhibited only minimal to moderate occlusal wear on the first permanent molars, as reflected in the presence of enamel faceting, progression to dentin exposure in the form of islands on the cusps, and the eventual coalescence of two dentin islands. Individuals with advanced occlusal wear of the first permanent molar, as reflected in the coalescence of three or more dentine islands, the formation of a single dentin basin covering the occlusal aspect of the tooth, or the eventual involvement of the root (e.g., as described by Dean et al., 1992; Phillips-Conroy et al., 2000; Welsch, 1967), were excluded from the study. Individuals showing evidence of osteoarthritis (as described by Schwartz, 1995; White, 1991) were also excluded.

Chronology of dental eruption and epiphyseal union. Due to the scarcity of data describing the chronology of dental eruption in *Hylobates lar*, and even less information

¹ It is noted here that tooth eruption is known to precede completion of root development, often by a substantial margin (Dean and Wood, 1981; Kuykendall, 1996). Thus, individuals assigned to the EPIPH age class may not yet have reached complete dental maturity, as defined by apical closure of the root.

concerning the timing of long bone epiphyseal union in all three taxa studied here, individuals were grouped into the biological age classes presented in Table 3-2 for statistical analysis of intraspecific age-related variability in bone microstructural features. However, where possible, estimation of chronological age is useful when considering the relationship of bone microstructure to other developmental events in life history, such as attainment of adult body size. Table 3-4 presents corresponding estimated chronological ages for each biological age class, based on available gingival emergence chronologies developed from captive animals. These times may underestimate gingival emergence times for wild-collected animals (Phillips-Conroy and Jolly, 1988; Zihlman et al., 2004). Based on observations of alveolar and gingival emergence in *Papio anubis* and *Pan troglodytes* discussed by Kelley and Smith (2003), it is also noted that alveolar emergence, as observed in the current study, may occur approximately three to five months earlier than the gingival emergence ages presented here.

What little is known of dental eruption chronologies for *Hylobates lar* suggests that they complete eruption of their deciduous dentition by six months of age (Rumbaugh, 1965; Smith et al., 1994). Data are particularly scarce for adults. In a histologic study of growth increments in the permanent mandibular teeth of a single specimen of *H. lar*, Dirks (1998) determined an age at death of 2.88 years for an individual having I₁, I₂ and M₁ in occlusion. (C₁, P₃, P₃ and M₂ were developing in their crypts.) Dirks (2003) later determined age at emergence of the third permanent molar in a wild-collected *H. lar* individual to be 6.1 years. Previous estimates based on wild-caught specimens of unknown age suggested that gibbons complete eruption of their permanent dentitions between their seventh and ninth years (Keith, 1931; Schultz, 1956,

1973).

Prediction equations based on brain mass generated by Smith et al. (1994) suggest this age for M3 emergence in gibbons may be later than expected given other anthropoids (i.e., non-hominoids) of similar body size. They reported an expected age at eruption of the first permanent molar at 1.28 years and age at completion of the permanent dentition at 4.80 years for *Hylobates lar*. Their prediction equations were dominated by New World and Old World monkeys, however. Given that gibbons show marked difference in other aspects of life history compared to Old World monkeys of similar body size (e.g., later ages at weaning, later ages at first reproduction, and longer interbirth intervals) (Brockelman et al., 1998; Harvey et al., 1987; Palombit, 1995), older ages at tooth emergence (as determined by Dirks, 1998, 2003) seem reasonable.

A dental eruption chronology for *Chlorocebus aethiops* was provided by Ockerse (1959), based on observations of an unspecified number of captive individuals of known age. Smith and colleagues (1994) called into the question the validity of Ockerse's data, based primarily on suggested inaccuracies in his proposed chronology for the deciduous dentition. Bolter and Zihlman (2003) later revised this chronology based on observations of gingival emergence in a captive colony (based on observations by L. Fairbanks, communicated by Bolter and Zihlman, 2003). Ages at emergence presented by Bolter and Zihlman (2003) are slightly later than predicted ages of eruption presented in Smith et al. (1994). The latter study predicted ages of eruption in *C. aethiops* for the deciduous dentition at 0.31 years of age, and for the first and last permanent teeth at 0.84 and 3.21 years of age, respectively.

Dental eruption schedules in *Pan troglodytes* have been the focus of several

studies based on captive animals of known age (Conroy and Mahoney, 1991; Kuykendall et al., 1992; Nissen and Riesen, 1964; and compiled by Smith et al., 1994). The permanent dentition chronology provided by Nissen and Riesen is shown in Table 4-3, since it includes observations of the third permanent molar in contrast to more recent chronologies. Zihlman and colleagues (2004) recently presented maxillary gingival emergence times for a sample of eighteen skeletonized individuals of known age from the wild. Their gingival emergence times were estimated from observed alveolar emergence, for comparison with chronologies based on captive animals. According to this, Zihlman and colleagues' (2004) estimated gingival emergence times in wild chimpanzees were later than those provided for captive animals, particularly for later-erupting teeth (e.g., M2 and M3). Ages of maxillary gingival emergence for wild chimpanzees as shown by Zihlman and colleagues (2004) are provided in Table 3-5.

To date, systematic studies of long bone epiphyseal union based on skeletal remains of known aged animals are rare in nonhuman primates, with a few exceptions (Cheverud, 1981; Kimura and Hamada, 1990). The majority of investigations of known-aged animals have used radiographic techniques in examinations of the appearance and union of primary and secondary ossification centers (reviewed in Watts, 1986). Among the taxa examined here, only chimpanzees have been the focus of such studies to my knowledge (Kerley, 1966b; Watts, 1971, 1986). In Kerley's (1966b) study, using both radiographic and histologic methods, chimpanzees were found to complete long bone epiphyseal union by fifteen years of age. This is consistent with a recent report by Zihlman and colleagues (2004) based on a cross-sectional sample of known-aged wild chimpanzees, in which the proximal humeral epiphysis (the last long bone epiphysis to

fuse) was reported to fuse between 13.4 and 16.5 years of age.

Description of the sample.

Details of the particular samples utilized in the current study are described below. Sample representation across biological age classes is provided in Table 3-6, and summary data concerning individuals comprising each species sample can be found in Appendix B.

Vervet monkeys (Chlorocebus aethiops). Midshaft femur and humerus bone samples from thirty-two individuals [26 immatures (12F, 14M) and 6 adults (3F, 3M)] were collected for the current study. These individuals belong to a larger sample of wild vervet monkeys collected in southwestern Uganda by Dr. Sherwood L. Washburn over a period of 35 days in 1947 (Bolter and Zihlman, 2003). This collection is curated as part of the Sherwood L. Washburn (SLW) collection by Dr. Adrienne L. Zihlman in the Department of Anthropology at the University of California at Santa Cruz. Because this collection was obtained over a restricted period of time and individuals are associated with body mass and other somatometric data, it has provided a unique and well-represented cross-sectional sample in which aspects of postnatal growth and development have been examined (e.g., Bolter and Zihlman, 2003). In the current study, the taxonomic designation of this sample follows recent systematic revision based on molecular data (Goodman et al., 2005; Tosi et al., 2003; Tosi et al., 2004).

White-handed gibbons (Hylobates lar). Midshaft femur and humerus bone samples from

twenty-eight individuals [22 immatures (10F, 12M) and 6 adults (3F, 3M)] were collected for the current study. Material was obtained from two lending sources: 1) Samples from fourteen individuals [8 immatures (2F, 6M) and 6 adults (3F, 3M)] were obtained from the Dr. Sherwood L. Washburn (SLW) collection, curated by Dr. Adrienne L. Zihlman of the Department of Anthropology at the University of California at Santa Cruz. 2) Samples from the remaining fourteen immature individuals (8F, 6M) were obtained from the Museum of Comparative Zoology, Harvard University (MCZ). All individuals were collected in the Chiangmai region of Thailand in association with the Asian Primate Expedition of 1937. *Hylobates lar* groups in this region were the focus of Carpenter's (1940) field study of this species, and some individuals in the collection have been the focus of previous research on skeletal biology and development by Schultz (1944). Individuals obtained from the MCZ, which span the deciduous through M3 dental eruption stages, have associated recorded body mass. However, body mass data were not available for individuals obtained from the SLW collection, which include most M3 individuals and all adults examined here.

Common chimpanzee (Pan troglodytes). Midshaft femur bone samples from thirteen individuals [10 immatures (5F, 1M, 4 unknown) and 3 adults (1M, 2 unknown)] were collected for the current study. Material was obtained from the Museum für Naturkunde der Humboldt Universität in Berlin (MNH). There is less geographic and temporal control in this sample. Individuals were collected in the early 1900s from Cameroon and Central Africa. Further, individuals available for the current study were not associated with known body mass and in many cases sex. Most individuals in this sample can be

attributed to *P. troglodytes troglodytes* following Groves (2001).

Additional notes concerning the sample. In a few cases, only the femur was present and available for study. Further, four specimens (*C. aethiops* SLW 72 femur, *H. lar* SLW 39 humerus, *H. lar* MCZ 41473 humerus, *P. troglodytes* MNH 83619 femur) showed abnormal histologic structure and evidence of possible pathology or fracture, such as hyper-vascularized regions indicating localized proliferative periosteal reactions (Schultz, 1993, 2003). The humerus of *H. lar* SLW39 showed no clear evidence of pathology, but it was strongly bowed and the pattern of cortical drift was unusual compared to other conspecifics. These elements were derived from individuals showing no obvious external evidence of skeletal pathology and the microstructural organization of the associated femur (for *H. lar* SLW 39 and MCZ 41473) or humerus (for *C. aethiops* SLW 72) appeared normal. In these cases, only the affected element was excluded from the study.

Dental emergence data were not available for three individuals included in this study: *C. aethiops* SLW 101, *H. lar* SLW 10 and *H. lar* SLW 10. All three individuals were assigned to the M3 age class on the basis of bone length and their incomplete long bone epiphyseal fusion status. Further, MNH 35526 was in questionable association with a skull having only first permanent molars erupted and unerupted second permanent molars. This latter individual was reassigned to the M2 age class on the basis of bone length and its incomplete epiphyseal fusion status. (Table 3-6).

BONE SAMPLING AND MEASUREMENT PROTOCOLS

Sampling location

The midshaft femur and humerus were utilized in this study. The midshaft femur

and humerus are commonly included in investigations of bone structural properties and histocompositional variables, making results of the current study more comparable to other published studies (e.g., Burr, 1992; Goldman et al., 2003; Schaffler and Burr, 1984; Schaffler et al., 1985). Further, sites located more proximally or distally along the diaphysis are subject to more destructive growth remodeling processes, such as metaphyseal remodeling in which the periosteal cortex is largely resorptive (pilot observations; Enlow, 1962, 1963).

With respect to this latter point, it is recognized that anatomical midshaft may not coincide with the true developmental midshaft. The proximal metaphysis of the femur and the distal metaphysis of the humerus, respectively, contribute disproportionately to the overall lengthening of these bones during growth (Scheuer and Black, 2000), and it is therefore possible that the sampling location relative to the developmental midshaft may be relocated in morphological space relative to other bone regions during growth (Enlow, 1962, 1963). (This is illustrated in Figure 2-2). Serial lengthwise sectioning of representative elements across age classes would allow for the accurate identification of developmental midshaft based on histological details. While such a destructive sampling protocol was not possible in the current study given the limitations of specimen availability, sampled regions affected by such growth remodeling processes should be recognizable on the basis of histological details characterized previously (Enlow, 1962, 1963).

Whole-bone measurements

Identification of the midshaft of long bones under investigation is usually based

on a measurement of bone length, which includes the diaphysis, metaphyses and epiphyses of a long bone (e.g., Ruff and Hayes, 1983). However, this traditional measure of bone length prevents the inclusion of specimens for which epiphyses are either unfused, broken or otherwise missing. In order to maintain consistency in our definition of midshaft between immature (unfused) and adult specimens, a measure of bone length was developed for the current study which excluded the epiphyses. Following an examination of adult and unfused long bones in a broad range of primates (strepsirrhines, platyrrhines and catarrhines), proximal and distal landmarks were chosen on the basis of their presence at all ages. *Bone length* was then defined as the distance between proximal and distal landmarks. (Figure 3-1).

Femur landmarks – posterior view (Scheuer and Black, 2000; White, 1991):

Proximal:	inferior-most point along the superior surface of the femoral neck, just medial to the greater trochanter metaphyseal surface
Distal:	mediolateral midpoint of the distal margin of the metaphysis or, if present, the mediolateral midpoint of the intercondylar line just superior to the intercondylar fossa

Humerus landmarks – posterior view (Scheuer and Black, 2000; White, 1991):

Proximal:	most superior point of the posterior shaft, just lateral to the site of the anatomical neck and defined by the posterior notch of the proximal epiphysis
Distal:	mediolateral midpoint of the distal margin of the olecranon fossa, just superior to the posterior trochlear articular surface if present

Bone length measurements were collected to 0.5 mm accuracy using a portable osteometric board (Paleo-Tech Concepts, Crystal Lake, IL.) fitted with a moveable laser diode attachment to facilitate landmark-based measurement. The femur and humerus were each positioned on the osteometric board anterior surface down, with the

longitudinal axis of the diaphysis aligned parallel to the long axis of the board. The longitudinal axis of the femur was defined proximally by the mediolateral midpoint of the proximal shaft just inferior to the lesser trochanter, and distally by the mediolateral midpoint of the distal shaft just superior to the expansion of the distal metaphysis. The longitudinal axis of the humerus was defined by the mediolateral midpoints of the shaft just inferior and superior to the expansions of the proximal and distal metaphyses, respectively. In *C. aethiops*, the proximal third of the humeral shaft is flexed posteromedially. Thus, the longitudinal axis of the humerus in *C. aethiops* was defined proximally by the mediolateral midpoint at the approximate midshaft location, and distally by the mediolateral midpoint of the distal shaft just superior to the expansion of the distal metaphysis.

Three independent bone length measurements were taken, in which the bone was removed and then repositioned on the osteometric board, and the average of these measurements was recorded as the final bone length. Midshaft was located at the 50% level of average bone length, and marked with a graphite pencil. The mediolateral midpoint at midshaft was marked with a graphite pencil, to maintain anteroposterior orientation. Where possible, the right-side element was sampled for the current study to maintain a common orientation for image analysis.

In a number of cases, long bones that were either cracked or missing a proximal or distal end were utilized in this study, in an effort to minimize the impact of the semi-destructive sampling protocol on valuable skeletal collections. In these situations, bone length and midshaft location were estimated from the complete contra-lateral element. (Only those damaged bones with an intact proximal or distal epiphysis and 50% or more

of the shaft length present were utilized, to ensure there was always a proximal or distal landmark present to use as a reference point for determination of midshaft.) In these instances, estimated midshaft location and anteroposterior orientation may contain slight estimation error. However, as suggested by Goldman's (2001) analyses of cross-sectional geometric properties and cortical microstructure (collagen fiber orientation) in the human midshaft femur, this variation should not significantly affect the outcome of analyses presented here.

Whole-bone photography

All femora and humeri utilized in the current study were photographed prior to sectioning in anterior, posterior and medial views using a Nikon Coolpix 995 digital camera. A millimeter scale was included in all photographs to permit their later use for whole-bone measurement. Digital photographs and whole-bone measurements (described above) of all long bones were archived on CD and provided to their respective lending institutions.

Removal of bone samples

A 0.5 – 1.0 cm (depending on bone size) block of bone was removed from the midshaft region of each *Chlorocebus aethiops* and *Hylobates lar* femur and humerus, using an Isomet slow-speed saw configured with a 4" diamond wafering blade (Beuhler Ltd., Lake Bluff, IL.). *Pan troglodytes* samples collected on-site at the Museum für Naturkunde in Berlin were removed using a portable 4" Faceter's saw configured with a 4" or 6" DiaLaser blade depending on bone diaphyseal breadth (Graves Company,

Pompano Beach, FL.). Bone samples were removed by producing two serial cuts, positioned 0.5 – 1.0 cm apart. The first cut was made 0.1 cm distal to midshaft, and the second 0.4 - 0.9 cm proximal to midshaft.

By gently striking the proximal block surface with the rotating saw blade, a small ca. 300 micron (μm) slot was made to preserve anteroposterior orientation. This was necessary as markings produced by graphite and ink frequently dissolved during the chemical preparation process. Further, this slot could be seen after sample embedding, thus preserving the orientation of the sample for later section mounting and imaging. Bone blocks were also labeled with specimen numbers using aluminum tags prior to chemical preparation.

Whole-bone reconstruction

To meet the specific requests of lending institutions providing material for this study, a protocol was developed for the reconstruction of sectioned long bones. (Appendix C). Prior to sectioning, the midshaft region of each long bone was coated with Exaflex Injection Type low-viscosity silicone impression material (GC Dental Production Corporation, Japan) to create a high-resolution mold encompassing the area to be removed. The impression material was allowed to dry for 5 minutes. To ensure that the orientation and form of the bone was maintained in the reconstruction, the inferior aspect of the bone was then pressed into a mound of Exaflex Putty Type O high-viscosity silicone putty (GC Dental Production Corporation, Japan) to create a rigid impression of the bone that could later be used for realignment of the sectioned proximal and distal halves. The two impression materials have an affinity for each other, leaving the high-

resolution mold spanning the midshaft region bonded to the low-resolution impression underneath. Following removal of the bone block from midshaft, the two bone ends were then refit into the mold, and a replica was created of the missing region using a clear low-viscosity, high-resolution epoxy resin (RS Epoxy Resin Two part quick set).

HISTOLOGICAL PREPARATION

Histological thin sections were prepared from the midshaft femur and humerus according to the method described by Goldman et al. (1999). Some modifications were made to accommodate the particular bone samples being used in the current study, and in an effort to improve specimen embedding and mounting. This method was developed for the preparation of histological thin sections that are compatible with imaging in both transmitted light and scanning electron microscopy modalities, and thus will permit a future correlative study of mineralization on these same specimens utilizing backscattered electron imaging in the scanning electron microscope.

Cleaning, preparation and embedding of bone samples

Specimen cleaning and dehydration. Bone blocks were cleaned of adhering soft tissue in an enzymatic detergent solution of 1% terg-a-zyne (Alconox, N.Y.) at 50°C with 24-hour changes over one to two weeks, depending on bone size. Daily ultrasonication and vacuuming improved infiltration and the efficiency of this solution in removing debris from small bone spaces, such as vascular canals. Samples were then dehydrated in a series of graded (50%, 70%, 95%, 100%) ethanol changes with frequent ultrasonication and vacuuming over a period of approximately one week. Following this, specimens were refluxed in a Soxhlet apparatus for one to two weeks (depending on bone size) in a

50:50 solution of isopropanol : heptane for further dehydrating and defatting.

Specimen embedding. Specimens were transferred to 100% acetone for approximately one hour, with ultrasonication and vacuuming, followed by a graded series (75:25, 50:50, 25:75) of acetone : polymethylmethacrylate (PMMA) changes prior to embedding. Specimens were transferred through three 24-hour changes of PMMA solution with 5% by volume styrene (a stabilizing agent) and 0.5g/500ml of azo-bisisobutyronitrile (a catalyst) added, with frequent ultrasonication and vacuuming to improve infiltration. Acetone was substituted for the methanol and xylene changes described by Goldman et al. (1999). Acetone acts as a solvent for PMMA, decreasing its viscosity and improving its infiltration into small bone spaces.

Specimens were then transferred to a final change of PMMA solution (with styrene and azo-bisisobutyronitrile) with ultrasonication and vacuuming, in which they were allowed to polymerize at room temperature. Polymerization and final curing of specimens was typically complete after two to three weeks, after which time the polymerized bone blocks were broken out of their glass bottles for preparation of histological thin sections.

Preparation of histological thin sections

Mounting of bone samples to glass slides. The distal ends of embedded bone blocks were ground flat to the midshaft section surface using a graded series of carbomide emery papers to 1200 grit on a Beuhler Handimet II (Beuhler Ltd., Lake Bluff, IL.). Blocks were then ultrasonicated to remove adhering grit particles and air dried before preparing

the surface for mounting. Blocks were placed with their prepared distal surface down into a thin film of acetonitrile for three minutes and then air-dried using compressed air. Acetonitrile solubilizes polymerized PMMA, thus creating topography on the block surface, which improves adhesion of the block to the mounting adhesive. The prepared surface of the bone itself was etched with Dentin Activator (Parkell, Farmingdale N.Y.), brushed only over the bone surface and left for 10 seconds before rinsing and air-drying with compressed air. This last step improves adherence of the bone itself to the mounting adhesive.

The surface of a glass histologic slide was also prepared to enhance bonding of the glass surface and the mounting adhesive. First, its surface was etched in 20% hydrofluoric acid for four minutes, followed by rinsing and air-drying. The surface of the glass slide was then treated with silane porcelain primer (Bisco, Itasca, IL.) for 30 seconds and then air-dried.

Final mounting of the bone block to the glass slide was done using Dentsply's Prime & Bond 2.1 NT Dual Cure and ProBond bonding agents (Dentsply International Inc., York, PA.). First, the prepared distal surface of the block was primed with a 50:50 mixture of Prime & Bond 2.1 NT bonding agent and Self Cure Activator. This mixture was continuously brushed across the surface of the block for a period of 30 seconds, and then allowed to air dry for five minutes. The primer was then cured using a visible light-curing gun for 30 seconds. ProBond adhesive was applied to the prepared surface of the glass slide, forming a small pool of adhesive covering the area to be occupied by the bone section. The prepared surface of the bone block was pressed down into the ProBond on the slide, held flat for 30 seconds with a weight, and light cured from above and below

the slide for two minutes according to the method of Goldman et al. (1999).

Occasionally, weak bonding of the bone block, the mounting adhesive and the glass slide would result in a mounted bone block (or prepared thin section) readily peeling away from the glass slide several days after mounting. Adding heat while air-drying (*e.g.*, with a hot air dryer) the silane porcelain primer applied to the glass slide and the Dentsply primers applied to the bone block improved the stability of the thin section over time.

Each bone block was mounted with its distal surface down and its anterior orientation positioned at the top of the slide. Additionally, the anterior orientation mark on the bone block was used to record the section orientation on the undersurface of the slide itself, so that histological details could be used to confirm orientation in acquired microscopy images of bone samples later.

Sectioning and final preparation of histological samples. Following mounting, the glass slide was affixed to a slide chuck, and the mounted bone block was cut away from the slide using a Beuhler Isomet slow-speed diamond blade saw (Beuhler Ltd., Lake Bluff, IL.) to leave a section thickness of approximately 130 μm . The section was then ground to 1200 grit using graded emery papers on a Beuhler Handimet II (Beuhler Ltd., Lake Bluff, IL.) at a final section thickness of $100 \pm 5 \mu\text{m}$.

Section thickness was determined optically using an Edge R400 Real-time 3D microscope. Using a 20X objective, the top and bottom of each section was identified, and the precision Z staging of this microscope enabled measurement of the intervening distance. After correcting this distance against the refractive index of the glass slide and

mounting medium, section thickness was calculated. Four to ten independent section thickness measurements were taken on each cross-section, depending on section size, and spanning the anterior, posterior, medial and lateral cortices to ensure an even thickness. Specimens were temporarily cover-slipped with ethylene glycol prior to imaging to improve image quality.

OVERVIEW OF SPECIMEN IMAGING AND ANALYSIS

All of the variables examined in this study could be readily viewed using a transmitted light microscope. Imaging of bone samples in both conventional transmitted light microscopy (LM) and circularly-polarized light microscopy (CPL) facilitated examination of the organization of bone cells and vascular canals, and the organization of the collagen fiber matrix, respectively. Acquired LM and CPL images were then imported into several commercially available software packages for image processing, image visualization and data acquisition. Following is a brief overview of imaging and image processing procedures. These procedures are further explained in Chapters 4-6, and they are outlined in Appendix D of this thesis.

Image acquisition

All images were acquired at low magnification using a Leica DMRX/E Universal microscope (Leica Inc., Wetzlar, Germany) configured with an automated Marzhauser stage (0.1m accuracy in 'X' and 'Y') and a circularly polarized light filter arrangement. Images were captured using a JVC KY55BU (5X objective; 1 pixel = 1.4970 μm) or JVC KY55BE (5X objective; 1 pixel = 1.2563 μm) color CCD camera, and were automatically transferred to a Syncroscopy Montage Explorer system (Synoptics Ltd.,

Cambridge, UK.). (The JVC KY55BU camera was upgraded to a JVC KY55BE model in the middle of this study.) The Syncroscopy Montage Explorer system relies on pattern-recognition algorithms to automate the montaging of large field-of-view, high-definition images in real-time. This capability overcomes the limitation of a restricted field-of-view in conventional imaging techniques, and produces a single image of an entire cross section of bone (up to 30,000 x 30,000 pixels in size) that retains original microscopic detail afforded by the optical conditions of the microscope. Further, it eliminates the need for post-acquisition manual stitching of adjacent single field-of-view images, which is both time consuming for large samples and results in the accumulation of mismatch errors between adjacent image frames. The ability to accurately acquire large field-of-view high-definition images of entire bone cross sections was critical to the successful completion of the analyses undertaken in this study.

Imaging in circularly-polarized light microscopy (CPL). Polarized light microscopy and imaging of anisotropic materials. The technique of polarized light microscopy facilitates examination of materials exhibiting optical anisotropy (Bromage et al., 2003; Slayter and Slayter, 1992). Anisotropic materials interact with light in a manner that is dependent upon their crystallographic axes, producing orientation-dependent changes in the refraction of light leaving the specimen. (Such materials are said to exhibit birefringence.) In contrast, isotropic materials exhibit the same optical properties irrespective of orientation.

Linear polarized light (LPL) microscopy is achieved by positioning two polarizing filters in the optical path (Bromage et al., 2003; Slayter and Slayter, 1992).

The first filter (the polarizer) is positioned between the specimen and the illumination source, and the second filter (the analyzer) is positioned between the specimen and the observer (i.e., the eyepiece objectives or the camera). The illumination source emits a beam of light oscillating in all planes perpendicular to the direction of light propagation (i.e., unpolarized). As the beam passes through the polarizer, light traveling in one vibration plane (i.e., the transmission axis) is allowed to pass to the specimen, while light oscillating in all other planes is absorbed. The analyzer is positioned with its transmission axis oriented 90 degrees with respect to the transmission axis of the polarizer. In the absence of an anisotropic material in the optical path, light exiting the polarizer is absorbed by the analyzer and no light passes through to the observer; the image therefore appears extinct (or dark). In this way, the crossed filter arrangement results in background extinction. If an anisotropic material is placed in the optical path, properties of that material interact with light exiting from the polarizer, resulting in a change in its vibration plane. In the latter case, the image appears bright, with variation in illumination intensity dependent on specimen orientation.

A disadvantage of LPL microscopy is that reduced light intensity occurs at each $45\text{-}225^\circ$ and $135\text{-}315^\circ$ of arc relative to the transmission axis of the polarizer, and regions of anisotropic materials in these positions (normally appearing bright in polarized light) appear extinct (Bromage et al., 2003). This is referred to as the “Maltese Cross” artifact. This artifact is eliminated by a circularly-polarized light (CPL) filter arrangement, in which two quarter wave retardation filters are placed in the optical path; one quarter wave filter is placed between each linear polarizing filter (polarizer and analyzer) and the specimen. Quarter wave retardation filters are positioned with their transmission axes

oriented at 45 degrees with respect to the adjacent polarizer or analyzer, and each effectively resolves light into two orthogonal components which are out of phase (by $\frac{1}{4}$ wave length) with one another. The result is circularly polarized light traveling in an elliptical vibration plane. As with LPL, quarter wave retardation filters above and below the specimen (producing a clockwise and counter-clockwise vibration axis, respectively) are crossed for background extinction (Bromage et al., 2003; Slayter and Slayter, 1992).

Collagen is an anisotropic material, and the collagen fiber matrix of bone therefore exhibits optical anisotropy when viewed under polarized light (Bromage et al., 2003). When collagen fibers are aligned exactly transversely with respect to the direction of light propagation through the specimen (or in the plane of section), there is a change in refraction of light from the specimen. Light is allowed to pass through the analyzer to the observer, resulting in maximum brightness. When collagen fibers are aligned exactly parallel with respect to the direction of light propagation (or perpendicular to the plane of section), there is no change in the refraction of light from the specimen. In this latter case, the collagen matrix *appears* isotropic; the vibration direction of light remains unchanged as it passes through the analyzer, and the specimen appears extinct. Collagen fibers having intermediate orientations exhibit intermediate brightness intensities.

Imaging procedures. Polarized light imaging of bone samples in this study was done using a Leica DMRX/E Universal microscope configured with a CPL filter arrangement in place, for the strict purpose of visualizing the organization of the collagen fiber matrix in bone to facilitate analyses of aspects of primary bone tissue organization (described further in Chapters 5) and secondary intracortical remodeling (described further in Chapter 6). Since the purpose of this study was not to acquire quantitative

gray-level information for analysis of collagen fiber orientation (e.g., Bromage et al., 2003; Goldman et al., 2003), analyses undertaken here would have been possible with the use of an LPL filter arrangement rather than CPL. However, as CPL eliminates the “Maltese Cross” artifact of LPL, visualization was further improved with this technique.

Further, CPL images acquired under standardized illumination conditions [described previously by Bromage et al. (2003)], while critical for the quantitative analysis of preferred collagen fiber orientation, were not appropriate for the current study. Pilot images of whole bone cross sections acquired from the study sample according to the method of Bromage et al. (2003) were frequently too dark for adequate visualization and interpretation of bone microstructural organization, owing to the minimal brightness of large areas of primary bone tissues in these samples having a preferentially longitudinal collagen fiber orientation. Thus, it was necessary to adjust the illumination conditions for CPL imaging depending on section brightness, so that subtle changes in primary bone tissue organization could be visualized.

Imaging in conventional transmitted light microscopy (LM). LM imaging was undertaken with the bottom linear polarizing filter of the CPL filter arrangement removed, creating conventional (i.e., unpolarized) transmitted light illumination. To improve LM image quality, individual image fields were corrected in Montage Explorer for illumination and color unevenness using shade correction functions as the montage was assembled.

Following acquisition, all images of left-side elements were horizontally flipped in Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.) to maintain a common

orientation for analysis.

Brief overview of image processing and analysis

LM and CPL images acquired using Syncroscopy's Montage Explorer system were imported into several commercially available software packages for image processing, image visualization and data acquisition. A brief overview of the various image processing and analysis procedures utilized in this study is presented here.

LM images were imported into Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.) for image processing to create a binary image mask, in which non-bone areas (*e.g.*, medullary canal, large resorption spaces) were deleted. This binary image was imported into NIH Image 1.63, a public domain image analysis program available through the National Institutes of Health, for the calculation of cross-sectional geometric properties (described further in Chapter 4).

LM and CPL images were imported into Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.), where they were superimposed for visualization purposes and areas representing distinct primary (described further in Chapter 5) and secondary (described further in Chapter 6) bone tissue types were manually traced and color-coded on separate layers (using the "Layers" option in Photoshop). Growth remodeling patterns and other features of primary bone microstructural organization (described further in Chapter 5) were also annotated in separate layers on these composite tissue "maps", facilitating the reconstruction of the growth history of each individual bone cross-section from its histological organization.

Composite tissue maps were converted to gray-scale images in Photoshop and

imported into Optimas 6.5 Image Analysis software (Media Cybernetics Inc., Silver Spring, MD.), where data relating to the distributions and proportions of primary bone tissue types (described further in Chapter 5) and secondary intracortical remodeling (described further in Chapter 6) were collected. A customized macro developed for Optimas 6.5 (Feik et al., 2000) divided each bone cross section into 16 radial sectors x 3 rings, from which quantitative gray-level data were extracted. This method permitted the quantitative analysis of regional variation in primary bone tissue types and secondary intracortical remodeling both within and between bone cross sections.

The various image processing and analysis techniques overviewed here enabled the enhanced visualization and quantification of variability in aspects of bone microstructure that reflect postnatal growth patterns and life history. They will be discussed in further detail in chapters 4-6 to follow.

TABLE 3-1 a. Summary of relevant life history characteristics of the study taxa.

Adult female body mass (kg)	Adult male body mass (kg)	Neonatal body mass (g) ¹	Maximum neonatal body mass growth velocity ²	Sexual dimorphism in body mass growth ²	Age at adult female body mass: captivity ² (wild)	Age at adult male body mass: captivity ² (wild)	Age at complete long bone epiphyseal union (wild)	Age at complete dentition (wild)
<i>C. aethiops</i>³								
5.6	7	f: 335.9 m: 334.9	~ 1 kg/yr.	Bimaturism; slight subadult growth spurt, most pronounced in males (~ 1 kg/yr.).	4 yrs. (~ 4 yrs.)	5.5 yrs (~ 6 yrs.)	> 38 mos.	38-67 mos.
<i>H. lar</i>⁴								
5.3	5.9	f: 407 m: 400	< 1.5 kg/yr.	Limited bimaturism and rate differences. Both sexes grow at a declining rate from birth.	5.75 yrs. (6 - 8 yrs.)	5.75 yrs. (6 - 8 yrs.)		6.1 yrs.
<i>P. troglodytes</i>⁵								
45.8	59.7	f: 1,814 m: 1,877	~ 3 kg/yr.	Minimal bimaturism. Males exhibit a pronounced subadult growth spurt (< 8 kg/yr.).	11.5-12 yrs. (12 yrs. +)	12-13 yrs. (15 yrs. +)	~ 15 yrs. (13.4-16.5 yrs.)	11.36 yrs. (12.4 yrs.)

¹ Source for neonatal body mass data summarized here: (Smith and Leigh, 1998).² While there are some reports based on wild individuals (e.g., Bolter and Zihlman, 2003; Pusey, 1978; Schultz, 1944; Turner et al., 1997), postnatal growth patterns have generally been best characterized for captive animals of known chronological age. Body mass growth data summarized here are from studies of captive animals by Leigh and colleagues (Leigh, 1992, 1996; Leigh and Shea, 1995, 1996).³ Additional sources for *C. aethiops* data summarized here: adult body mass (Fedigan & Fedigan, 1988); age at adult body mass in wild animals (Horrocks, 1986; Fedigan & Fedigan, 1988); age at epiphyseal union and complete dentition (Bolter and Zihlman, 2003).⁴ Additional sources for *H. lar* data summarized here: adult body mass (Carpenter, 1940); age at adult body size in wild animals (Brockelman et al., 1998; Leighton, 1987); age at M3 emergence (Dirks, 2003).⁵ Additional sources for *P. troglodytes* data summarized here: adult body mass (Smith and Jungers, 1997; based on *P. t. troglodytes*); age at adult body size in wild animals (Goodall, 1986; Pusey, 1978); age at epiphyseal union (Kerley, 1966; Zihlman et al. 2004); age at complete dentition (based on maxillary M3 emergence, Smith et al., 1994; Zihlman et al. 2004).

TABLE 3-1 b. Adult locomotor behaviors of the study taxa. Frequencies of different locomotor behaviors practiced (as a percentage of total locomotor time) are presented in parentheses.

Taxon	Locomotor behavior (% of total locomotor time)
<i>C. aethiops</i> ¹	quadrupedal walking or running (53.9%), climbing (29.5%), and leaping (9.6%)
<i>H. lar</i> ²	brachiation (56%), climbing (21%), leaping (15%), and bipedalism (8%)
<i>P. troglodytes</i> ³	knuckle-walking quadrupedalism (85.6 - 96.5%), quadrumanous climbing / scrambling (3.5 - 11.1%), suspensory behaviors (0.5 - 1.5%), bipedalism (0.2 - 1.2%)

Adapted from Ruff (2002: Table 2.).

¹ Source for locomotor frequencies: (Rose, 1979).

² Source for locomotor frequencies: (Fleagle, 1980).

³ Source for locomotor frequencies: (Doran, 1996; Doran & Hunt, 1994). Ranges represent variation in common chimpanzees reported among sexes, localities and subspecies.

TABLE 3-2. Biological age classes used in the current study.

Age Classes	Alveolar emergence/epiphyseal union criteria
DECID. (<i>infancy</i>)	alveolar emergence of one or more deciduous teeth
M1 (<i>juvenile 1</i>)	alveolar emergence of one or more permanent 1st molars
M2 (<i>juvenile 2</i>)	alveolar emergence of one or more permanent 2nd molars
M3 (<i>juvenile 3</i>)	alveolar emergence of one or more permanent 3rd molars
EPIPH. (<i>adult</i>)	Fully erupted permanent dentition, long bone epiphyseal union complete.

TABLE 3-3. Biological age classes based on Bolter & Zihlman, 2003.

Age Classes	Alveolar emergence/epiphyseal union criteria
1	alveolar emergence of one or more deciduous teeth
2	alveolar emergence of one or more permanent 1st molars
3	alveolar emergence of one or more permanent incisors
4	alveolar emergence of one or more permanent 2 nd molars
5	alveolar emergence of one or more permanent premolars
6	alveolar emergence of one or more permanent canines
7	alveolar emergence of one or more permanent 3 rd molars
8	All teeth fully erupted and long bone epiphyseal union complete.

TABLE 3-4. Ages of standard gingival emergence¹ and completion of long bone epiphyseal union in captive animals, and corresponding biological age classes.

Age class	<i>C. aethiops</i> ²	<i>H. lar</i> ³	<i>P. troglodytes</i> ⁴	
			Females	Males
DECID	6 - 115 days	≤ 6 mo.	0.25 (0.14, 0.38) - 1.02 (0.64, 1.35) yrs.	
M1	12 -14 mo.	< 2.88 yrs.	3.19 yrs. (2.67 - 3.75)	3.33 yrs. (3.00-3.58)
M2	26 - 31 mo.		6.45 yrs. (5.92-7.33)	6.47 yrs. (5.58-7.00)
M3	38 - 67 mo.	6.1 yrs.	10.71 yrs. (9.00-13.08)	10.27 yrs. (9.00 - 11.08)
EPIPH			≤15 yrs. ⁵	

¹ It is noted that alveolar emergence, as observed in the current study, may occur 3-5 months earlier than gingival emergence in the permanent dentition (Kelley and Smith, 2003).

² (Bolter and Zihlman, 2003)

³ (Dirks 1988, 2003; Smith et al., 1994)

⁴ (Kuykendall et al., 1992; Nissen and Riesen, 1964; compiled by Smith et al., 1994) Deciduous dentition: Median (5th, 95th percentile), combined sexes. Permanent dentition: Mean (range). Dental emergence chronologies are shown for mandibular teeth here.

⁵ (Kerley, 1966). Combined sexes.

TABLE 3-5. Estimated ages of standard gingival emergence and completion of long bone epiphyseal union in wild chimpanzees based on Zihlman et al. (2004)¹.

Age class	<i>P. troglodytes</i>
DECID (dC)	≤ 1.5 yrs.
M1	4.1 yrs.
M2	8.2 - 8.4 yrs.
M3	12.4 yrs.
EPIPH	13.4 - 16.5 yrs.

¹ Standard gingival emergence was estimated from observed alveolar emergence in the maxillary dentition.

TABLE 3-6. Sample representation across biological age classes (femur/humerus).

Sex	Total <i>N</i>	DECID	M1	M2	M3	EPIPH	Immature (unknown) ¹
<i>Cercopithecus aethiops</i>							
F	15/13	1/1	5/5	2/1	4/3	3/3	0/0
M	16/17	4/4	1/1	4/5	3/3	3/3	1/1 ²
<i>Hylobates lar</i>							
F	13/12	2/2	2/2	2/1	2/2	3/3	2/2 ³
M	15/14	2/2	1/1	4/4	5/4	3/3	0/0
<i>Pan troglodytes</i>							
F	5	1	0	1	3	0	0
M	2	0	0	1	0	1	0
unk.	5	1	1	2 ⁴	0	1	0

This table does not include specimens that were excluded from the study on the basis of abnormal histology. See text.

¹ Includes individuals lacking associated dental emergence data.

² SLW 101 – Assigned to M3 age class for analysis, based on bone length and epiphyseal union.

³ SLW 10 & 35 – Assigned to M3 age class for analysis, based on bone length and epiphyseal union.

⁴ MNH 35526 femur – Questionable association with skull having only permanent M1s erupted and unerupted M2s; reassigned to M2 age class based on bone length.

FIGURE 3-1 a. Proximal and distal landmarks for determination of femur length.
LEFT: juvenile femur. *RIGHT:* adult femur. Scale bar = 2 cm.



FIGURE 3-1 b. Proximal and distal landmarks for determination of humerus length.
LEFT: juvenile humerus. *RIGHT:* adult humerus. Scale bar = 2 cm.



CHAPTER 4.

CROSS-SECTIONAL GEOMETRY

INTRODUCTION

Postnatal ontogeny is a time of dramatic increases in body mass and linear dimensions, and acquisition of adult postural and locomotor skills. Behavioral studies of primates in natural settings have demonstrated marked age-related variability in the frequencies and modes of positional behaviors practiced, and in the types and diameters of substrates used, among infant, juvenile and adult primates (Crompton, 1983; Doran, 1992, 1997; Dunbar, 1989; Dunbar and Badam, 1998; Grand, 1977; Rawlins, 1976, 1977; Turnquist, 1986; Turnquist and Wells, 1994; Wells and Turnquist, 2001). These changes are also accompanied by marked changes in body composition and limb mass distribution (Bolter and Zihlman, 2002; Grand, 1977; Raichlen, 2005a, 2005b; Turnquist and Wells, 1994).

Development of long bone structure is influenced by both systemic and local mechanical factors. With respect to the latter, the skeleton is suggested to adapt to variable mechanical loadings associated with increases in body size and changes in positional behavior by making appropriate adjustments in the quantity and distribution (i.e., in structural properties) of bone. Such adjustments are accomplished by growth remodeling at periosteal and endosteal surfaces, and they are proposed to maintain peak *in vivo* bone strains within acceptable limits (reviewed in Chapter 2 of this thesis). Bone structural properties and their relationship to skeletal function in primates has been the focus of many studies, which have relied on the application of engineering beam theory

to the analysis of long bone cross-sectional geometry. Indeed, long bone cross-sectional geometry has been shown to vary with respect to locomotor behavior across a broad range of adult primates (e.g., Burr et al., 1981; Burr et al., 1989b; Carlson, 2005; Demes and Jungers, 1993; Ruff, 1987, 1989, 2002; Ruff and Runestad, 1992; Schaffler et al., 1985; Terranova, 1995b). Less well understood, however, is the manner in which adult patterns of diaphyseal geometry are acquired during postnatal ontogeny.

The current study addresses this issue by examining ontogenetic variation in cross-sectional geometry at the midshaft femur and humerus of *Chlorocebus aethiops* (vervet monkey) and *Hylobates lar* (white-handed gibbon), and at the midshaft femur of *Pan troglodytes* (common chimpanzee). Growth patterns of body mass and other dimensions, as well as adult locomotor behavioral repertoires, have been previously characterized for these taxa (Bolter and Zihlman, 2003; Doran and Hunt, 1994; Fleagle, 1980; Hamada et al., 1996; Hunt, 1992; Leigh, 1996; Leigh and Shea, 1995; Rose, 1979; Turner et al., 1997), and locomotor ontogeny in natural settings has been the focus of studies in common chimpanzees (Doran, 1992, 1997). The study of cross-sectional geometry provides a means of quantifying ontogenetic changes in bone size and shape which have mechanical relevance. This provides an important context for microstructural analyses to follow in Chapters 5 and 6 of this thesis.

BACKGROUND

Analyses of cross-sectional geometry can provide insight into the manner in which bone architecture in load-bearing limb elements responds to variable loadings associated with growth in body size, and the development of adult locomotor behaviors

(Burr et al., 1989a; Heinrich et al., 1999; Heinrich et al., 1993; Ruff, 2003a, 2003b; Sumner and Andriacchi, 1996). However, few researchers have examined the ontogeny of long bone diaphyseal cross-sectional geometry in primates. The following is a review of cross-sectional geometric principles and analysis. This is followed by a brief account of prior investigations of long bone cross-sectional geometry in nonhuman primates, focusing on catarrhines.

Principles of cross-sectional geometry

The application of engineering beam theory to the structural analysis of long bone cross-sections provides a useful means to quantify aspects of their size and shape which have mechanical relevance (Huiskes, 1982). Long bone diaphyses having circular or elliptical geometries can be modeled as hollow cylindrical beams, and they are suitable for simple engineering analysis based on principles describing relationships between beam geometry and strength or rigidity. Accordingly, several cross-sectional geometric properties, which characterize the amount and distribution of bone material around a given cross section, are proportional to the resistance of long bones to the stresses and strains engendered by different kinds of loading (Biewener, 1992; Ruff, 1989; Ruff and Hayes, 1983a; Wainwright et al., 1976). These properties are reviewed below.

The resistance of a cylindrical beam to pure axial tensile or compressive loads is determined primarily by the cross-sectional area of bone tissue in the section, or *cortical area (CA)*. Body mass has a significant influence on the axial loads experienced by skeletal elements (e.g., Ruff, 1989; Swartz and Biewener, 1992), and is discussed briefly below. However, while axial strains are engendered by most loading regimes, more

complex loads due to bending and torsion are thought to be the predominant sources of strain in most elements during locomotion (Biewener, 1991, 1992; Lanyon and Rubin, 1984; Rubin, 1984).

According to beam theory, a hollow cylindrical beam subjected to pure bending will experience its maximum tensile strains on its outer convex surface and its maximum compressive strains on its outer concave surface (Biewener, 1992; Ruff and Hayes, 1983a; Wainwright et al., 1976). (Figure 4-1 a). The central axis of the beam about which no deformation occurs is called the *neutral axis*. Tensile and compressive strains experienced by material within the bone are proportional to their distance from the neutral axis. If bone material is distributed economically and optimally, greater structural reinforcement is expected in the plane where maximal bending loads occur. The most critical cross-sectional geometric property for estimating section strength or rigidity when subjected to bending is the *second moment of area (I)*, which is also referred to as the area moment of inertia or cross-sectional moment of inertia. The second moment of area takes into account both the amount of bone material and its distribution relative to the neutral axis. It can be calculated by summing the squared orthogonal distance (y) of each unit area (dA) of material in a cross-section from its neutral axis, according to the following equation (Biewener, 1992; Wainwright et al., 1976). (Figure 4-1 b).

$$I = \int y^2 dA \quad (\text{units: mm}^4)$$

Because it is proportional to the squared distance of each unit area of bone from the neutral axis, section rigidity under bending increases rapidly as bone is positioned at a greater distance from the neutral axis (i.e., as a result of bone deposition at the periosteal

surface).

The second moment of area can be calculated relative to any anatomical or arbitrary axis. The *maximum and minimum second moments of area* ($I_{\max}$ and $I_{\min}$) are calculated with respect to the major and minor principal axes of the cross section, respectively, which are (mutually) perpendicular. $I_{\max}$ and $I_{\min}$ represent the planes (or directions) of greatest and least bending rigidity in the section, respectively. The angle theta (θ) specifies the orientation of the plane of greatest bending rigidity, and is usually measured counter-clockwise from the medio-lateral plane (Ruff and Hayes, 1983a).

$I_{\max}$ and $I_{\min}$ frequently do not correspond to anatomically-defined planes of interest, however, and second moments of area about medio-lateral and antero-posterior axes (I_x and I_y , respectively) may also be calculated (e.g., Burr et al., 1989a; Ruff, 2002; Schaffler et al., 1985). I_x , for instance, indicates bending rigidity in the anteroposterior plane, about the mediolateral axis.

The ratio $I_{\max} / I_{\min}$ provides an index of circularity of a cross section. Cross-sections approaching circularity have two principle axes which are approximately equal, yielding a ratio of 1. A departure from circularity in elliptical sections, where $I_{\max}$ is maximized and $I_{\min}$ is minimized, would minimize overall strains in long bones in which the predominant loading regime is unidirectional bending and in the plane of $I_{\max}$. A circular cross-section would better resist bending loads occurring in each and every plane, equally (Biewener, 1992; Ruff and Hayes, 1983a).

Long bones are also frequently subject to torsional loads, in which shear strains are generated about the neutral axis. The *polar moment of area*, J , provides a measure of bone strength under torsional loading in cylindrical cross-sections (Daegling, 2002). J

can be calculated by summing any two second moments of area calculated for orthogonal axes (e.g., $I_{\max}$ and $I_{\min}$). J also provides a measure of average or overall bending strength, since it incorporates second moments of area about orthogonal planes.

The magnitude of bending strains are proportional to their distance from the neutral axis of the bone, and a more relevant estimate of strength is the *section modulus*, Z (e.g., Ruff, 2002). Section modulus takes into account the perpendicular distance from the bone's neutral axis to the outer perimeter (d_y), where maximum bending strains are predicted to occur. Z can be calculated about any axis according to the formula (Ruff, 2002; Terranova, 1995a; Wainwright et al., 1976):

$$Z_x = I_x / d_y \quad (\text{units: mm}^3)$$

The polar section modulus, Z_p , is a measure of torsional strength, and can be estimated by dividing the polar moment of area, J , by the average of the radial distances (d) in two orthogonal axes. Since it is based on J , the polar section modulus can also be interpreted as a summary estimate of average bending strength (Ruff, 2002).

Limitations of cross-sectional geometric analyses. Cross-sectional geometric analyses are less applicable to bone regions having extensive amounts of cancellous bone, such as the ends of long bones (Burr and Piotrowski, 1982; Ruff, 1983), or to cross-sections departing substantially from circularity (Biewener, 1992; Daegling, 2002; Ruff, 1989). Structural analyses of bone based on beam theory are also limited in their assumption that the material properties of the structure are homogeneous and isotropic (Biewener, 1992; Ruff, 1989). In reality, bone material properties are both heterogeneous and anisotropic (Ascenzi and Bonucci, 1967, 1968, 1972; Currey, 1953; Martin et al., 1998; Reilly and

Burnstein, 1975), although the influence of such variation in material properties on structural analyses is generally assumed to be insignificant. It has also been shown that axes of greatest structural rigidity (as reflected in the orientation of $I_{\max}$) do not always coincide with experimentally-determined axes where the greatest bending loads occur (Demes et al., 2001; Demes et al., 1998; Lieberman et al., 2004). Further, in some animals, rather than economizing bone material, bone may be distributed in such a manner as to increase load predictability and minimize resistance in the plane in which bending normally occurs (Lanyon and Rubin, 1984; Rubin, 1984). The extent to which these factors influence strength estimates derived from beam theory principles in different elements is not well understood (Lieberman et al., 2004).

Despite these limitations, application of engineering beam theory to the structural analysis of primate limb bones provides a useful means of quantifying variation in cross-sectional shape which may relate to the pattern of loads to which limb bones are subjected. The extent to which many of the above factors influence estimates of bone strength derived from beam theory can be considered in future studies where cross-sectional geometric properties are considered in combination with micro-structural and experimental (e.g., *in vivo* strain analysis) data. To date, however, such studies are sorely lacking.

Influence of body size on cross-sectional geometry

Body mass has an important influence on diaphyseal cross-sectional geometry. As a major component of mechanical loads, the magnitude of forces exerted on the skeleton varies in proportion to body mass ($\propto L^3$). However, as discussed above,

the ability of a bone to resist axial loads varies in proportion to cross-sectional area ($\propto L^2$). If geometric similarity is maintained, loads on the skeleton are expected to increase with increasing body size ($\propto L^{3/2}$). Thus, cross-sectional geometric properties are predicted to scale with positive allometry to avoid failure with increased body size and mechanical loading (Biewener, 1990, 1991).

The limb bones of mammals across a large size range are observed to scale close to geometric similarity (e.g., Alexander et al., 1979; Bertram and Biewener, 1990), however, and the mechanisms by which similar peak bone strains and safety factors are maintained are suggested to vary with increasing size (Biewener, 1983, 1990, 1991; Rubin and Lanyon, 1984). Animals of medium size (0.1 – 300 kg), are predicted to reduce forces exerted on the skeleton mainly by altering limb posture (e.g., using more extended limb postures), thereby increasing the mechanical advantage of limb musculature, and exhibiting only slight positive allometry. Animals of larger size are suggested to increase positive allometric scaling and reduce locomotor “performance”.

Anthropoid primates show similar general scaling trends as observed among other medium-sized mammals, exhibiting isometry or slight positive allometry of geometric properties with body mass in most analyses (Jungers and Burr, 1994; Jungers et al., 1998; Ruff, 1987; Schaffler et al., 1985). Based on a study of cercopithecines by Polk (2002), postural adjustments similar to those observed in other mammals are suggested to contribute to the relatively constant scaling relationships observed between cross-sectional geometric variables and body size, despite differences in limb proportions. Nevertheless, deviations from this general scaling trend are observed, such as the significant negative allometry of hindlimb geometric properties with body mass in

orangutans (Ruff, 1987, 1990), and are suggested to reflect mechanical adaptations to specialized positional behaviors (also *Presbytis* in Schaffler et al., 1985).

Previous investigations of diaphyseal cross-sectional geometry in catarrhines

Numerous researchers have examined structural properties of long bone diaphyses in an attempt to assess the influence of body size (discussed above) and skeletal function on diaphyseal geometry in nonhuman primates (e.g., for the latter see Burr et al., 1981; Burr et al., 1989b; Carlson, 2002, 2005; Demes and Jungers, 1993; Matsumura et al., 2002; Ohman, 1993; Ruff, 1987, 1989, 2002; Ruff and Runestad, 1992; Schaffler et al., 1985; Terranova, 1995a; Terranova, 1995b). However, cross-sectional geometric properties have received comparatively little attention in studies of postnatal ontogeny. The following briefly reviews investigations that have been undertaken thus far, particularly focusing on nonhuman catarrhine primates.

Burr and colleagues (Burr et al., 1981; Burr et al., 1989b) examined cross-sectional geometric properties at five different sections levels along the femoral and humeral shafts in adult representatives of three species of macaques. They reported that bending rigidity (as reflected in $I_{\max}$) was lowest at midshaft compared to other section levels in the femur of terrestrial macaques (*Macaca mulatta* and *M. nemestrina*). In contrast, the more arboreal *M. fascicularis* was characterized by femora having greatest bending rigidity (as reflected in $I_{\max}$, $I_{\min}$ and J) at midshaft, and reduced cross-sectional moments proximally and distally along the shaft. Burr and colleagues (1989b) suggested that this “barrel-shaped” pattern characteristic of more arboreal species represents a structural adaptation to long slender limb elements, reducing the chance of failure where

bending stresses are likely to be greatest. Interestingly, while differences in the pattern of bending rigidity in the femur across section levels and among species were consistent with locomotor adaptations, the humerus showed less variation in diaphyseal geometry among taxa characterized by different locomotor specializations (Burr et al., 1989b).

Schaffler and colleagues (1985) examined scaling of cross-sectional geometric properties and variation in femur and humerus strength proportions in several Old World monkey taxa (*Macaca nemestrina*, *M. fascicularis*, and *Presbytis cristata*) and *Hylobates lar*. They demonstrated that the ratio of average humeral:femoral bending rigidity varied in accordance with functional differentiation of the limbs during locomotor behaviors. Leaf monkeys were characterized by the lowest humeral: femoral strength ratio (0.609), while gibbons had the highest (0.917). Interestingly, some degree of hindlimb dominance was evident in all taxa examined, despite the fact that the forelimb is the primary propulsive and weight-bearing element during brachiation in gibbons. Ruff (2002) later examined inter-limb strength proportions in more catarrhine taxa. His results were consistent with those of Schaffler and colleagues (1985); orangutans exhibited the greatest relative humeral strength, while colobines exhibited the lowest. However, he also noted substantial overlap in inter-limb strength proportions among African great apes and cercopithecines.

Schaffler and colleagues (1985) examined more subtle differences the manner in which loads are distributed in the femoral and humeral diaphyses, as reflected in the ratio of second moments of area in mediolateral and anteroposterior planes (I_{ML} / I_{AP}). Femora and humeri were shown to be significantly more adapted to resist bending loads in the mediolateral plane in arboreal quadrupeds (*Macaca fascicularis* and *Presbytis cristata*)

and *Hylobates lar*, although the midshaft humerus of *Macaca nemestrina* also exhibited slight reinforcement in the mediolateral plane.

Ruff (1989) identified an association between relative anteroposterior bending rigidity in the femoral shaft and frequency of leaping behaviors in four primate species. Taxa which practiced more frequent leaping (including *Galago senegalensis*) were characterized by increased anteroposterior bending strength in the proximal femoral shaft. In a later study, Ruff and Runestad (1992) demonstrated a size-related trend towards increasing relative mediolateral bending rigidity in primates, which they suggested is related to proposed postural mechanisms for maintaining similar peak strain levels during locomotor behaviors across animals of varying body size.

Ohman (1993) examined several cross-sectional geometric properties at the midshaft femur and humerus in hominoids, including *H. lar*, *P. troglodytes*, *Gorilla gorilla* and *Homo sapiens*. Structural patterns identified in his study were evaluated in light of their functional implications for *Australopithecus afarensis*. Matsumura and colleagues (2002) examined cross-sectional geometric properties at thirteen different sections levels along the femoral and humeral shafts in adult common chimpanzees, and compared these patterns to humans.

Carlson (Carlson, 2002, 2005) examined subspecific variation in femur and humerus diaphyseal geometry in relation to observed frequencies of positional behaviors in an extensive sample of African apes, including *G. gorilla*, *P. troglodytes* and *P. paniscus*. Results of his study provide general support for an association between increased total frequency of arboreal locomotor behavior and more circular cross-sectional geometries as reflected in $I_{\max} / I_{\min}$. This pattern was most apparent in the

femoral diaphysis and less so in the humerus, and it was also observed when sexes were compared within subspecies. This relationship was hypothesized to reflect more variable load distributions in the limb skeleton of animals practicing more frequent arboreal locomotor behaviors, compared to behaviors associated with more stereotyped limb movements, as may occur during terrestrial quadrupedalism. However, patterns of association between principle moments of area indices across femoral and humeral regions of interest and specific arboreal locomotor behaviors (e.g., arboreal scrambling, quadrupedal climbing, suspensory behaviors) were less straightforward in the combined sample, and when genera and subspecies were considered separately. In a similar study of *P. troglodytes* individuals from Taï Forest (*P. t. verus*) and Mahale (*P. t. schweinfurthii*) with known positional behavior profiles, Carlson and colleagues (2006) also failed to find clear relationships between specific arboreal locomotor behaviors and diaphyseal shape.

Ontogenetic variation in cross-sectional geometry of nonhuman primates. Cross-sectional geometric properties have been incorporated into a number of studies of postnatal ontogeny in humans. Many of these studies have focused on characterizing normal patterns of growth in cortical area and, more recently, other estimates of bone strength, during postnatal development (e.g., Frisancho et al., 1970; Ruff et al., 1994; Schoenau et al., 2001; Sumner and Andriacchi, 1996). Studies have also explicitly examined the influence of variables such as body mass and muscle strength on the development of diaphyseal cross-sectional geometry (e.g., Moro et al., 1996; Ruff, 2003a; Schoenau, 1998; Schoenau et al., 2000; van der Meulen et al., 1996). In an

interesting pair of recent studies by Ruff (Ruff, 2003a, 2003b), growth velocities of diaphyseal geometric properties at the midshaft femur and humerus of humans were shown to be strongly associated with both body size growth velocities and important behavioral shifts in the development of bipedal walking.

However, the development of long bone cross-sectional geometry in nonhuman primates has received comparatively little attention. Kikuchi (2003) described ontogenetic variation in cortical and subperiosteal areas as part of a larger study examining parameters of bone mineral density at the distal radius in captive Japanese macaques and common chimpanzees. Burr and colleagues (1989a) examined growth-related variation in bending rigidity at three section levels of femur, tibia and humerus diaphyses in captive free-ranging *Macaca mulatta*. In the latter study, sex-specific patterns of growth in femoral and humeral I_{AP} were largely consistent with reported growth patterns for other body dimensions (Watts and Gavan, 1982). Additionally antero-posterior bending rigidities increased at a significantly faster rate with respect to body mass in males compared to females, which Burr and colleagues (1989a) interpreted as a demand for increased anteroposterior strength at midshaft in rapidly-growing males. However, no significant sex differences in shape were identified, suggesting that the manner in which the limbs are loaded is similar among the sexes.

Ruff (2003b) examined ontogenetic variation in femoral / humeral strength proportions (i.e., Z_p) in a cross-sectional sample of *Papio cynocephalus*. Strength proportions showed less pronounced change during postnatal ontogeny in baboons compared to humans, consistent with less pronounced change in limb function during locomotor ontogeny and an absence of the marked functional differentiation of the limbs

in adult locomotor behaviors. Femoral / humeral strength proportions in baboons increased slightly until 2.5 years of age, after which time they stabilized. Relative femoral strength was slightly higher than humeral strength after this time, but humeral strength did not show the significant decline in later ontogeny present in humans.

Summary of nonhuman primate studies. Studies discussed above have demonstrated variability among nonhuman primates in long bone diaphyseal geometric properties, in accordance with species variation in locomotor behaviors. However, ontogeny of diaphyseal cross-sectional geometry has received relatively little attention. The few studies that have been undertaken thus far indicate that ontogenetic variation in diaphyseal geometry reflects the influence of both growth-related increases in body size and behavioral changes as immatures acquire adult postural and locomotor skills.

RESEARCH OBJECTIVES

The principal objective of this study is to use cross-sectional geometric parameters to quantify ontogenetic variability in diaphyseal size and shape (i.e., bone structural properties), to provide a context for interpretation of ontogenetic and regional variability in microstructural features at the midshaft femur and humerus. The mechanical significance of ontogenetic variation in structural properties at the midshaft femur and humerus of taxa examined here is also considered. This study has two specific aims.

1) *To characterize patterns of growth in individual cross-sectional geometric parameters (CA, I_{max}, J, Z_x, Z_y, and Z_p) at the midshaft femur and humerus.* Growth-

related variation in cross-sectional geometric parameters is considered in light of postnatal growth patterns for other body dimensions (i.e., body mass and bone length), as described in the current study and reported previously by other investigators.

2) *To describe ontogenetic variation in diaphyseal cross-sectional proportions (i.e., shape) at the midshaft femur and humerus.* Ontogenetic variation is described in several parameters relating to the distribution of bone material around single cross-sections, and which are hypothesized to reflect relative bending rigidity about principle and anatomical axes: 1) index of section modulus about anatomical axes (Z_y / Z_x), reflecting relative mediolateral bending rigidity, 2) theta (θ), the orientation of greatest bending rigidity, and 3) principle moment of area index ($I_{\max} / I_{\min}$), reflecting relative bending rigidity in principle axes and providing a measure of section circularity.

Characterization of cross-sectional growth patterns and development of cross-sectional shape at the midshaft femur and humerus will provide a context for studies of primary bone tissue types and secondary intracortical remodeling to follow in Chapters 5 and 6 of this thesis. Further, given the small and cross-sectional nature of samples utilized in the current study, this provides an opportunity to determine whether current results are consistent with earlier reports on cross-sectional geometry in the study taxa, and whether samples utilized here exhibit similar characteristics of postnatal growth as reported previously for other body dimensions.

MATERIALS AND METHODS

This study was conducted as part of a larger examination of cortical bone micro-structural development at the midshaft femur and humerus in a comparative sample of

Chlorocebus aethiops (vervet monkeys, $N=32$), *Hylobates lar* (white-handed gibbons, $N=28$) and *Pan troglodytes* (common chimpanzees, $N=12$; femur only). Table 4-1 lists the most common locomotor behaviors characterizing adult members of each taxon in order of their relative frequencies (reflected in % of total locomotor time spent in each behavior).

Materials

Sample selection. As described in Chapter 3 of this thesis, several criteria were prioritized in the selection of collections from which specimens would be taken for the current study: 1) only collections comprising wild-caught individuals from restricted geographic areas were assessed, to reduce potential variability in growth and development among individuals living under different ecological constraints (e.g., Altmann and Alberts, 1987; Hamada et al., 1996; e.g., Phillips-Conroy and Jolly, 1988; Zihlman et al., 2004); 2) collections were prioritized that comprised an equal distribution of individuals among different age classes throughout the postnatal growth period (discussed below), and that had an equal distribution of males and females and associated body mass data; 3) only collections comprising immature individuals and non-geriatric adults were included in the selection process. Geriatric adults were excluded on the basis of tooth wear and evidence of osteoarthritis. Individuals showing obvious evidence of skeletal fractures and pathologies (as described in White, 1991) were also avoided.

Both the invasive nature of the sampling protocol required for histological processing and the importance of the above selection criteria meant that the taxonomic range and appropriate samples available for this study were limited. Though it was

generally possible to meet criteria 1-3 for the *Chlorocebus aethiops* and *Hylobates lar* samples, many *Hylobates lar* individuals selected are missing associated body mass data. Further, many criteria were difficult to meet when selecting appropriate specimens for the *Pan troglodytes* sample. However, given what little is known of *P. troglodytes*, the sample available for this study still provides invaluable preliminary data on ontogenetic variability in long bone diaphyseal geometry.

Biological age classes. In the absence of chronological age information for individuals examined in the current study, it was necessary to rely on other criteria to estimate biological age. Dental alveolar emergence was chosen as the most appropriate criterion for estimating biological age in the current study for several reasons (e.g., Smith, 1989; Smith, 1992). For statistical analysis in the current study, individuals were classified into five biological age classes, presented in Table 4-2 and described in detail in Chapter 3 of this thesis.

At times, it was desirable to further refine this biological age classification, for the purpose of establishing the relative ages of individuals within biological age classes (particularly within age classes based on molar emergence) as defined above and for visualizing growth-related increases in variables over biological time *within* taxa. In these instances, individuals were further grouped into eight biological age classes according to the classification provided in Bolter and Zihlman (2003) for *C. aethiops* and presented in Table 4-3. Use of this biological age classification system also allowed comparisons between the current study and previous studies of growth and development undertaken on other wild *C. aethiops* samples (Bolter and Zihlman, 2003; Turner et al.,

1997). This biological age classification was also applied to *P. troglodytes*, which shares the same sequence of dental eruption (Smith et al., 1994).

Tooth eruption and long bone epiphyseal union status were assessed according to the methods of Wintheiser et al. (1977) and Bolter and Zihlman (2003). Individuals continue skeletal growth after full eruption of the dentition (e.g. personal observations, and Bolter and Zihlman, 2003; Watts, 1990). Thus, individuals were only considered adult when they had reached both dental and skeletal maturity, defined by complete permanent dentitions and complete fusion of all long bone epiphyses, respectively. Among adults, geriatric individuals were excluded on the basis of extreme tooth wear or evidence of osteoarthritis as described by White (1991).

Description of the sample. Table 4-4 provides details about sample representation across biological age classes in the current study. Two specimens warrant mention here. First, *P. troglodytes* MNH 35526 was questionably associated with a skull having only first permanent molars erupted and unerupted second permanent molars. This specimen was reassigned to the M2 age class based on bone length and incomplete epiphyseal fusion. Due to the uncertainty regarding dental emergence status of this individual, this specimen was excluded from LOWESS regression analyses in which individuals were grouped into biological age classes according to the classification provided by Bolter and Zihlman (2003). Secondly, anteroposterior orientation for the midshaft humerus of *C. aethiops* SLW 21 was lost during processing. This specimen was excluded from analysis of shape variables dependent upon specimen orientation (e.g., Zx, Zy, theta). Further detailed information about individuals comprising each species sample can be found in Chapter 3

and in Appendix B.

Sample location

Identification of the midshaft of long bones under investigation is usually based on a measurement of bone length, which includes the epiphyses of a long bone (e.g., Ruff and Hayes, 1983a). However, this traditional measure of bone length prevents the inclusion of specimens for which epiphyses are either unfused, broken or otherwise missing. To maintain consistency in our definition of midshaft between immature (unfused) and adult specimens, a measure of bone length was developed for the current study which excluded the epiphyses. Bone length measurements and orientations were completed as described in Chapter 3 of this thesis.

Image and data acquisition

Since a goal of this study was to examine the relationship between the development of diaphyseal geometry and bone microstructural features, geometric properties were measured on the same thin sections examined as part of the histologic analyses described in Chapters 5 and 6 of this thesis. The sample removal, preparation, and imaging protocols are described in further detail in Chapter 3 and Appendix D of this thesis.

Images were acquired in conventional transmitted light illumination (LM) using a Leica DMRX/E Universal microscope (Leica Inc., Wetzlar, Germany) configured with a 5X objective and a JVC KY55BU (5X objective; 1 pixel = 1.4970 μm) or JVC KY55BE (5X objective; 1 pixel = 1.2563 μm) color CCD camera. Images were automatically

transferred to a Syncroscopy Montage Explorer system (Synoptics Ltd., Cambridge, UK.), which relies on pattern-recognition algorithms to automate the montaging of large field-of-view, high-definition images in real-time.

Image montages of whole bone cross-sections were acquired in conventional transmitted light illumination (LM) and then imported into Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA.) for image processing to create a binary image mask. Non-bone areas (e.g., medullary canal, large Haversian canals and other resorption spaces) were edited out and converted to background color. The image was converted to binary, using the 'Threshold' function and a final 'Dust and Scratches' filter (radius = 2 pixels), such that bone area appeared black and background and other non-bone areas appeared white.

The resulting binary image was reduced to 6.25% (*C. aethiops*, *H. lar*) or 3.125% (*P. troglodytes*) of its original size and imported into NIH Image 1.63, a public domain image analysis program available through the National Institutes of Health, for the calculation of cross-sectional geometric properties using a macro ('MOMENTMACRO') made available by Dr. Christopher Ruff (<http://www.hopkinsmedicine.org/FAE/mmamcro.htm>). The following cross-sectional geometric parameters were examined in this analysis:

CA:	Cortical area (mm ²)
MA:	Medullary canal area (mm ²)
TA:	Total subperiosteal area (mm ²)
I _{max} :	Maximum second moment of area (mm ⁴)
I _{min} :	Minimum second moment of area (mm ⁴)
I _{max} / I _{min} :	Principle second moments of area index, a measure of section circularity
Ø:	Angle theta, the orientation of the plane of greatest bending rigidity

	($I_{\max}$), measured counter-clockwise from the mediolateral axis
J:	Polar second moment of area (mm^4)
Z_x :	Section modulus about the x axis (mm^3), an estimate of anteroposterior bending strength
Z_y :	Section modulus about the y axis (mm^3), an estimate of mediolateral bending strength
Z_p :	Polar section modulus (mm^3), calculated from J_{xy} / (mean of d_x and d_y)

As reviewed above, many of these parameters have been shown to exhibit growth trajectories that are consistent with body size growth patterns. These parameters also provide a useful means of quantifying ontogenetic variation in cross-section size and shape which have mechanical relevance. CA is a measure whole cortex size, and is proportional to section rigidity under axial loading. MA and TA were also examined, as these have an influence on CA. Other geometric variables examined here are proportional to section rigidity in bending ($I_{\max}$, $I_{\min}$, Z_x , and Z_y) or torsion (J and Z_p). J and Z_p are also often used as a summary estimate of average section strength in bending. Their indices ($I_{\max} / I_{\min}$ and Z_y / Z_x) and the variable $\emptyset$ provide a means of quantifying variation in cross-sectional shape, which may relate to regional variation in microstructural features examined in later chapters.

Visualization of growth-related changes in geometric properties

Nonparametric locally-weighted least squares (LOWESS) regression was used to characterize growth-related changes in individual properties across age classes. This is a nonparametric smoothing technique. It fits a line to a bivariate distribution in sequential steps by fitting a weighted linear regression function to a local window of points centered on a single “target” point. The size of this window is referred to as the “tension” value; a window with a tension value of 0.25 contains the target point at its center and the

surrounding 25% of the data points across the range of x values. A tension value of 1.0 results in a linear least squares fit through the entire distribution, while a tension value approaching 0.0 preserves local curvilinear associations between x and y variables. Shifting of this window along the x axis results in a LOWESS fit through the entire distribution (Efron and Tibshirani, 1991; Leigh, 1992, 1996).

LOWESS plots were generated in Statistica 6.0 (Statsoft, Inc.) software package, using a tension value of 0.25. In *C. aethiops* and *P. troglodytes*, the x axis was scaled to estimated age in months, based on the dental emergence chronologies communicated in Bolter and Zihlman (2003, based on information provided by L. Fairbanks) and Smith et al. (1994). Use of estimated chronological age as the independent variable where possible provides a more accurate representation of the relative ages of individuals within each species sample for visualization purposes. For *C. aethiops*, estimated age was calculated as the midpoint of the age of emergence for each dental stage reported in Bolter and Zihlman (2003). Due to the lack of information regarding the age at completion of epiphyseal union in this species, the maximum age at emergence of the M3 was used as a rough approximation of the minimum age at adulthood. For *P. troglodytes*, estimated age in months was calculated as the mean age at emergence in males and females for the deciduous dentition and mandibular permanent molars as reported in Smith et al. (1994). For premolars (age class 5), mean age at emergence of maxillary teeth was used, as maxillary premolars emerge earlier on average than mandibular premolars. An age of 15 years was used as a minimum age at adulthood in chimpanzees, based on approximate timing of completed epiphyseal union (Kerley, 1966).

To compare growth-related changes in body mass, bone length and geometric

properties, data were normalized by dividing individual values by the maximum age group mean (e.g., Sumner and Andriacchi, 1996). Body mass data was recorded for all but a single individual in the *C. aethiops* sample, and for 14 *H. lar* individuals. In the latter taxon, body mass was available for all individuals in the DECID, M1, and M2 age classes, and for two females in the M3 age classes. Mean adult body masses provided by Schultz (1944) were used to calculate normalized values for those individuals with associated body mass in the current study sample, as a percentage of the adult means for males and females, 5.7 kg and 5.3 kg, respectively. These data provided by Schultz are based on a larger sample of *H. lar* collected from northern Thailand.

The shape of normalized mean plots and LOWESS plots was influenced by small and unequal sample sizes, particularly in the younger age classes for *H. lar* and in *P. troglodytes*. Further, the assumption that growth begins at deciduous tooth emergence and ends at fusion of the proximal humerus (the last long bone epiphysis to fuse, and the criterion for assigning individuals to the ‘adult’ age class) also had a large influence on the shape of growth trajectories in the youngest and oldest age classes. Despite these limitations, however, LOWESS plots and normalized mean plots provide a useful means of characterizing growth-related change in body mass, bone length and cross-sectional geometric properties in the current sample. Results of this study will also be compared to other studies in the literature where possible.

Statistical comparisons among groups

Prior to analysis, assumptions of normality and homoscedasticity were tested using the Kolmogorov-Smirnov test and Levene’s homogeneity of variance test.

Because data did not meet assumptions for parametric statistical analysis in many cases, all statistical analyses were performed using nonparametric techniques. All statistical analyses were performed in Statistica version 7.1 software package. Results were considered statistically significant at a probability level of $p \leq 0.05$.

Contrasts among group medians were made using Kruskal-Wallis (for three or more independent samples) and Mann-Whitney (for two independent samples) tests. Age and sex were factors in these analyses. Contrasts among groups were also made using Kolmogorov-Smirnov (for two independent samples) and Median tests (for three or more independent samples), which test for overall differences in the shape and location of distributions among groups (Sokal and Rohlf, 1995). Results of Mann-Whitney and Kruskal-Wallis tests are presented here. Sex contrasts in body mass, diaphyseal length and geometric variables in *C. aethiops* and *H. lar* were restricted to the M3 and EPIPH age classes due to unequal sex representation and small sample sizes in the youngest age classes. Statistical contrasts among sexes were not possible in the current sample of *P. troglodytes*. Element contrasts for *C. aethiops* and *H. lar* were conducted using Wilcoxon matched pairs tests for dependent samples.

Nonparametric one-sample Wilcoxon tests were employed to test for significant departures in indices (Z_y / Z_x and $I_{\max} / I_{\min}$) from 1.0 (indicating circularity) within age classes. This was undertaken in Statistica 7.1 using a Wilcoxon matched pairs design, with the null hypothesis of 1.0 assigned to a second constant variable.

RESULTS

Cross-sectional geometry raw data are provided in Appendix E for all individuals

included in this study. Descriptive statistics for each taxon, age class and sex for body mass, bone length, and cross-sectional geometric variables are also provided.

Growth-related variation in body mass, bone length and geometric properties

***C. aethiops*.** Figure 4-2 a shows growth of males and females in absolute body mass, bone length and geometric properties of the femur plotted against estimated age in months. The shapes of LOWESS plots for $I_{\max}$, J, and section moduli were similar to each other. Thus, only Z_p is shown here. Visual inspection revealed that male and female growth patterns were similar in early ontogeny, and diverged in the M2 age class. Females showed a continuation of steady growth following M2 emergence (with the possible exception of body mass), while males can be described to undergo a period of more rapid growth between the M2 and M3 age classes in many variables. All variables showed some evidence of continued growth between the M3 and EIPPH age classes in males, although growth appeared to be more substantial for body mass and CA during this time. Females, in contrast, evidenced little or no growth after M3 emergence in most variables examined.

As shown in Figure 4-2 b, growth trends in bone length and geometric properties of the femur and humerus were similar in *C. aethiops*, despite the greater length of the femur across all age classes. There was a substantial degree of overlap in femur and humerus geometric properties at all ages.

Figure 4-2 c shows ontogenetic variation in TA, CA, and MA. TA showed similar growth trends as described for CA above. The marked increase in CA between the M3 and EIPPH age classes in males appears to reflect in large part a decrease in MA.

Females, in contrast, showed only a slight decrease in MA during this time. That the LOWESS plot for TA in females also showed a slight decrease between the M3 and EIPPH age classes suggests that this trend may reflect sampling bias.

Examples of individuals from each age class are presented in Figure 4-3 a and b.

Statistical contrasts between sexes and between elements. Femur length was significantly longer than humeral length in the M1 – EIPPH age classes, while results for geometric properties were variable. (Table 4-5). While several contrasts revealed significantly greater geometric properties in the femur compared to the humerus, scatterplots showed substantial overlap in geometric properties among elements in all age classes. (Figure 4-2 b). All contrasts among sexes in the pooled M3 –EIPPH subsample were statistically-significant except MA, reflecting the larger body size and long bone dimensions in males compared to females. (Table 4-6).

H. lar. Figure 4-4 a shows growth of males and females in absolute body mass, femur length and geometric properties plotted against biological age class. As growth trajectories for $I_{\max}$, J and section moduli were similar to each other, only Z_p is shown here. First, although LOWESS plots for males were slightly elevated above those for females, growth trajectories appeared to be largely overlapping throughout postnatal growth for all variables examined. Second, bone length appeared to increase steadily across biological age classes, before leveling off following the M3 age class. Geometric properties, in contrast, showed evidence of continued growth through the EIPPH age class.

As shown in Figure 4-4 b, growth trends in bone length and geometric properties

were similar for the femur and humerus in *H. lar*, despite the greater length of the humerus across all age classes. There was a substantial degree of overlap in femur and humerus geometric properties at all ages.

Figure 4-4 c shows ontogenetic variation in TA, CA, and MA. TA showed similar growth trends as described above for other geometric properties. MA continued to increase throughout all age classes examined.

Examples of individuals from each age class are presented in Figure 4-5 a and b.

Statistical contrasts between sexes and between elements. Significant contrasts between elements are presented in Table 4-7. Bone length was found to differ significantly among elements in the M2 - EIPPH age classes, reflecting the longer humeri compared to femora. Element contrasts in geometric properties were largely non-significant, except CA in the M3 and EIPPH age classes. Femoral CA was slightly larger than humeral CA in these age classes, although scatterplots showed substantial overlap. (Figure 4-4 b). There were no statistically-significant sex contrasts in the M3 – EIPPH subsample. (Table 4-8).

P. troglodytes. Figure 4-6 a shows growth of the combined-sex sample in bone length and geometric properties of the femur, plotted against estimated age in months. As growth trajectories for $I_{\max}$, J and section moduli were similar to each other, only Z_p is shown here. The intervals between the M1 and M2 age classes can be characterized by relatively slower growth compared to the preceding and following periods, particularly for $I_{\max}$, J and section moduli. Whether rapid growth between the M2 and M3 age classes continued through the EIPPH age class is unclear, as the two individuals in this age class

exhibited considerable variation in geometry (but not length).

Figure 4-6 b shows ontogenetic variation in TA, CA, and MA. TA showed similar periods of more rapid growth between the DECID – M1 and between the M2 - M3 age classes compared to the intervening period. However, TA also showed evidence of substantial growth following M3 emergence. MA also continued to increase through the EPIPH age class.

Examples of individuals from each age class are presented in Figure 4-7.

Normalized growth patterns in C. aethiops, H. lar, and P. troglodytes. Figure 4-8 a-c shows growth in body mass, femur and humerus length and geometric properties across age classes in *C. aethiops*, *H. lar* and *P. troglodytes* (femur only), respectively, with values normalized to the maximum age group mean. Plots for normalized section moduli (Z_x , Z_y and Z_p) were very similar, with little spread between them. The same was true for plots of normalized J and I_{max} , which were typically displaced below section moduli. Plots for normalized body mass, bone length, CA, J and Z_p are shown here.

Inspection of these plots reveals that in *C. aethiops* and *H. lar*, cross-sectional geometric properties tracked growth in body mass more closely than bone length. For example, in the combined-sex *C. aethiops* sample, individuals had attained approximately 70% of their adult femur and humerus lengths and only 35-40% of their adult body mass in the M1 age class. Also at this age, femoral and humeral CAs were approximately 40% of their adult size, and other geometric properties were between 20 – 30% of their adult dimensions. Section moduli shared nearly identical normalized growth patterns, which were largely superimposed on the body mass trajectory. Sex differences in growth in

body mass, bone length and geometric properties were evident in *C. aethiops*, and reflect the larger absolute size attained and the longer growth period in males. (Figure 4-8 a). *H. lar* showed no evidence of sex differences in normalized growth patterns. In *P. troglodytes*, elevated growth rates for geometric properties following M2 emergence were particularly evident when normalized values were considered.

Ontogenetic variation in diaphyseal proportions.

Development of diaphyseal strength proportions was examined using an index of relative mediolateral bending rigidity (Z_y / Z_x); the angle, $\emptyset$, representing the orientation of $I_{\max}$; and the principle moments of area index ($I_{\max} / I_{\min}$). As there were no significant contrasts among sexes within taxa in shape proportions (Table 4-9) and clear trends were not apparent from visual inspection of the data, results are reported for combined-sex analyses.

Relative bending rigidity in anatomically-defined axes: Z_y / Z_x . The index Z_y / Z_x was used as a means of examining ontogenetic variation in mediolateral / anteroposterior bending rigidity at the midshaft femur and humerus. Figure 4-9 plots raw values and age class means for *C. aethiops*, *H. lar* and *P. troglodytes*, respectively. Generally, unidirectional trends in Z_y / Z_x were observed across age classes within taxa. Nonparametric one sample Wilcoxon tests were employed to detect significant departures in age group medians from 1.0, indicating section circularity.

C. aethiops. Kruskal-Wallis test detected no statistically-significant variation in Z_y / Z_x among age classes for the midshaft femur and humerus. (Table 4-10). As shown

in Figure 4-9, Z_y / Z_x decreased slightly with age at the midshaft femur, from a DECID age class mean of 1.07 to an EPIPH age class mean of 0.98. No significant deviations from section circularity were detected (Table 4-11). At the midshaft humerus, Z_y / Z_x increased with age from a DECID age class mean of 1.03 to an EPIPH age class mean of 1.12, indicating slight preferential resistance to bending in the mediolateral plane. Results of statistical analysis detected significant deviation from section circularity in the total sample, and the M2 and EPIPH age classes when considered separately. (Table 4-11).

H. lar. Kruskal-Wallis test detected statistically-significant variation in Z_y / Z_x among age classes for the midshaft femur and humerus. (Table 4-12). As shown in Figure 4-9, femur midshaft Z_y / Z_x increased with age, from a DECID age class mean of 0.96 to an EPIPH age class mean of 1.07. The midshaft humerus also increased in mediolateral bending strength with age, from a mean index of 0.85 in the DECID age class to a mean of 1.07 in the EPIPH age class. Results of statistical analysis were significant for midshaft femur and humerus Z_y / Z_x in the oldest age classes, indicating slight preferential resistance to mediolateral bending. (Table 4-13).

P. troglodytes. Statistical analysis was not undertaken in the *P. troglodytes* sample, due to small and unequal sample sizes across age classes. However, age trends in femur Z_y / Z_x can be appreciated in Figure 4-9. Femur midshaft Z_y / Z_x increased in early ontogeny, from a DECID age class mean of 0.92 and reaching an EPIPH age class mean of 1.04.

Orientation of the principle axis of greatest bending rigidity: Theta (θ). As can be

appreciated in Figure 4-10, the axis of greatest bending rigidity did not always coincide with anatomical axes. In *C. aethiops*, the orientation of $I_{\max}$ at the midshaft femur was predominantly anteromedial - posterolateral, and rotated slightly towards the anteroposterior plane in later ontogeny. At the midshaft humerus, $I_{\max}$ was predominantly located in or near the anterolateral – posteromedial plane. At the midshaft femur of *P. troglodytes*, the orientation of $I_{\max}$ was roughly coincident with the anteroposterior anatomical plane in DECID individuals, and it rotated to a more medio-lateral or anteromedial - posterolateral orientation in older individuals. *H. lar* showed more variation in the position of $I_{\max}$ compared to other taxa examined here, particularly at the midshaft femur. At the midshaft humerus, the orientation of $I_{\max}$ was roughly coincident with the anteroposterior anatomical plane in DECID individuals, and rotated to a predominantly mediolateral or anterolateral - posteromedial orientation during later ontogeny.

Principle moments of area (PMA) index: $I_{\max} / I_{\min}$. Because the orientation of maximum bending rigidity did not always coincide with anatomical axes, the PMA index was also examined as an indicator of relative bending rigidity in principle axes at the midshaft femur and humerus. Figure 4-11 plots raw values and age class means for *C. aethiops*, *H. lar* and *P. troglodytes*, respectively. Nonparametric one sample Wilcoxon tests were employed to detect for significant deviations in age group medians from 1.0, indicating section circularity.

C. aethiops. Overlap among age classes in PMA index at the midshaft femur can be appreciated in Figure 4-11. An overall age-related increase in PMA index was

apparent at the midshaft humerus, from a DECID age class mean of 1.47 to an EPIPH age class mean of 1.73. However, Kruskal-Wallis test detected no statistically-significant contrasts among age classes in either element. (Table 4-14). Table 4-15 reports results of one sample Wilcoxon tests. All contrasts were statistically-significant, indicating preferential resistance to bending in the major axis. This departure from circularity was pronounced at the midshaft humerus, as can be appreciated in Figure 4-11.

H. lar. Overlap among age classes in PMA index at the midshaft femur can be appreciated in Figure 4-11. A decrease in PMA index was apparent at the midshaft humerus, from a DECID age class mean of 1.44 to an M1 age class mean of 1.15. Thereafter, PMA index exhibited no change. Kruskal-Wallis also detected statistically-significant variation among age classes at the midshaft humerus. (Table 4-16). Table 4-17 reports results of one sample Wilcoxon tests. Results for the total sample and for older age classes were statistically-significant.

P. troglodytes. Statistical analysis was not undertaken in this sample. Overlap among age classes in PMA index can be appreciated in Figure 4-11. PMA ratios were typically between 1.05 – 1.35 for the midshaft femur.

DISCUSSION

Growth of body mass, bone length and geometric properties.

Prior studies have demonstrated that ontogenetic variation in femur and humerus cross-sectional geometric properties reveals many of the same growth trends as observed in other body dimensions, such as periods of growth acceleration and sexually-dimorphic patterns of growth (Burr et al., 1989a; Ruff, 2003a). A similar conclusion is reached in

the current study. Nonparametric LOWESS regression was used to characterize growth – related variation in body mass (where possible), bone length and cross-sectional geometric properties at the midshaft femur and humerus of *C. aethiops* and *H. lar*, and at the midshaft femur of *P. troglodytes*. This variation is considered in light of earlier reports of postnatal growth in these taxa.

Chlorocebus aethiops. Nonparametric statistical analysis revealed significant contrasts in body mass, bone length and geometric properties among sexes in the M3 – EPIPH age classes, reflecting the absolutely larger size of adult males compared to females in all variables studied. Sexual dimorphism in growth patterns was also evident. Growth patterns in body mass, linear and geometric variables were similar among males and females until M2 emergence (estim. 26 - 31 months, Bolter and Zihlman, 2003), after which time they diverged. Following M2 emergence, males exhibited a period of more rapid growth compared to females, lasting through M3 emergence (estim. 38 - 67 months, Bolter and Zihlman, 2003). Males continued growth in most variables examined, particularly body mass and cortical area, until completion of long bone epiphyseal union. Females, on the other hand, appeared to complete most of their growth by M3 emergence. Growth patterns of the femur and humerus showed similar trends, despite the significantly greater length of the femur across all age classes.

Growth patterns for variables examined here are consistent with Bolter and Zihlman's (2003) examination of growth in body mass and musculoskeletal dimensions in the larger collection from which the current sample was derived. By M3 emergence, they reported that female vervets had reached 97% of their mean adult body mass, while

males had reached only 80% of their mean adult body mass. A similar pattern of dimorphism was observed for bone length. The dental emergence chronology for vervets communicated in Bolter & Zihlman (2003, provided by L. Fairbanks) suggests an age range of 38 – 67 months for M3 emergence. However, they also noted that M3s tend to erupt at approximately 4.5 years of age in males, and 5 years of age in many females. Thus, while dental maturation is estimated to occur slightly later in females compared to males, body mass and skeletal measurements apparently reach their adult sizes relatively earlier.

Growth patterns observed in the current sample are also consistent with other reports on postnatal growth in *C. aethiops*. Prior research on body mass growth in captive animals (Leigh, 1992, 1996) and wild populations from Kenya (Turner et al., 1997) and the West Indies (Horrocks, 1986), has shown that the larger adult size of males is attained primarily as a result of their longer growth durations, though they also exhibit slightly faster growth rates compared to females. For instance, Leigh (1992:35, Table 2) reported ages at cessation of body mass growth in captive vervets as 4.00 years for females and 5.50 years for males. Males and females in his sample were characterized by similar maximum growth rates of approximately 1 kg / year, following birth. However, their growth trajectories diverged at approximately 2 years of age, after which time females showed a deceleration in growth, while males exhibited a slight but definable growth spurt (Leigh, 1996:462, Figure 4). Current results and Bolter and Zihlman (2003) suggest a similar pattern of bimaturism and slightly elevated male growth rates in wild-collected Ugandan vervets.

The relative timing of this divergence in male and female growth patterns in the

current sample is also generally consistent with other published accounts of vervet growth (Horrocks, 1986; Turner et al., 1997). In a larger cross-sectional study of wild vervets across four different sites in Kenya, Turner et al. (1997) found that male and female growth patterns for body mass and other dimensions diverged following eruption of the central incisors [corresponding to Bolter and Zihlman's (2003) age class 3]. Males in their study continued rapid growth thereafter and showed evidence of growth in most variables into adulthood (defined by the presence of completely erupted permanent dentitions), while females reached most of their adult size by canine emergence [corresponding to Bolter and Zihlman's (2003) age class 6]. Interestingly, female body mass was particularly variable across sites, which they suggested relates to the proximity of some sites to cultivated areas and tourism. Given the lack of individuals of age class 3 and the presence of only a single female belonging to age class 6 in the current sample, findings presented here are generally consistent with Turner et al. (1997).

Horrocks (1986) reported that male and female growth patterns diverged after 1.5 years, which appears to be consistent with Leigh (1996: 462, Fig. 4), and that females completed body mass growth by the end of their fifth year. This age at completion of female body mass growth is slightly later than suggested by the current study and other reports (Leigh, 1992; Turner et al., 1997), and may reflect population variability in adult female body mass. Alternatively, this may result from the fact that individuals in Horrocks' (1986) study were of known chronological age, while studies of wild-caught animals (Bolter and Zihlman, 2003; Turner et al., 1997) relied on dental eruption chronologies based on captive animals to estimate age. Primates living in the wild have been shown to complete dental maturation and growth in other body dimensions later

than individuals living in captive environments (Altmann and Alberts, 1987; Hamada et al., 1996; Phillips-Conroy and Jolly, 1988; Zihlman et al., 2004). It is possible that dental development is also slightly delayed in wild vervets relative to their captive counterparts, and estimated ages reported here are younger than actual chronological ages of individuals in the sample.

Hylobates lar. As dental emergence chronologies are not well-characterized for *Hylobates* species, growth was plotted against biological age class. Several patterns emerged in the current study. Male and female growth trajectories were largely overlapping across age classes for all variables examined, and this was supported by statistical analysis. Many variables exhibited continued growth following M3 emergence, until completion of long bone epiphyseal union and possibly thereafter. Also, growth patterns of femur and humerus geometric properties showed similar trends, despite the statistically-significantly longer humerus compared to the femur in M2 – EPIPH age classes.

This lack of sexual dimorphism in bone cross-sectional growth is consistent with reports of postnatal growth in other body dimensions in gibbons (Leigh, 1992, 1996). However, the lack of known chronological or estimated age for individuals in the current study makes direct comparisons difficult. Much of what we know of the growth patterns of *H. lar* is based on examinations of body mass growth in captive animals of known chronological age. Leigh (1992) reported a lack of bimaturism and significant growth rate differences among males and females. Captive gibbons experience a steady linear decline in growth velocity from a maximum rate of less than 1.5 kg / year following birth,

reaching adult body mass at approximately 5.75 years of age (Leigh, 1992; Leigh and Shea, 1995).

Gibbons in the wild also reach adult body size at approximately 6 years of age (Gittins and Raemaekers, 1980; Leighton, 1987). However, the current study sample shows evidence of continued growth in long bone geometric properties following M3 emergence, which was estimated by Dirks (2003) to occur at 6.1 years of age in a wild-collected individual from Thailand. In the absence of body mass data for many older individuals examined in the current study, it cannot be determined whether observations reported here reflect a later age at attainment of adult body mass compared to other wild populations. It is interesting to note that Brockelman and colleagues (1998) reported that *H. lar* individuals in Khao Yai National Park do not attain adult size until approximately 8 years of age. The later age at body size maturation in this latter population is also associated with a high population density and delayed dispersal of individuals from their natal groups.

Pan troglodytes. In *P. troglodytes*, bone length and geometric properties was examined in the combined-sex sample. Inspection of LOWESS plots and mean plots of normalized values revealed that the period following M2 emergence (estimated at 6.45 - 6.47 yrs., Smith et al., 1994) can be characterized by more rapid growth in geometric properties compared to earlier ontogeny. Whether the trend of rapid growth between M2 and M3 emergence (the latter estimated at 10.27 - 10.71 yrs., Smith et al., 1994) continues thereafter is difficult to discern. However, results indicate continued growth until completion of long bone epiphyseal union.

Postnatal growth of *P. troglodytes* has been the focus of a number of previous examinations in captive animals of known chronological age (e.g., Gavan, 1953; Hamada et al., 1996; Leigh and Shea, 1996; Watts and Gavan, 1982). The larger adult size of male chimpanzees is attained through sexually dimorphic patterns of growth. Leigh and Shea (Leigh and Shea, 1995, 1996) reported that male and female growth patterns for body mass diverge at approximately 7.5-8 years of age, after which time males exhibit a pronounced subadult growth spurt, reaching a growth velocity of just under 8 kg / year. Although female growth rates also appear to be elevated during this time compared to earlier in ontogeny, they do not exhibit a distinct peak. This increase in growth velocity in males and females is initiated at approximately 5.5 years. A consistent and distinct growth spurt does not appear to characterize linear dimensions in all individuals studied (Hamada and Udono, 2002). Further, there is minimal bimaturism in common chimpanzees, with males and females in captivity reaching adult body mass at approximately 12-13 years and 11.5-12 years, respectively (Leigh and Shea, 1995).

In the current sample, evidence of relatively rapid growth in geometric properties was suggested for the period between M2 emergence, at approximately 6.5 years of age, and M3 emergence, between 10 and 11 years of age (based on Smith et al., 1994). This timing coincides with a definable body mass growth spurt in males and elevated growth rates in females described for captive animals (Leigh and Shea, 1995, 1996). Observations of the current study also suggest that growth in diaphyseal geometric dimensions may continue between M3 emergence and completion of long bone epiphyseal union (estimated to occur by approximately 15 years of age, Kerley, 1966). While this is consistent with reported ages at body mass growth cessation for captive

animals, it does not exclude the possibility that diaphyseal growth continues thereafter. Kikuchi (2003) also reported that cortical area at the distal radius exhibited linear growth until approximately 16-17 years and 12-13 years of age in male and female captive common chimpanzees, respectively.

As in many other catarrhines, development in wild chimpanzees is delayed relative to their captive counterparts (Hamada et al., 1996). For example, Pusey (1978) suggested that chimpanzees living in the wild at Gombe don't reach adult body mass until after 15 years of age. If ages in the current study are estimated based on the recent dental and skeletal chronology provided by Zihlman and colleagues (2004) for wild common chimpanzees, faster growth in geometric properties in the current sample is estimated to occur between roughly 8.3 and 12.4 years of age (corresponding to the M2 and M3 age classes, respectively), with growth continuing at least until completion of long bone epiphyseal union by approximately 13.4 - 16.5 years of age.

Comparisons of growth in body mass, bone length and geometric properties.

Normalized mean plots were generated to permit comparison of growth-related changes in body mass, bone length and geometric properties in each taxon. Results demonstrated that growth in geometric properties lagged behind growth in bone length, and more closely paralleled growth in body mass. While growth in bone length slowed following M3 emergence, geometric properties showed evidence of continued and often substantial increases in size between the M3 and EIPH age classes. This trend was most pronounced in *H. lar* and in *C. aethiops* males. (Although, in the latter, growth in geometric properties following M3 emergence may reflect, in part, a decrease in MA

dimensions). *C. aethiops* females were an exception to this pattern, in a manner that is consistent with bimaturism in this species (Leigh, 1992). Although body mass data were not available for *P. troglodytes*, this taxon showed a more linear growth pattern for bone length, apparent through the EIPPH age class.

This close correspondence between growth trajectories for cortical area and other geometric properties with growth trajectories for body mass, and a dissociation between growth in bone length and diaphyseal geometry, has been reported previously (e.g., Frisancho et al., 1970; Ruff, 2003a; Ruff et al., 1994; Sumner and Andriacchi, 1996). Subperiosteal expansion and increases in cortical area have been shown to continue into the third or fourth decade of life in humans, well after attainment of complete dental maturity and epiphyseal union, thus increasing cortical robusticity relative to length in older juveniles and young adults (Ruff and Hayes, 1983b; Ruff et al., 1994). This apparent decoupling of bone length and geometric properties during growth is suggested to reflect a primary influence of loads due to body mass on growth-related increases in diaphyseal geometry (Moro et al., 1996; Ruff et al., 1994; van der Meulen et al., 1996). However, non weight-bearing elements also show this pattern. This suggests that other factors, such as growth in muscle mass, are also important (Ruff, 2003a; Sumner and Andriacchi, 1996). For example, while growth velocities for femoral strength (i.e., as measured by geometric properties) in humans are highly correlated with body size growth velocities, humeral strength velocities are more highly correlated with muscle area growth velocities (Ruff, 2003a). Also, cortical area and other measures of diaphyseal strength in the human radius are highly correlated with forearm muscle area and grip strength (Schoenau, 1998; Schoenau et al., 2000).

Element contrasts in growth of geometric properties

Growth trends for geometric properties were similar among elements in *C. aethiops* and *H. lar* examined here, despite significant contrasts in bone length. Both taxa showed substantial overlap in geometric properties among elements. However, statistically-significant contrasts were identified in some comparisons, with femoral strength properties being greater than humeral strength properties. These observations are summarized and discussed briefly below.

In *C. aethiops*, statistical analyses revealed significantly stronger femoral diaphyses compared to humeral diaphyses in several geometric properties. However, results were variable across age classes. Slightly increased femoral strength is consistent with previous reports on other cercopithecines (Ruff, 2002; Schaffler et al., 1985), where it has been attributed to the importance of the femur in propulsion during frequent hindlimb-dominated behaviors, such as quadrupedalism and leaping. It also reflects a general primate trend of “hindlimb dominance. Compared to other mammals, primates have been characterized as having greater peak vertical and propulsive forces generated in the hindlimbs compared to the forelimbs during quadrupedal locomotion (Demes et al., 1994; Kimura, 1985, 1992; Kimura et al., 1979; Reynolds, 1985; Schmitt and Hanna, 2004). Primates also exhibit variation in this pattern. Among anthropoids, hindlimb dominance tends to be most pronounced in arboreal species. In the latter, lower peak vertical force components and increased limb differentiation compared to terrestrial species is suggested to reflect the importance of reducing stress and maintaining mobility in the forelimb (Demes et al., 1994; Kimura, 1985, 1992; Kimura et al., 1979; Reynolds,

1985; Schmitt and Hanna, 2004).

It has been suggested that reduced peak forces on arboreal supports is accomplished primarily by adoption of a compliant gait, characterized by increased limb protraction, joint yield and contact time (Schmitt, 1999, 2003b; Schmitt and Hanna, 2004). Vervet monkeys, which reportedly spend approximately 80% of their locomotor time on arboreal substrates (Rose, 1979), experience significantly higher peak vertical and accelerating forces in the hindlimbs compared to the forelimbs (i.e., more limb differentiation) when walking across a level platform compared to more terrestrial cercopithecines (Demes et al., 1994). Interestingly, vervets also appear to use more compliant gaits both on the ground and on simulated arboreal supports, and peak vertical forces experienced by the forelimbs are similar on both substrates (Schmitt, 1999, 2003b).

In *H. lar* examined here, femoral diaphyses were revealed to have significantly larger cortical areas compared to humeri in the M3 and EIPPH age classes. This pattern is consistent with previous reports on *H. lar* (Ruff, 2002; Schaffler et al., 1985). It is interesting that gibbons would also reflect some degree of hindlimb dominance, given that the forelimb is the dominant propulsive element during brachiation. However, as noted by Schaffler and colleagues (1985), increased relative femoral strength may reflect the importance of the hindlimb in support and propulsion during non-suspensory positional behaviors, such as climbing, leaping and bipedalism (Fleagle, 1980).

Variation in diaphyseal cross-section proportions

Development of diaphyseal strength proportions was examined using an index of

relative mediolateral bending rigidity (Z_y / Z_x). An index greater than 1.0 indicates a midshaft geometry characterized by increased bending rigidity in the mediolateral plane relative to the anteroposterior plane. However, in many cases, the axis of greatest bending rigidity ($I_{\max}$) did not correspond to anatomically-defined axes. Therefore, the orientation of $I_{\max}$ (represented by the angle, $\emptyset$) and the principle moments of area (PMA) index ($I_{\max} / I_{\min}$) were also reported for descriptive purposes.

Bending rigidity in mediolateral (ML) and anteroposterior (AP) planes: Z_y / Z_x .

Results reported here are generally consistent with earlier findings. In *C. aethiops*, statistical analyses revealed no significant contrasts among age classes in Z_y / Z_x at the midshaft femur and humerus. However, a slight age-related decline in relative ML bending rigidity was visually apparent at the midshaft femur, reaching an EIPPH age class mean of 0.98 and indicating a circular geometry. At the midshaft humerus, Z_y / Z_x increased from a DECID age class mean of 1.03 to an EIPPH age class mean of 1.12. Statistical analyses revealed a significant departure from circularity in the EIPPH age class, indicating preferential resistance to ML bending.

C. aethiops has not been the focus of previous examination. However, in a study of other Old World monkey species, Schaffler and colleagues (1985) reported increased relative ML bending rigidity (as reflected in I_{ml}/I_{ap}) at the midshaft femur and humerus of adult *Macaca fascicularis* and *Presbytis cristata*, and at the midshaft humerus of *M. nemestrina*. Burr and colleagues (Burr et al., 1981; Burr et al., 1989b) reported slightly increased ML bending strength at the midshaft femur of *Macaca* sp., coincident with increased ML bone density (Burr, 1979). However, while *C. aethiops* observed here also

showed increased ML bending rigidity at the midshaft humerus, the pattern observed for the midshaft femur was more similar to that reported for the more terrestrial macaque species, *M. nemestrina* (Schaffler et al., 1985). Both taxa are characterized by a femoral midshaft cross-section equally well adapted to bending in either (AP or ML) plane.

Whether these differences in femoral structural proportions are biologically significant is not entirely clear; the midshaft femora of arboreal and terrestrial Old World monkey taxa examined in the current and previous investigations are all characterized by a circular geometry overall, with mean I_{ml}/I_{ap} (equivalent to I_y/I_x in the current study) usually of 1.11 or less (Burr et al., 1981; Schaffler et al., 1985).

In *H. lar*, results indicated a statistically-significant increase in relative ML bending rigidity at the midshaft femur and humerus across age classes, with both elements reaching an EIPPH age class means of 1.07. This departure from circularity was statistically significant in the oldest age classes. Schaffler and colleagues (1985) and Ruff and Runestad (1992) also reported slightly increased rigidity in ML bending for adult *H. lar*. However, in the former study, relative ML bending rigidity as reflected in second moments of area was higher at the midshaft humerus ($I_{ml}/I_{ap} = 1.22$) than reported here.

At the midshaft femur of *P. troglodytes*, an increase in Z_y/Z_x was apparent between the DECID and M1 age classes. However, a maximum age class mean index of 1.06 indicated a circular geometry overall, with slight reinforcement in the ML plane. A pattern of increased ML bending rigidity has been reported previously for adult animals (Carlson, 2002; Ruff, 1989; Ruff and Runestad, 1992). Results of the current study are also consistent with Ohman's (1993) observations of I_{max} and the plane of maximum

cortical thickness, which tended to show a predominantly ML orientation.

In summary, with the exception of the midshaft femur of *C. aethiops*, most taxa examined here exhibited slightly increased ML bending rigidity at the midshaft femur and humerus. This pattern resulted from an age-related increase in ML bending rigidity during postnatal ontogeny, and was most pronounced at the midshaft humerus of *C. aethiops*. Other taxa and elements examined exhibited a fairly circular geometry overall; the difference between ML and AP bending rigidity was typically below 20% (reflected in a Z_y / Z_x index of 0.8 - 1.2). Increased ML bending rigidity has been reported previously for several anthropoid primates, particularly taxa characterized by greater use of arboreal substrates (Burr et al., 1981; Burr et al., 1989b; Carlson, 2002; Ohman, 1993; Schaffler et al., 1985). However, the absence of available *in vivo* strain data for most primates examined, and particularly for behaviors other than quadrupedalism (but see Swartz et al., 1989), has meant that relating this structural pattern to any specific aspect of the locomotor-induced mechanical environment has been difficult.

Ruff and colleagues (Ruff, 1989; Ruff and Runestad, 1992) reported a size-related trend in relative ML bending rigidity of the femoral shaft among adult primates, which they suggested relates to proposed mechanisms for maintaining similar peak strain levels during locomotor behaviors across animals of varying body size. Peak bone strain levels are maintained in larger-bodied animals during dynamic locomotor behaviors through postural adjustments, such as utilizing more extended limb postures, which increase the effective mechanical advantage of limb musculature and minimize loads (Biewener, 1983, 1990, 1991; Polk, 2002; Rubin and Lanyon, 1984). However, Ruff and Runestad (1992) proposed that such changes in limb angulation, if they occur predominantly in a

parasagittal plane, would lead to the reduction of only AP bending loads. Among primates examined by them, Old World monkeys and gibbons possessed fairly circular midshaft femoral geometries with slight reinforcement in ML bending (as reflected in I_y/I_x), while increased ML bending rigidity was most pronounced in large-bodied great apes.

Strain gauge analysis is the most direct means of determining the *in vivo* loading environment of limb elements during locomotor behaviors. The few *in vivo* strain analyses undertaken thus far have demonstrated that significant ML bending forces may be generated during quadrupedal locomotion in nonhuman primates. In a study of rhesus macaques moving quadrupedally across a level platform, bending occurred in the ML plane at the midshaft ulna and in the anteromedial - posterolateral plane in the midshaft tibia (Demes et al., 2001; Demes et al., 1998). Demes and colleagues suggested that bending patterns observed at these skeletal sites reflect both limb kinematics (particularly, the inclination of the substrate reaction force resultant relative to limb position) and local muscle forces. Interestingly, however, planes of greatest structural reinforcement at the midshaft ulna and tibia (as reflected in cross-sectional geometry) of these animals did not coincide with observed planes of *in vivo* bending in their study (Demes et al., 2001; Demes et al., 1998).

In vivo strain analysis of the midshaft humerus of gibbons during brachiation demonstrated a predominantly tensile loading regime, combined with torsion and AP bending (Swartz et al., 1989). This latter finding is not in agreement with slightly increased structural rigidity in the ML plane, as observed in the current study and also reported by Schaffler and colleagues (1985). This may reflect the recent realization that

the relationship between diaphyseal cross-sectional geometry and *in vivo* strains is not always as straightforward as theoretical models predict (Demes et al., 2001; Demes et al., 1998; Lieberman et al., 2004). In some animals, bone may be preferentially distributed in the orthogonal plane, to increase load predictability and minimize resistance in the plane in which bending normally occurs (Lanyon and Rubin, 1984). Alternatively, slightly increased ML bending rigidity at the midshaft femur and humerus in gibbons may reflect loading regimes associated with other positional behaviors (e.g., climbing), for which *in vivo* strains have not yet been systematically characterized. Unfortunately, *in vivo* strain data are not available for *P. troglodytes* or other great apes.

Recent force-platform analyses characterizing external forces on the limbs have also indicated the prominence of ML – directed forces generated during primate locomotion (Carlson et al., 2005; Demes et al., 2006; Schmitt, 2003a). For instance, Schmitt (2003a) demonstrated that the substrate reaction force (SRF) resultant of the forelimb was ML-inclined during quadrupedal walking in several Old World and New World monkey taxa, and that this varied with the type of substrate used. The magnitude of ML -directed SRFs was usually lower on a simulated arboreal substrate compared to a level platform (as also reported for lemurids by Carlson et al., 2005). Interestingly, however, both *C. aethiops* and *M. mulatta* were found to be partial exceptions in Schmitt's (2003a) study, in that both experienced forelimb SRFs on simulated arboreal substrates which were generally higher than for other primates examined, and similar in magnitude to SRFs experienced on the ground. However, he did not examine SRFs in the hindlimb. An earlier study by Kimura and colleagues (1979) demonstrated a transverse force component in the fore- and hind-limbs during quadrupedal locomotion in

several anthropoid taxa, including both great apes and Old World monkeys.

As suggested by Dickinson and colleagues (2000), forces generated orthogonal to the direction of travel may be important both for balance and stability of locomotion, as well as for maneuverability. In nonhuman primates, Demes and colleagues (2006) reported that ML forces are considerably higher during turning behaviors (i.e., during changes in the direction of travel) than during straight-line travel in ring-tailed lemurs and patas monkeys moving quadrupedally on both platforms and simulated arboreal substrates. Further, Carlson (2006; pers. comm.) demonstrated that ML forces increase with the degree of limb abduction in other lemurids, independently of locomotor speed. These observations may have implications for understanding slightly increased ML bending rigidity in limb diaphyses of primates engaging in other behaviors which involve abducted limb postures, such as occurs during many arboreal locomotor behaviors (e.g., brachiation, vertical climbing and scrambling, the latter involving more variably-oriented limb positions, Hunt et al., 1996; Isler, 2005).

Orientation of I_{max} and the principle moments of area (PMA) index. Recent investigations have tended to focus on geometric properties measured in anatomically-defined (i.e., AP or ML) axes or average estimates of bending rigidity, such as J and Z_p , as these can more readily be related to predicted patterns of limb loadings associated with species-specific locomotor behaviors (e.g., Ruff, 1987; Ruff, 1989, 2002; Ruff and Runestad, 1992; Schaffler et al., 1985). There exist fewer comparative data on the orientation of I_{max} (represented by the angle, $\emptyset$) and principle moments of area proportions (measured by I_{max} / I_{min}). In the current study, strength proportions exhibited

stronger evidence of unidirectional bending when PMA index was considered, compared to patterns described above for Z_y / Z_x . These observations are discussed briefly below, in light of available evidence. Because $\emptyset$ and PMA index are descriptors of diaphyseal shape and are hypothesized to have mechanical relevance, they may also relate in some way to regional variation in microstructural features examined in later analyses.

The midshaft humerus of *C. aethiops* in the current study was characterized by an $I_{\max}$ predominantly oriented in an anterolateral – posteromedial plane across all age classes, consistent with the pattern reported previously for *Macaca* sp. (Burr et al., 1989b). Further, PMA index deviated substantially from circularity (reaching an EPIPH age class mean of 1.73), indicating a structural adaptation for unilateral bending in this plane. At the midshaft femur of *C. aethiops*, $I_{\max}$ was located in a predominantly anteromedial - posterolateral plane throughout ontogeny, and rotated to a slightly more AP orientation in several individuals of the M3 and EPIPH age classes. This likely contributes to the decline in relative ML bending strength observed with age, as discussed above. This pattern may be attributed to the prominence of the linea aspera along the posterolateral aspect of the diaphysis, as was also noted previously for the proximal femur diaphysis of *Macaca* sp. (Burr et al., 1981; Burr et al., 1989b). Alternatively, increased rigidity in the AP plane may reflect the importance of the femur in propulsion during quadrupedalism, as well as other positional behaviors such as leaping (e.g. Burr et al., 1982; Demes and Jungers, 1993; Terranova, 1995a).

H. lar exhibited a more variable position of $I_{\max}$ (represented by $\emptyset$) and a fairly circular geometry overall, particularly at the midshaft femur. At the midshaft humerus, individuals of DECID age class were characterized by an $I_{\max}$ located in a predominantly

AP plane and a PMA index indicating more pronounced structural reinforcement for unidirectional bending, while older age classes were characterized by a reduction in PMA index and a shift in $I_{\max}$ to an anterolateral or ML position. Results for older age classes were broadly consistent with prior reports on examinations of adult gibbons, as discussed above (Ruff and Runestad, 1992; Schaffler et al., 1985).

In *P. troglodytes*, the orientation of $I_{\max}$ at the midshaft femur was roughly coincident with the AP plane in the DECID age class, while it varied between a more or less ML orientation and an anteromedial - posterolateral orientation in older age classes. Ohman (1993) and Carlson (2002) reported a predominantly medio-lateral orientation of $I_{\max}$ at the midshaft femur in adult common chimpanzees, though considerable variability was observed in this parameter and in the distribution of cortical thickness (for the latter, see Ohman, 1993: Figures 52 and 66, respectively). PMA indices reported in the current study are generally consistent with earlier reports (Carlson, 2002, 2005; Ohman, 1993), though midshaft femur PMA ratios reported here were slightly lower than those reported by Carlson (Carlson, 2002, 2005).

The more variable position of $I_{\max}$ in gibbons and at the midshaft femur of common chimpanzees compared to vervet monkeys may reflect arboreal locomotor behaviors other than quadrupedalism characteristic of the former taxa (Doran, 1992; Doran and Hunt, 1994; Fleagle, 1980). In an *in vivo* strain analysis of the forelimb of four brachiating gibbons, Swartz and colleagues (1989) demonstrated that the midshaft humerus experienced a predominantly tensile loading regime, with torsion and bending in the AP plane also indicated. While individuals observed in the current study exhibited slight structural reinforcement in the *ML* plane, Swartz and colleagues also noted that

individuals in their study experienced “...considerable variability in strain pattern and peak magnitude among swings...” during brachiation (Swartz et al., 1989: 270). Further, Demes and colleagues (2001) reported more variation in strain pattern observed in the tibial midshaft of a single *M. mulatta* during climbing compared to walking across a level platform.

Thus, as noted by Carlson (Carlson, 2002, 2005), the available *in vivo* strain evidence seems to suggest that some arboreal locomotor behaviors may impose more variably-oriented bending loads on supporting limb elements compared to locomotor behaviors involving more stereotyped limb movements, such as occurs during terrestrial quadrupedalism. Bending loads engendered during arboreal locomotion are predicted to be multi-directional, as individuals navigate three-dimensionally complex substrates. In accordance with this, Carlson (2002) observed greater variability in the position of $I_{\max}$ at three sites along the femur and humerus diaphyses in *P. troglodytes* subspecies compared to *G. gorilla* subspecies, associated with increased arboreality in the former. Carlson (2005) also reported that increased frequency of total arboreal behaviors was associated with increased cross-section circularity ($I_{\min}$ approaches $I_{\max}$, as reflected in a decrease in PMA index) in the femur and humerus of African great apes. However, while arboreal walking tended to exhibit one of the weakest correlations with PMA index in *Pan*, consistent relationships between specific non-quadrupedal arboreal locomotor behaviors (*i.e.*, scrambling, quadrumanous climbing, combined suspensory behaviors) and PMA index were not apparent across all femur and humerus regions of interest in all groups. As discussed above, further research to characterize *in vivo* loading patterns associated with arboreal positional behaviors other than quadrupedalism is clearly needed.

Ontogenetic trends in diaphyseal cross-section proportions. Significant ontogenetic variability in diaphyseal proportions was apparent within taxa, and young infants and juveniles frequently did not exhibit the same diaphyseal proportions characteristic of their adult counterparts. An age-related increase in relative mediolateral bending rigidity (Z_y/Z_x) was observed at the midshaft femur and humerus of most taxa examined here, although trends were not always statistically-significant. This pattern may reflect an increase in relative mediolateral bending rigidity associated with growth-related increases in body size, in a manner that is consistent with adult size trends observed among primates by Ruff and colleagues (Ruff, 1989; Ruff and Runestad, 1992) and discussed above. Exceptions to this pattern, evident at the midshaft femur of *C. aethiops*, may reflect the importance of the femur in propulsion in quadrupedalism and other locomotor behaviors, and the influence of local sites of muscle attachment on diaphyseal geometry.

In *C. aethiops*, age-related trends in diaphyseal proportions (i.e., decreasing Z_y/Z_x at the midshaft femur and increasing Z_y/Z_x at the midshaft humerus) were accompanied by little change in the orientation of the axis of greatest bending rigidity ($I_{\max}$). (A slight anteroposterior shift in $I_{\max}$ was visually apparent in older age classes at the midshaft femur, although overlap among age classes was considerable.) Observations of $I_{\max}$ in *C. aethiops* suggest that positional behaviors practiced during early and late ontogeny may not generate qualitatively different loading patterns on these skeletal sites, while trends in Z_y/Z_x may reflect an increased frequency of adult positional behaviors as individuals develop locomotor competence and increased loadings associated with growth in body size (e.g., as suggested for dinosaurs by Heinrich et al., 1993).

However, marked variability in the orientation of $I_{\max}$ was observed at the midshaft femur and humerus of *H. lar* and the midshaft femur of *P. troglodytes*, both within and among age classes. Nevertheless, with the exception of the midshaft femur of *H. lar*, these observations reflect a ML shift in the location of $I_{\max}$ from a more AP orientation in the DECID age class, and a consequent increase in relative ML bending rigidity which appears most marked during early ontogeny. Whether this variation in the orientation of maximum bending rigidity during early ontogeny reflects changes in loading patterns associated with important qualitative shifts in the attainment of adult behavioral skills (e.g., as described by Doran, 1992) is unclear, however.

Studies of positional behavior ontogeny in primates in natural settings have documented marked changes in the modes and frequencies of positional behaviors practiced (Crompton, 1983; Doran, 1992, 1997; Dunbar and Badam, 1998; Wells and Turnquist, 2001). Initial locomotor behaviors of young infants tend to be less coordinated and frequently occur on more stable substrates compared to adults. Further, infant and juvenile primates engage in an expanded repertoire of positional behaviors, many of which are associated with play and/or occur more frequently on arboreal substrates, compared to their adult conspecifics. Despite the greater variety of positional behaviors practiced during early ontogeny, however, species-typical adult postural and locomotor preferences emerge very early in development and are evident in the behaviors of older infants. For example, rhesus macaque infants and juveniles are characterized by a variety of locomotor behaviors, such as galloping, leaping, climbing hopping, and suspensory behaviors (Dunbar and Badam, 1998; Wells and Turnquist, 2001). Yet, quadrupedalism emerges as the most common positional behavior practiced among

infants, and its frequency increases with age. A similar pattern is observed in bonnet macaques. In contrast, hopping and leaping behaviors are more frequent among langur infants (Dunbar and Badam, 1998). Similar taxonomic variability in the acquisition of adult positional behaviors has also been observed among African apes (Doran, 1992, 1997), reflecting the influence of body size and the development of species-typical locomotor behaviors from early ontogeny.

It is interesting to note that despite greater variability in the positional behavior repertoires of infants compared to adult conspecifics, DECID individuals in the current study frequently did not exhibit diaphyseal geometries indicative of more variable or multidirectional bending loads compared to individuals in older age classes. Qualitative assessment indicates that the orientation of maximum bending rigidity at the midshaft femur and humerus appeared *less* variable among individuals of the DECID age class compared to older conspecifics at the midshaft humerus of *H. lar* and the midshaft femur of *P. troglodytes*. Further, the midshaft humerus of DECID *H. lar* individuals was characterized by a less circular geometry, as reflected in a larger PMA index, compared to older age classes.

Recent observations indicate that the relationship between diaphyseal cross-sectional geometry and mechanical loading patterns associated with dynamic locomotor behaviors is not as straightforward as theoretical models based on engineering beam principles predict (Demes et al., 2001; Demes et al., 1998; Lanyon and Rubin, 1984; Lieberman et al., 2004). Further, the extent to which diaphyseal geometry, particularly during early postnatal ontogeny, is determined by genetic (e.g., see Lovejoy et al., 2003) versus epigenetic factors is not well understood. Mechanical influences on diaphyseal

shape are likely to become more pronounced as development proceeds (Carter and Beaupré, 2001; Carter et al., 1996; van der Meulen et al., 1993). In order to more fully understand the implications of ontogenetic variability in diaphyseal cross-sectional geometry for understanding mechanical loading patterns during behavioral development, additional studies characterizing *in vivo* strains associated with positional behaviors other than quadrupedalism and brachiation, and involving more variable limb kinematics, are clearly needed. Studies of both adult and immature primates will also be important.

SUMMARY

The main objective of the current study was to use cross-sectional geometric analysis to quantify ontogenetic variability in attributes of bone size and shape at the midshaft femur and humerus of *Chlorocebus aethiops* and *Hylobates lar*, and at the midshaft femur of *Pan troglodytes*. This provides an important context for interpretation of ontogenetic and regional variability in microstructural features examined in Chapters 5 and 6 of this thesis.

First, patterns of growth-related variation in cross-sectional geometric dimensions were characterized using LOWESS regression. Growth patterns were also characterized in relative terms, normalized to the maximum (i.e., adult) age class mean. Results were considered in light of postnatal growth patterns reported for other body dimensions (i.e., body mass and linear dimensions). Results were consistent with earlier reports indicating that growth in long bone cross-sectional geometry most closely parallels body mass growth and lags behind growth in bone length. Further, cross-sectional geometric dimensions examined here exhibited broadly similar characteristics of postnatal growth

as reported previously for other body dimensions, such as sexually-dimorphic growth patterns in *C. aethiops*, largely overlapping growth trajectories for males and females in *H. lar*, and a period of relatively rapid growth during later ontogeny in *P. troglodytes*. Variation in primary bone microstructure examined in Chapter 5 is also expected to reflect these growth trends.

Secondly, ontogenetic variation was described in several parameters relating to the distribution of bone material around single cross-sections (i.e., cross-sectional shape), and which are hypothesized to reflect relative bending rigidity about principle and anatomical axes. Results were consistent with earlier reports on long bone cross-sectional geometry in the study taxa. However, as prior studies have focused largely on adults, this study also contributes new information concerning ontogenetic variation in long bone cross-sectional geometry. For example, an age-related increase in relative mediolateral bending rigidity was apparent at most skeletal sites, with the exception of the femur of *C. aethiops*, which showed the opposite pattern. This age-related trend in relative mediolateral bending rigidity was accompanied by little change in the orientation of $I_{\max}$ through ontogeny in *C. aethiops*. However, considerable variability in $I_{\max}$ orientation was observed within and among age classes of *H. lar* and *P. troglodytes*. With the exception of the midshaft femur of *H. lar*, these latter observations reflect a medio-lateral shift in the location of $I_{\max}$ from a more antero-posterior orientation in the DECID age class, and a consequent increase in relative medio-lateral bending rigidity which appeared most marked during early ontogeny. The significance of these results is considered in light of available evidence concerning *in vivo* strain patterns and loads generated during locomotor behaviors in adult primates, and in terms of its implications

for understanding variability in limb loadings associated with positional behavior
ontogeny.

TABLE 4-1. Adult locomotor behaviors of the study taxa. Frequencies of different locomotor behaviors practiced (as a percentage of total locomotor time) are presented in parentheses.

Taxon	Locomotor behavior (% of total locomotor time)
<i>C. aethiops</i> ¹	quadrupedal walking or running (53.9%), climbing (29.5%), and leaping (9.6%)
<i>H. lar</i> ²	brachiation (56%), climbing (21%), leaping (15%), and bipedalism (8%)
<i>P. troglodytes</i> ³	knuckle-walking quadrupedalism (85.6 - 96.5%), quadrumanous climbing / scrambling (3.5 - 11.1%), suspensory behaviors (0.5 - 1.5%), bipedalism (0.2 - 1.2%)

Adapted from Ruff (2002: Table 2, p. 308).

¹ Source for locomotor frequencies: (Rose, 1979).

² Source for locomotor frequencies: (Fleagle, 1980).

³ Source for locomotor frequencies: (Doran, 1996; Doran & Hunt, 1994). Ranges represent variation in common chimpanzees in locomotor frequencies reported among sexes, localities and subspecies.

TABLE 4-2. Biological age classes used for statistical analysis.

Age Class	Alveolar emergence/epiphyseal union criteria
DECID.	alveolar emergence of one or more deciduous teeth
M1	alveolar emergence of one or more permanent 1st molars
M2	alveolar emergence of one or more permanent 2nd molars
M3	alveolar emergence of one or more permanent 3rd molars
EPIPH.	fully erupted permanent dentition, long bone epiphyseal union complete

TABLE 4-3. Biological age classes based on Bolter & Zihlman, 2003.

Age Class	Alveolar emergence/epiphyseal union criteria
1	alveolar emergence of one or more deciduous teeth
2	alveolar emergence of one or more permanent 1st molars
3	alveolar emergence of one or more permanent incisors
4	alveolar emergence of one or more permanent 2 nd molars
5	alveolar emergence of one or more permanent premolars
6	alveolar emergence of one or more permanent canines
7	alveolar emergence of one or more permanent 3 rd molars
8	fully erupted permanent dentition, long bone epiphyseal union complete.

TABLE 4-4. Sample representation across biological age classes for analysis of cross-sectional geometry (femur/humerus).

Sex	Total <i>N</i>	DECID.	M1	M2	M3	EPIPH.	Immature (unknown) ¹
<i>Chlorocebus aethiops</i>							
F	15/13	1/1	5/5 ²	2/1	4/3	3/3	0/0
M	16/17	4/4	1/1	4/5	3/3	3/3	1/1 ³
<i>Hylobates lar</i>							
F	13/12	2/2	2/2	2/1	2/2	3/3	2/2 ⁴
M	15/14	2/2	1/1	4/4	5/4	3/3	0/0
<i>Pan troglodytes</i>							
F	5	1	0	1	3	0	0
M	2	0	0	1	0	1	0
unk.	5	1	1	2 ⁵	0	1	0

¹ Includes individuals lacking associated dental emergence data.

² SLW 21 Humerus – Orientation lost during processing. This specimen was excluded from analyses of shape variables.

³ SLW 101 – Assigned to M3 age class for analysis, based on bone length and epiphyseal union.

⁴ SLW 10 & 35 – Assigned to M3 age class for analysis, based on bone length and epiphyseal union.

⁵ MNH 35526 femur – Questionable association with skull having only permanent M1s erupted and unerupted M2s; reassigned to M2 age class based on bone length.

TABLE 4-5. Wilcoxon matched pairs tests: bone length and geometric properties between ELEMENTS in *C. aethiops*.
Significant contrasts in bold ($p \leq 0.05$).

Variable	N _F	N _H	N	T	Z	p
DECID						
LTH	5	5	5	0	2.0226	0.04312
CA	5	5	5	5	0.6742	0.50018
J	5	5	5	3	1.2136	0.22492
lmax	5	5	5	4	0.9439	0.34523
Zx	5	5	5	5	0.6742	0.50018
Zy	5	5	5	1	1.7529	0.07962
Zp	5	5	5	1	1.7529	0.07962
M1						
LTH	6	6	5	0	2.0226	0.04312
CA	6	6	5	1	1.7529	0.07962
J	6	6	5	1	1.7529	0.07962
lmax	6	6	5	7	0.1348	0.89274
Zx	6	6	5	0	2.0226	0.04312
Zy	6	6	5	0	2.0226	0.04312
Zp	6	6	5	0	2.0226	0.04312
M2						
LTH	6	6	5	0	2.0226	0.04312
CA	6	6	5	0	2.0226	0.04312
J	6	6	5	0	2.0226	0.04312
lmax	6	6	5	3	1.2136	0.22492
Zx	6	6	5	0	2.0226	0.04312
Zy	6	6	5	0	2.0226	0.04312
Zp	6	6	5	0	2.0226	0.04312
M3						
LTH	8	7	7	0	2.3664	0.01796
CA	8	7	7	8	1.0142	0.31050
J	8	7	7	7	1.1832	0.23672
lmax	8	7	7	8	1.0142	0.31050
Zx	8	7	7	5	1.5213	0.12819
Zy	8	7	7	10	0.6761	0.49896
Zp	8	7	7	7	1.1832	0.23672
EIPPH						
LTH	6	6	6	0	2.2014	0.02771
CA	6	6	6	0	2.2014	0.02771
J	6	6	6	5	1.1531	0.24886
lmax	6	6	6	2	1.7821	0.07474
Zx	6	6	6	0	2.2014	0.02771
Zy	6	6	6	8	0.5241	0.60018
Zp	6	6	6	3	1.5724	0.11585

TABLE 4-6. Mann-Whitney and Kolmogorov-Smirnov tests: body mass, bone length and geometric properties between SEXES in *C. aethiops* (M3-EIPPH subsample). Significant contrasts in bold ($p \leq 0.05$).

actinops (the E1.1.1 subsample). Significant contrasts in bold (p<0.05).														
Mann-Whitney								Kolmogorov-Smirnov						
Variable	N _F	N _M	Rank Sum F	Rank Sum M	U	Z	p-level	Max Neg Diff.	Max Pos Diff.	p-level	Mean F	Mean M	S.D. F	S.D. M
FEMUR														
MASS	7	6	29.0	62.0	1.0	-2.85714	0.00428	-0.85714	0.00000	p < .025	3353.29	5360.00	448.280	1160.276
LTH	7	7	28.0	77.0	0.0	-3.13050	0.00175	-1.00000	0.00000	p < .005	116.09	136.28	4.073	7.252
TA	7	7	32.0	73.0	4.0	-2.61939	0.00881	-0.71429	0.00000	p < .10	53.95	71.37	5.574	15.411
CA	7	7	30.0	75.0	2.0	-2.87494	0.00404	-0.85714	0.00000	p < .025	28.78	43.18	2.922	8.545
MA	7	7	50.0	55.0	22.0	-0.31944	0.74939	-0.42857	0.28571	p > .10	25.17	28.19	4.241	9.359
J	7	7	31.0	74.0	3.0	-2.74717	0.00601	-0.71429	0.00000	p < .10	364.69	705.44	71.438	300.448
lmax	7	7	31.0	74.0	3.0	-2.74717	0.00601	-0.71429	0.00000	p < .10	197.49	377.17	38.469	155.279
Zx	7	7	30.0	75.0	2.0	-2.87494	0.00404	-0.85714	0.00000	p < .025	40.88	69.86	6.144	21.956
Zy	7	7	32.0	73.0	4.0	-2.61939	0.00881	-0.71429	0.00000	p < .10	42.75	68.22	5.782	20.246
Zp	7	7	30.0	75.0	2.0	-2.87494	0.00404	-0.85714	0.00000	p < .025	83.64	138.09	11.458	42.049
HUMERUS														
MASS	6	7	22.0	56.0	1.0	-2.72218	0.00649	-0.83333	0.00000	p < .05	3345.17	5360.00	490.502	1160.276
LTH	6	7	21.0	70.0	0.0	-3.00000	0.00270	-1.00000	0.00000	p < .005	96.27	112.01	3.398	4.301
TA	6	7	25.0	66.0	4.0	-2.42857	0.01516	-0.85714	0.00000	p < .025	51.21	67.93	3.313	13.516
CA	6	7	22.0	69.0	1.0	-2.85714	0.00428	-0.85714	0.00000	p < .025	27.26	41.41	2.501	8.112
MA	6	7	38.0	53.0	17.0	-0.57143	0.56771	-0.40476	0.14286	p > .10	23.95	26.52	3.112	7.658
J	6	7	24.0	67.0	3.0	-2.57143	0.01013	-0.85714	0.00000	p < .025	340.88	667.19	42.578	271.686
lmax	6	7	24.0	67.0	3.0	-2.57143	0.01013	-0.85714	0.00000	p < .025	212.82	423.79	27.529	179.226
Zx	6	7	21.0	70.0	0.0	-3.00000	0.00270	-1.00000	0.00000	p < .005	35.76	60.48	2.444	15.687
Zy	6	7	25.0	66.0	4.0	-2.42857	0.01516	-0.85714	0.00000	p < .025	41.26	69.53	5.068	23.642
Zp	6	7	25.0	66.0	4.0	-2.42857	0.01516	-0.85714	0.00000	p < .025	77.39	130.78	6.877	39.883

TABLE 4-7. Wilcoxon matched pairs tests: bone length and geometric properties between ELEMENTS in *H. lar*.
Significant contrasts in bold ($p \leq 0.05$).

Variable	N _F	N _H	N	T	Z	p
DECID						
LTH	4	4	4	0	1.8257	0.06789
CA	4	4	4	3	0.7303	0.46521
J	4	4	4	1	1.4606	0.14413
lmax	4	4	4	1	1.4606	0.14413
Zx	4	4	4	1	1.4606	0.14413
Zy	4	4	4	3	0.7303	0.46521
Zp	4	4	4	1	1.4606	0.14413
M1						
LTH	3	3	3	0	1.6036	0.10881
CA	3	3	3	3	0.0000	1.00000
J	3	3	3	2	0.5345	0.59298
lmax	3	3	3	3	0.0000	1.00000
Zx	3	3	3	2	0.5345	0.59298
Zy	3	3	3	2	0.5345	0.59298
Zp	3	3	3	3	0.0000	1.00000
M2						
LTH	6	5	5	0	2.0226	0.04312
CA	6	5	5	2	1.4832	0.13801
J	6	5	5	7	0.1348	0.89274
lmax	6	5	5	5	0.6742	0.50018
Zx	6	5	5	4	0.9439	0.34523
Zy	6	5	5	5	0.6742	0.50018
Zp	6	5	5	6	0.4045	0.68583
M3						
LTH	9	8	8	0	2.5205	0.01172
CA	9	8	8	2	2.2404	0.02506
J	9	8	8	15	0.4201	0.67442
lmax	9	8	8	13	0.7001	0.48384
Zx	9	8	8	17	0.1400	0.88864
Zy	9	8	8	14	0.5601	0.57540
Zp	9	8	8	14	0.5601	0.57540
EIPPH						
LTH	6	6	6	0	2.2014	0.02771
CA	6	6	6	0	2.2014	0.02771
J	6	6	6	4	1.3628	0.17296
lmax	6	6	6	3	1.5724	0.11585
Zx	6	6	6	3	1.5724	0.11585
Zy	6	6	6	4	1.3628	0.17296
Zp	6	6	6	3	1.5724	0.11585

TABLE 4-8. Mann-Whitney and Kolmogorov-Smirnov tests: bone length and geometric properties between SEXES in *H. lar* (M3-EPIPH subsample).

			Mann-Whitney					Kolmogorov-Smirnov						
Variable	N _F	N _M	Rank Sum F	Rank Sum M	U	Z	p-level	Max Neg Diff.	Max Pos Diff.	p-level	Mean F	Mean M	S.D. F	S.D. M
FEMUR														
LTH	7	8	43.5	76.5	15.5	-1.44659	0.14801	-0.63	0.00	p > .10	178.70	186.01	5.7853	10.7373
TA	7	8	42.0	78.0	14.0	-1.62019	0.10519	-0.46	0.00	p > .10	70.68	77.68	8.2847	6.8982
CA	7	8	50.0	70.0	22.0	-0.69437	0.48745	-0.36	0.09	p > .10	46.40	51.16	7.3325	7.9382
MA	7	8	49.0	71.0	21.0	-0.81009	0.41789	-0.50	0.21	p > .10	24.28	26.52	4.5100	5.8692
J	7	8	43.0	77.0	15.0	-1.50446	0.13246	-0.48	0.00	p > .10	709.84	852.62	174.0891	172.7679
lmax	7	8	44.0	76.0	16.0	-1.38873	0.16492	-0.43	0.00	p > .10	377.27	451.84	100.6802	108.9106
lmax/lmin	7	8	60.0	60.0	24.0	0.46291	0.64343	-0.13	0.23	p > .10	1.13	1.12	0.0721	0.0873
Zx	7	8	42.0	78.0	14.0	-1.62019	0.10519	-0.45	0.00	p > .10	69.09	80.32	12.9766	11.3471
Zy	7	8	44.0	76.0	16.0	-1.38873	0.16492	-0.43	0.00	p > .10	72.88	85.04	13.3200	14.9551
Zp	7	8	43.0	77.0	15.0	-1.50446	0.13246	-0.48	0.00	p > .10	141.97	165.39	26.1679	26.2420
HUMERUS														
LTH	7	7	50.0	55.0	22.0	-0.31944	0.74939	-0.43	0.29	p > .10	206.87	215.41	7.0308	15.2786
TA	7	7	44.0	61.0	16.0	-1.08609	0.27744	-0.43	0.00	p > .10	72.98	78.03	7.9365	8.2984
CA	7	7	42.0	63.0	14.0	-1.34164	0.17971	-0.57	0.00	p > .10	42.77	47.36	5.1286	6.3806
MA	7	7	51.0	54.0	23.0	-0.19166	0.84801	-0.14	0.14	p > .10	30.21	30.67	5.6202	6.1199
J	7	7	44.0	61.0	16.0	-1.08609	0.27744	-0.29	0.00	p > .10	707.42	824.83	147.6397	182.5659
lmax	7	7	44.0	61.0	16.0	-1.08609	0.27744	-0.43	0.00	p > .10	381.34	435.29	86.2569	99.6871
lmax/lmin	7	7	57.0	48.0	20.0	0.57499	0.56530	-0.14	0.43	p > .10	1.17	1.11	0.0995	0.0276
Zx	7	7	42.0	63.0	14.0	-1.34164	0.17971	-0.43	0.00	p > .10	67.83	77.57	11.1146	12.1781
Zy	7	7	46.0	59.0	18.0	-0.83054	0.40623	-0.29	0.00	p > .10	72.58	80.96	12.4772	14.8663
Zp	7	7	43.0	62.0	15.0	-1.21387	0.22480	-0.43	0.00	p > .10	140.64	158.56	23.4103	26.8755

TABLE 4-9. Mann-Whitney and Kolmogorov-Smirnov tests: I_{max}/I_{min} and Z_y/Z_x between SEXES in *C. aethiops* and *H. lar* (M3-EPIPH subsamples).

ER II (15 Subsamples)														
Variable	N _F	N _M	Mann-Whitney					Kolmogorov-Smirnov						
			Rank Sum F	Rank Sum M	U	Z	p-level	Max Neg Diff.	Max Pos Diff.	p-level	Mean F	Mean M	S.D. F	S.D. M
<i>C. aethiops</i>														
FEMUR														
I _{max} /I _{min}	7	7	55.0	50.0	22.0	0.31944	0.74939	-0.14286	0.28571	p > .10	1.18	1.16	0.063	0.061
Z _y /Z _x	7	7	64.0	41.0	13.0	1.46942	0.14172	0.00000	0.57143	p > .10	1.05	0.98	0.089	0.042
HUMERUS														
I _{max} /I _{min}	6	7	33.0	58.0	12.0	-1.28571	0.19854	-0.54762	0.14286	p > .10	1.66	1.74	0.095	0.179
Z _y /Z _x	6	7	47.0	44.0	16.0	0.71429	0.47505	-0.09524	0.33333	p > .10	1.15	1.13	0.123	0.130
<i>H. lar</i>														
FEMUR														
I _{max} /I _{min}	7	8	60.0	60.0	24.0	0.46291	0.64343	-0.12500	0.23214	p > .10	1.13	1.12	0.072	0.087
Z _y /Z _x	7	8	55.0	65.0	27.0	-0.11573	0.90787	-0.35714	0.37500	p > .10	1.06	1.06	0.043	0.059
HUMERUS														
I _{max} /I _{min}	7	7	57.0	48.0	20.0	0.57499	0.56530	-0.14286	0.42857	p > .10	1.17	1.11	0.099	0.028
Z _y /Z _x	7	7	60.0	45.0	17.0	0.95831	0.33790	-0.14286	0.28571	p > .10	1.07	1.04	0.059	0.045

TABLE 4-10. Kruskal-Wallis and Median tests: Zy/Zx among AGE CLASSES in *C. aethiops*.

Element	df	N	Kruskal-Wallis		Median Test	
			H	p	Chi-square	p
Femur	4	31	6.5414	0.1622	8.443194	0.0766
Humerus	4	29	4.0313	0.4018	3.255102	0.5161

TABLE 4-11. One-sample Wilcoxon tests: Zy/Zx by age class in *C. aethiops*. Null hypothesis ($Zy/Zx=1$) indicates section circularity. Significant contrasts in bold ($p \leq 0.05$).

Element	Age class	One-sample Wilcoxon			
		N	T	Z	p
FEMUR	TOTAL sample	31	149	1.9401	0.0524
	DECID	5	1	1.7529	0.0796
	M1	6	3	1.5724	0.1159
	M2	6	8	0.5241	0.6002
	M3	8	12	0.8402	0.4008
	EPIPH	6	5	1.1531	0.2489
HUMERUS	TOTAL sample	29	32	4.0111	0.0001
	DECID	5	5	0.6742	0.5002
	M1	5	1	1.7529	0.0796
	M2	6	1	1.9917	0.0464
	M3	7	3	1.8594	0.0630
	EPIPH	6	0	2.2014	0.0277

TABLE 4-12. Kruskal-Wallis and Median tests: Zy/Zx among AGE CLASSES in *H. lar*. Significant contrasts in bold ($p \leq 0.05$).

Element	df	N	Kruskal-Wallis		Median Test	
			H	p	Chi-square	p
Femur	4	28	11.1724	0.0247	6.666667	0.1546
Humerus	4	26	13.347	0.0097	10.36667	0.0347

TABLE 4-13. One-sample Wilcoxon tests: Zy/Zx by age class in *H. lar*. Null hypothesis ($Zy/Zx=1$) indicates section circularity. Significant contrasts in bold ($p \leq 0.05$).

Element	Age class	One-sample Wilcoxon			
		N	T	Z	p
FEMUR	TOTAL sample	28	91	2.3544	0.0186
	DECID	4	0	1.6036	0.1088
	M1	3	1	1.0690	0.2851
	M2	6	9	0.3145	0.7532
	M3	9	4	2.1917	0.0284
	EPIPH	6	0	2.2014	0.0277
HUMERUS	TOTAL sample	26	140	0.9016	0.3673
	DECID	4	0	1.8257	0.0679
	M1	3	2	0.5345	0.5930
	M2	5	3	1.2136	0.2249
	M3	8	5	1.8204	0.0687
	EPIPH	6	1	1.9917	0.0464

TABLE 4-14. Kruskal-Wallis and Median tests: I_{max}/I_{min} among AGE CLASSES in *C. aethiops*. Significant contrasts in bold ($p \leq 0.05$).

Element	df	N	Kruskal-Wallis		Median Test	
			H	p	Chi-square	p
Femur	4	31	8.0347	0.0903	8.276354	0.0820
Humerus	4	29	7.0929	0.1311	10.08227	0.0391

TABLE 4-15. One-sample Wilcoxon tests: I_{max}/I_{min} by age class in *C. aethiops*. Null hypothesis (I_{max}/I_{min}=1) indicates section circularity. Significant contrasts in bold ($p \leq 0.05$).

Element	Age class	One-sample Wilcoxon			
		N	T	Z	p
FEMUR	TOTAL sample	31	0	4.8599	0.0000
	DECID	5	0	2.0226	0.0431
	M1	6	0	2.2014	0.0277
	M2	6	0	2.2014	0.0277
	M3	8	0	2.5205	0.0117
	EPIPH	6	0	2.2014	0.0277
HUMERUS	TOTAL sample	29	0	4.7030	0.0000
	DECID	5	0	2.0226	0.0431
	M1	5	0	2.0226	0.0431
	M2	6	0	2.2014	0.0277
	M3	7	0	2.3664	0.0180
	EPIPH	6	0	2.2014	0.0277

TABLE 4-16. Kruskal-Wallis and Median tests: I_{max}/I_{min} among AGE CLASSES in *H. lar*. Significant contrasts in bold ($p \leq 0.05$).

Element	df	N	Kruskal-Wallis		Median Test	
			H	p	Chi-square	p
Femur	4	28	9.3305	0.0534	5.777778	0.2164
Humerus	4	26	9.7707	0.0445	5.033333	0.2839

TABLE 4-17. One-sample Wilcoxon test: I_{max}/I_{min} by age class in *H. lar*. Null hypothesis (I_{max}/I_{min}=1) indicates section circularity. Significant contrasts in bold ($p \leq 0.05$).

Element	Age class	One-sample Wilcoxon			
		N	T	Z	p
FEMUR	TOTAL sample	28	0	4.6226	0.0000
	DECID	4	0	1.8257	0.0679
	M1	3	0	1.6036	0.1088
	M2	6	0	2.2014	0.0277
	M3	9	0	2.6656	0.0077
	EPIPH	6	0	2.2014	0.0277
HUMERUS	TOTAL sample	26	0	4.4573	0.0000
	DECID	4	0	1.8257	0.0679
	M1	3	0	1.6036	0.1088
	M2	5	0	2.0226	0.0431
	M3	8	0	2.5205	0.0117
	EPIPH	6	0	2.2014	0.0277

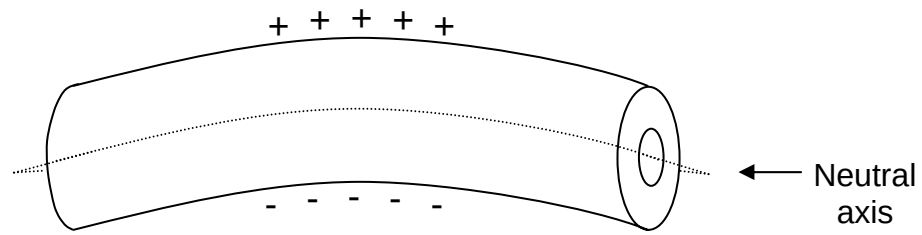


FIGURE 4-1a. Schematic of a beam subjected to bending. In a beam subjected to bending about a horizontal neutral axis, as shown here, maximum tensile strains (+++) will be experienced on its outer convex surface (above), and maximum compressive strains (---) will be experienced on its outer concave surface (below).

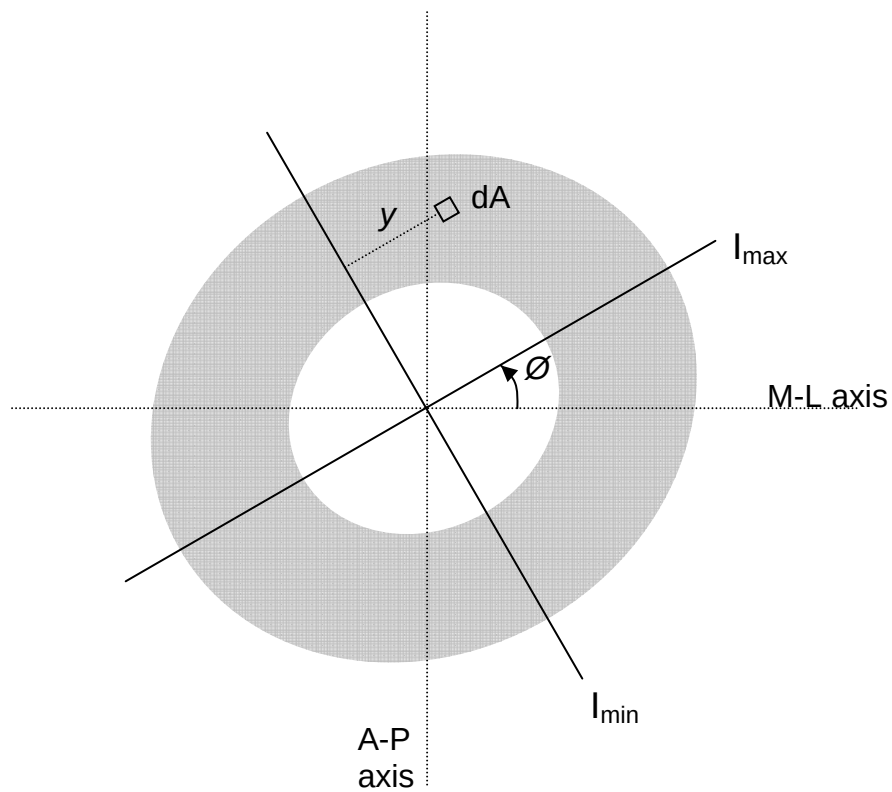


FIGURE 4-1b. Calculation of cross-sectional geometric properties.

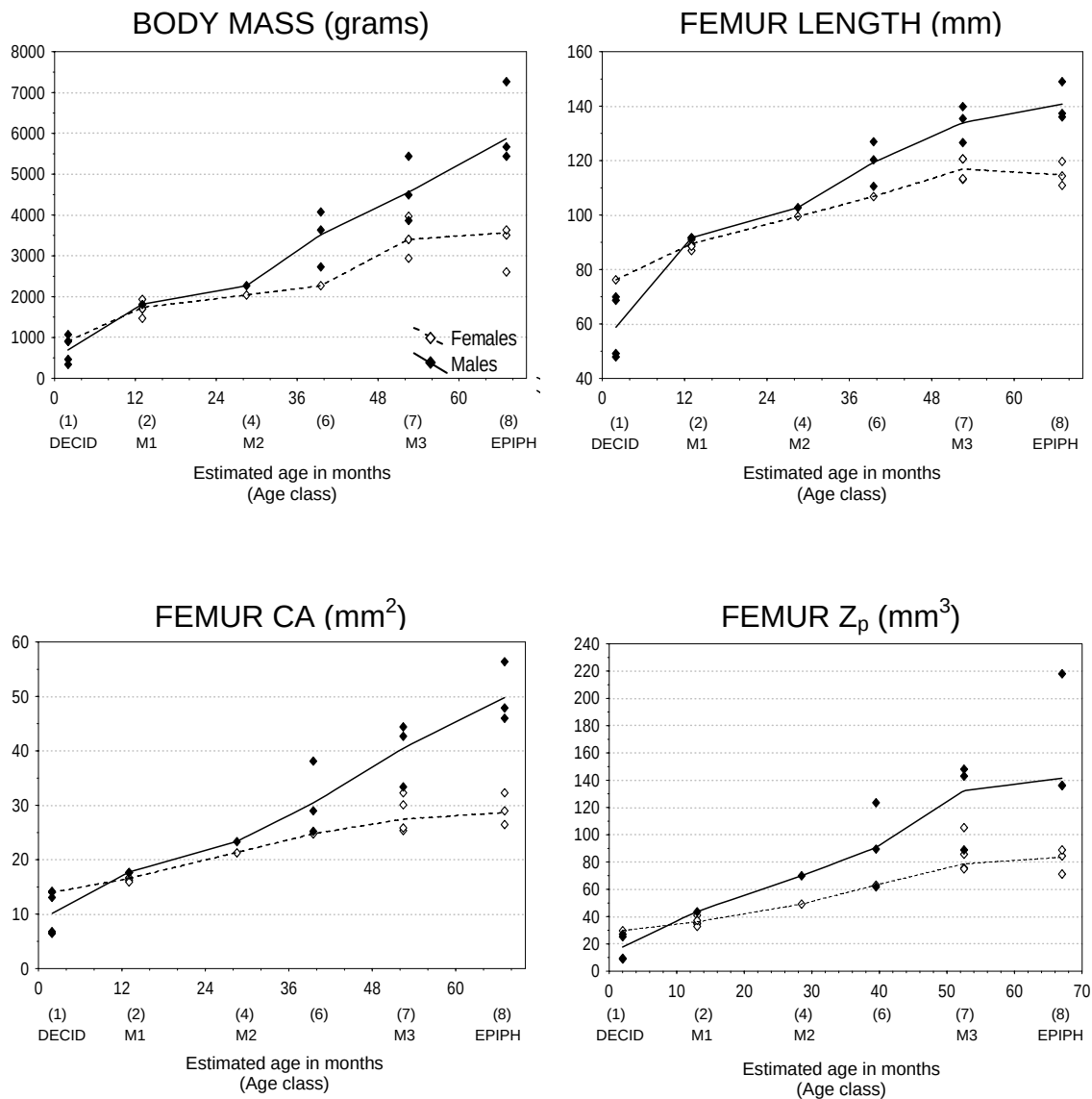


FIGURE 4-2 a. LOWESS plots of growth in *C. aethiops* body mass, femur length and geometric properties: sex contrasts. Plotted by estimated age (months) and biological age class.

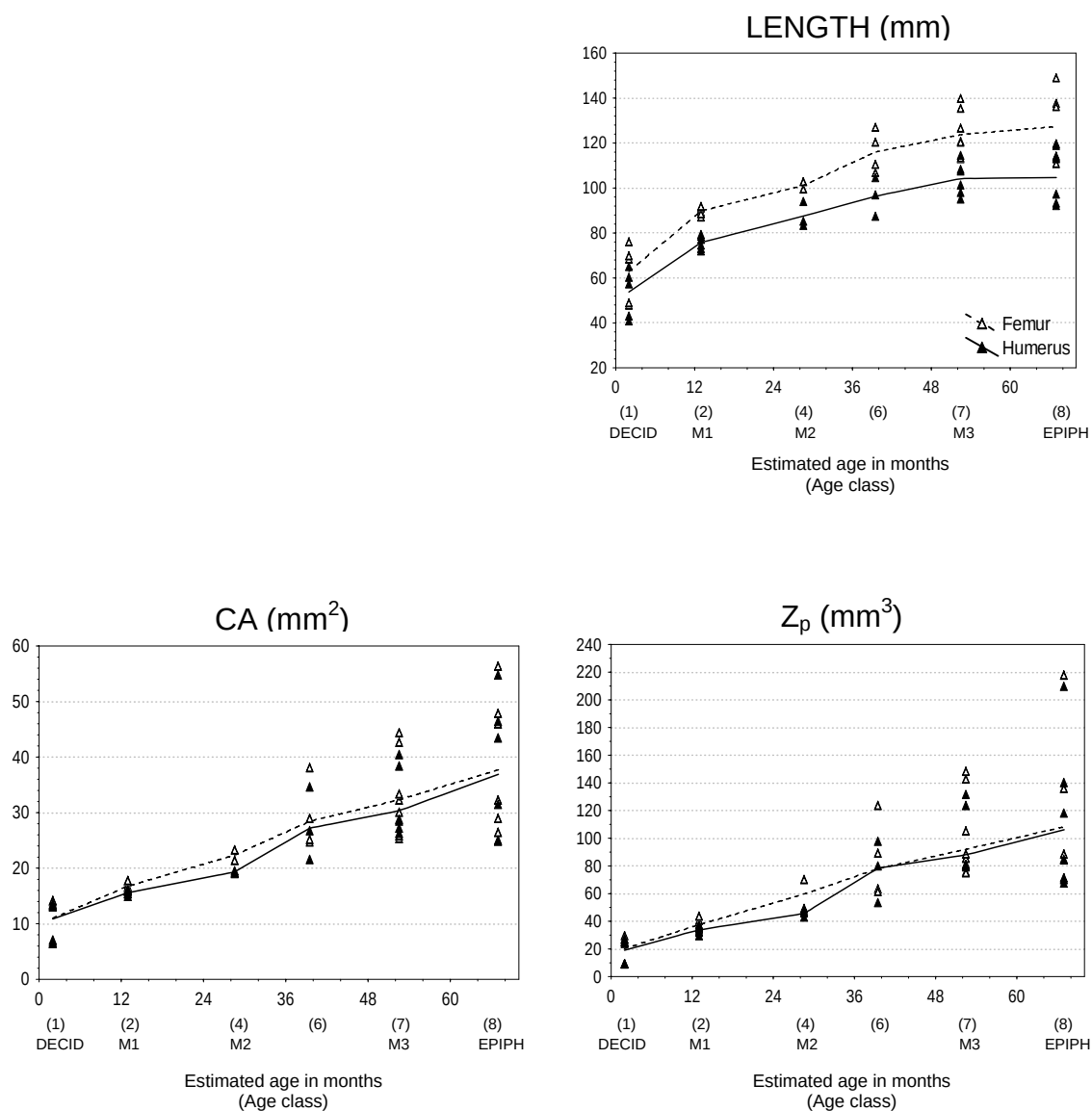


FIGURE 4-2 b. LOWESS plots of growth in *C. aethiops* bone length and geometric properties: element contrasts. Plotted by estimated age (months) and biological age class in the combined-sex sample.

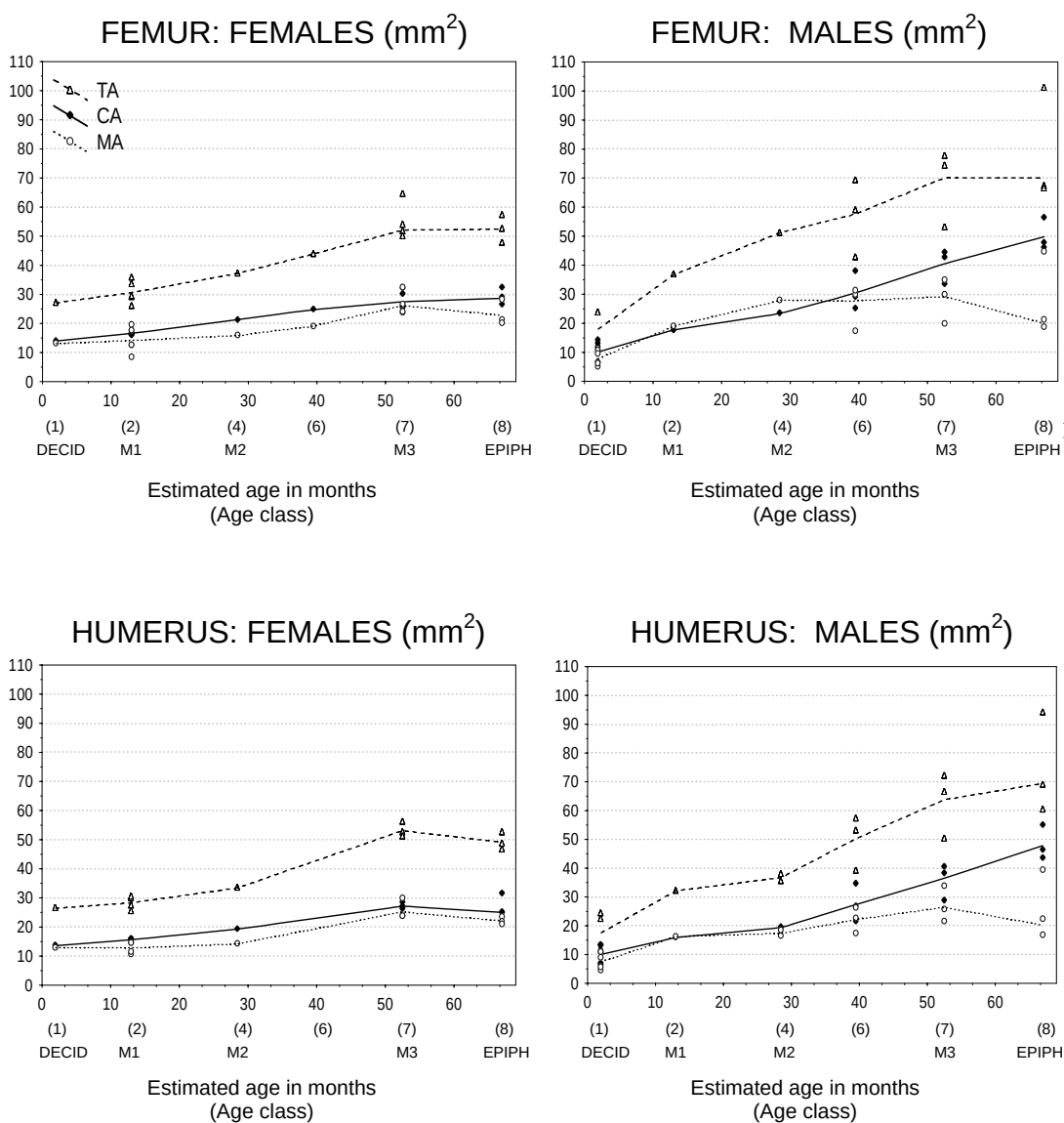


FIGURE 4-2 c. LOWESS plots of TA, CA, and MA at the midshaft femur and humerus of *C. aethiops*, by sex. Plotted by estimated age and biological age class.

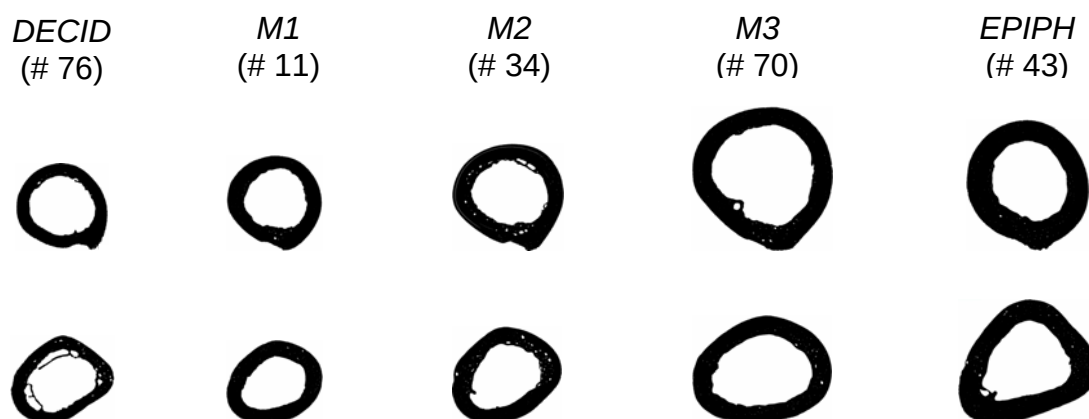


FIGURE 4-3 a. Examples of cross-sectional geometry from each age class: *C. aethiops* females. *Top Row:* midshaft femur. *Bottom Row:* midshaft humerus. Images are scaled to 3 % of original pixel dimensions. Intracortical porosity in these images is due to the formation of secondary osteons, described in Chapter 6.

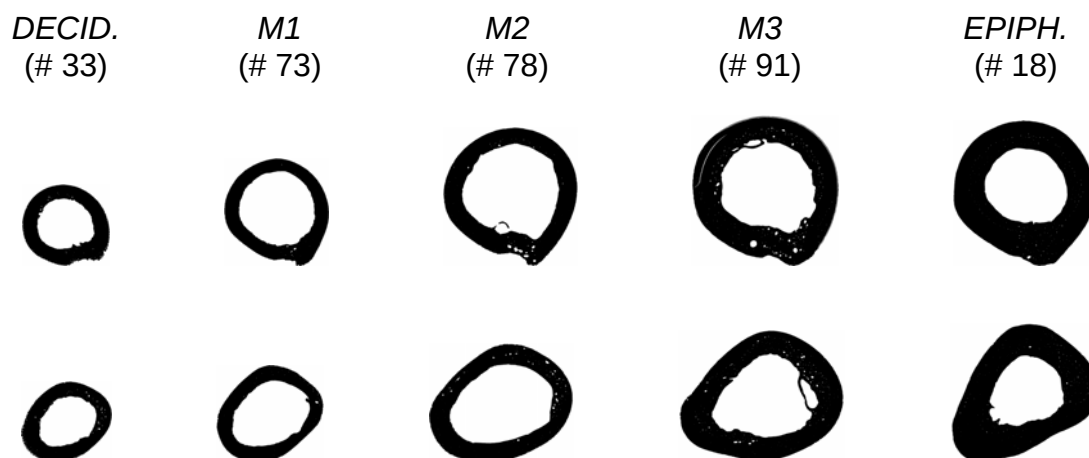
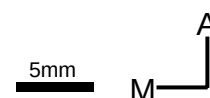


FIGURE 4-3 b. Examples of cross-sectional geometry from each age class: *C. aethiops* males. *Top Row:* midshaft femur. *Bottom Row:* midshaft humerus. Images are scaled to 3 % of original pixel dimensions. Intracortical porosity in these images is due to the formation of secondary osteons, described in Chapter 6.



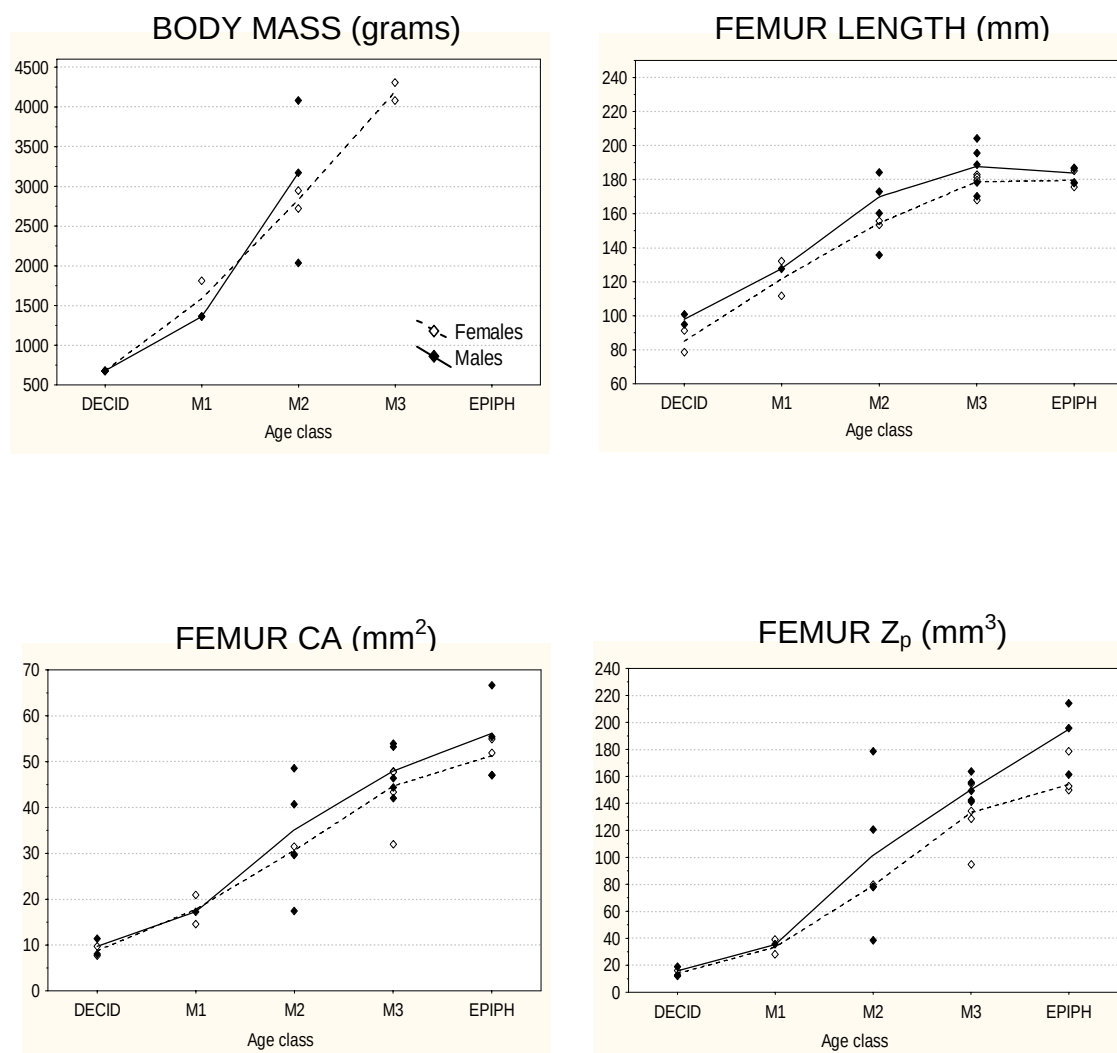


FIGURE 4-4 a. LOWESS plots of growth in *H. lar* body mass, femur length and geometric properties: sex contrasts. Plotted by biological age class.

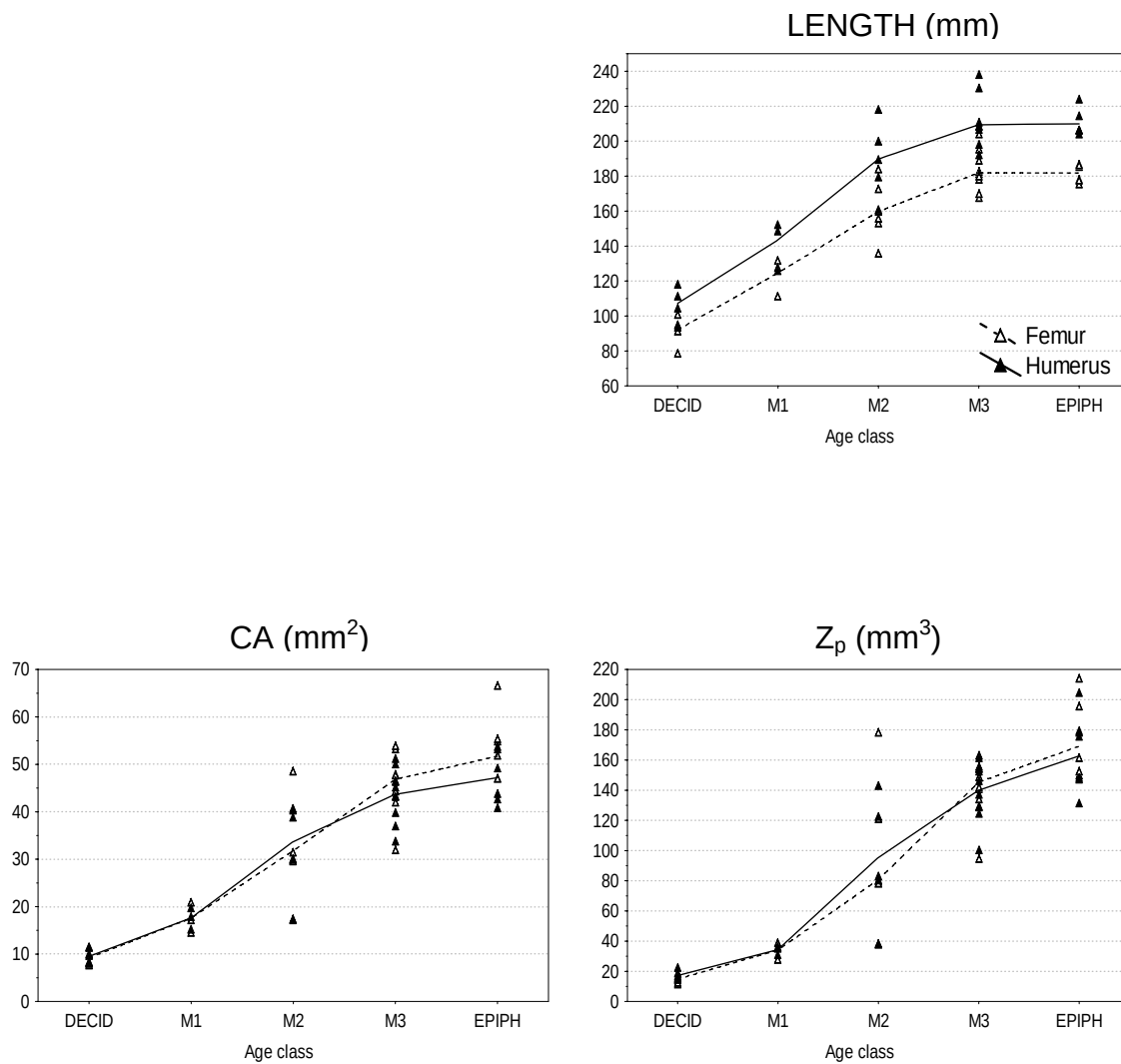


FIGURE 4-4 b. LOWESS plots of growth in *H. lar* bone length and geometric properties: element contrasts. Plotted by biological age class in the combined-sex sample.

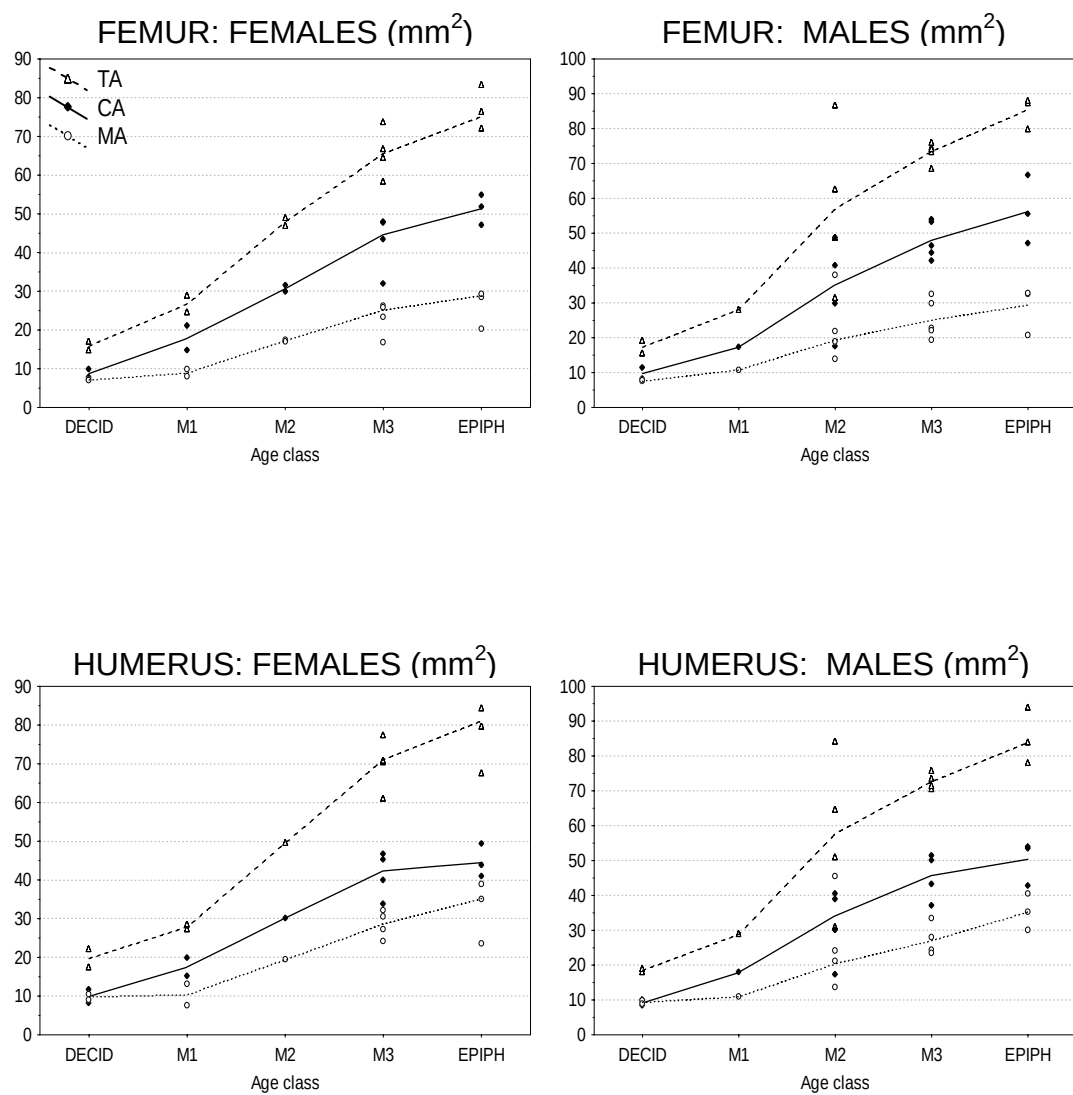


FIGURE 4-4 c. LOWESS plots of TA, CA, and MA at the midshaft femur and humerus of *H. lar*, by sex. Plotted by biological age class.

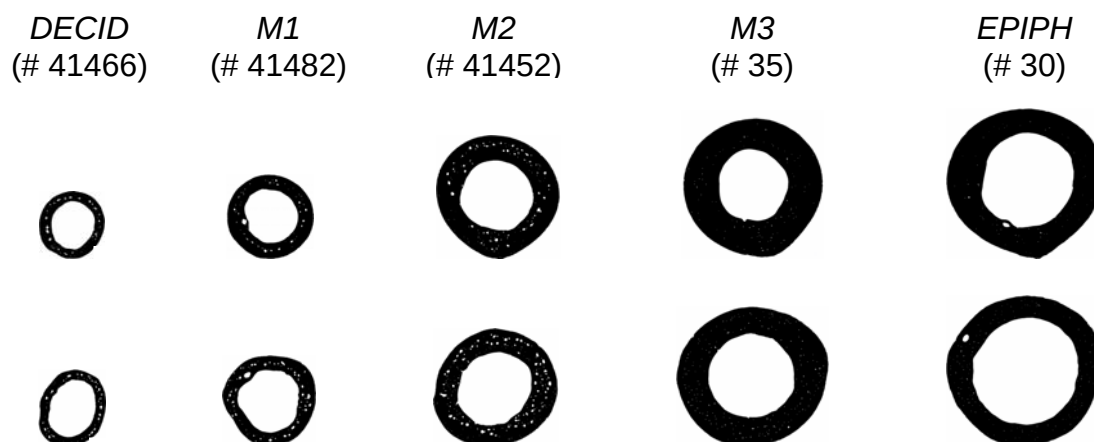


FIGURE 4-5 a. Examples of cross-sectional geometry from each age class: *H. lar* females. *Top Row:* midshaft femur. *Bottom Row:* midshaft humerus. Images are scaled to 3 % of original pixel dimensions. Intracortical porosity in these images is due to the formation of secondary osteons, described in Chapter 6.

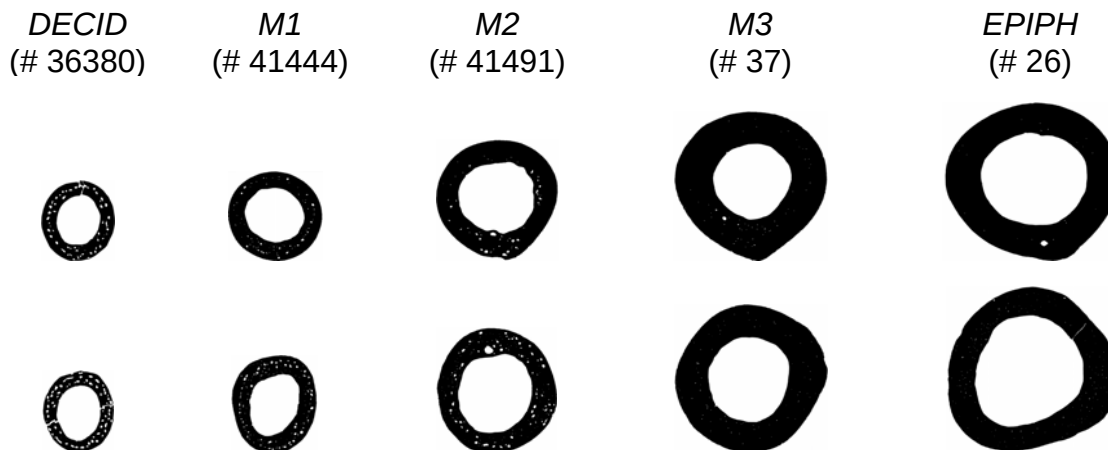
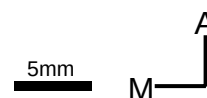


FIGURE 4-5 b. Examples of cross-sectional geometry from each age class: *H. lar* males. *Top Row:* midshaft femur. *Bottom Row:* midshaft humerus. Images are scaled to 3 % of original pixel dimensions. Intracortical porosity in these images is due to the formation of secondary osteons, described in Chapter 6.



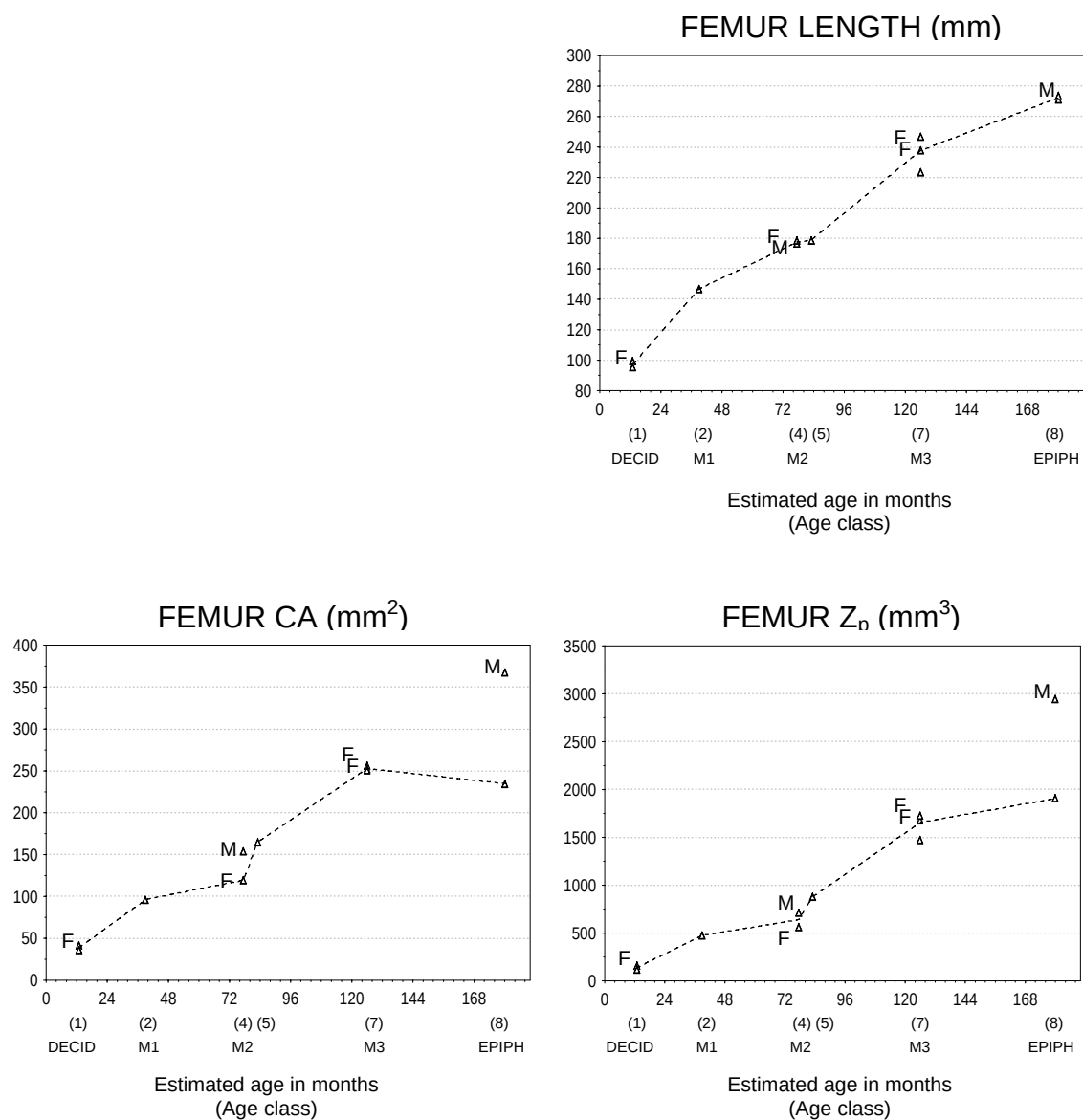


FIGURE 4-6 a. LOWESS plots of growth in *P. troglodytes* femur length and geometric properties. Plotted by estimated age (months) and biological age class. Sex is noted where known: F=female, M=male.

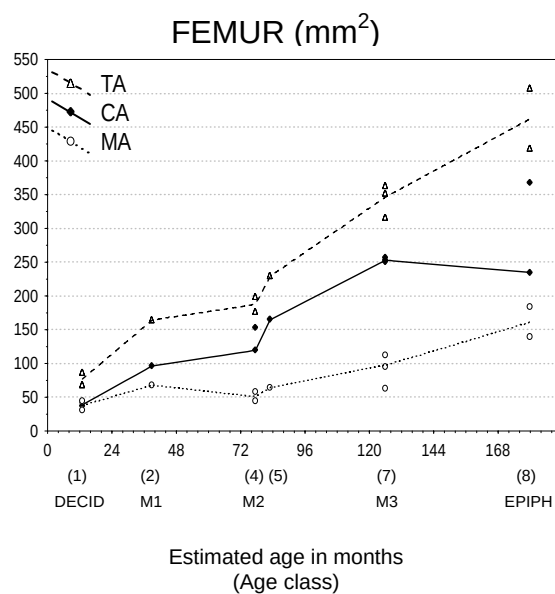


FIGURE 4-6 b. LOWESS plots of TA, CA, and MA at the midshaft femur of *P. troglodytes*. Plotted by estimated age and biological age class.

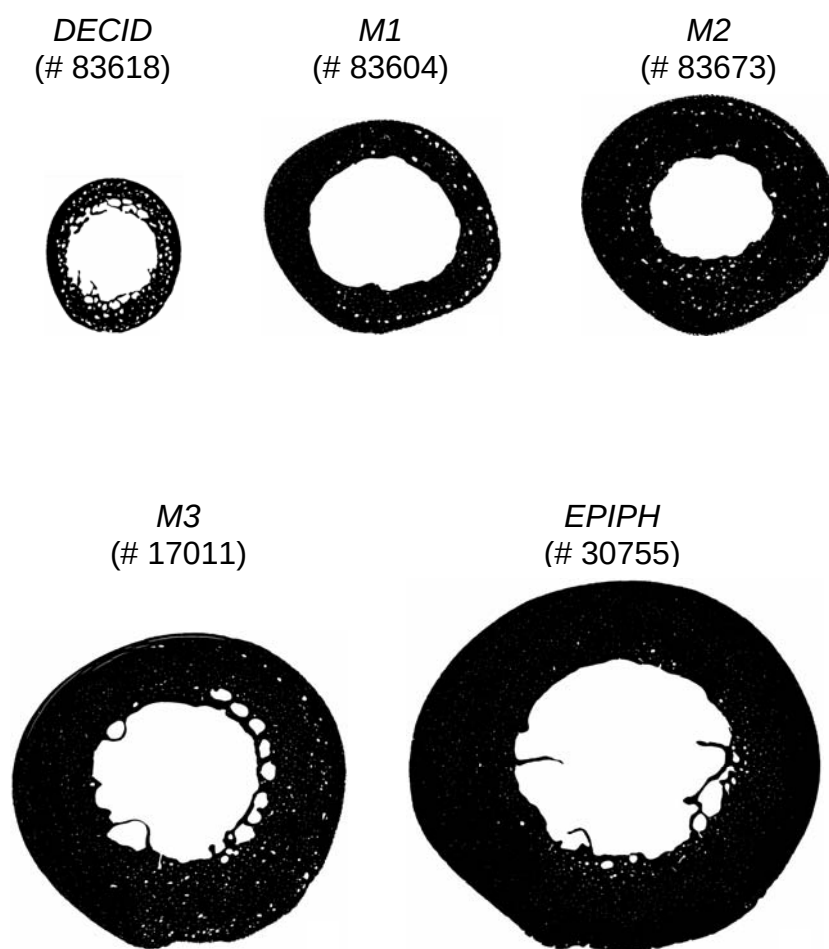
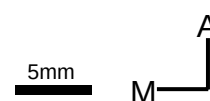


FIGURE 4-7. Examples of cross-sectional geometry from each age class: *P. troglodytes* midshaft femur. Images are scaled to 3 % of original pixel dimensions. Intracortical porosity in these images is due to the formation of secondary osteons, described in Chapter 6.



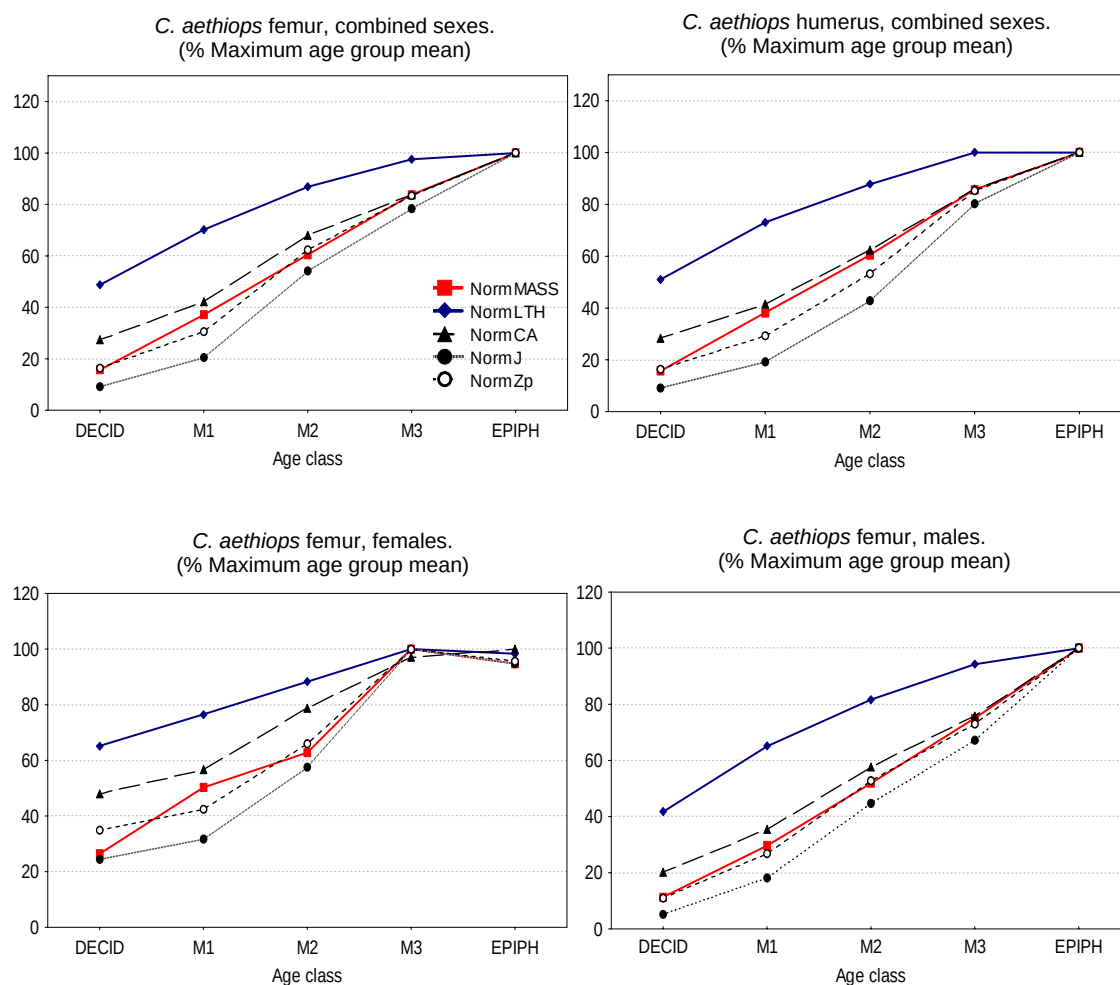


FIGURE 4-8 a. Mean plots of normalized growth of body mass, bone length and geometric properties: *C. aethiops*. (Top) Combined-sex sample, femur and humerus. (Bottom) Females and males, femur. Individual values were normalized against the maximum age group mean.

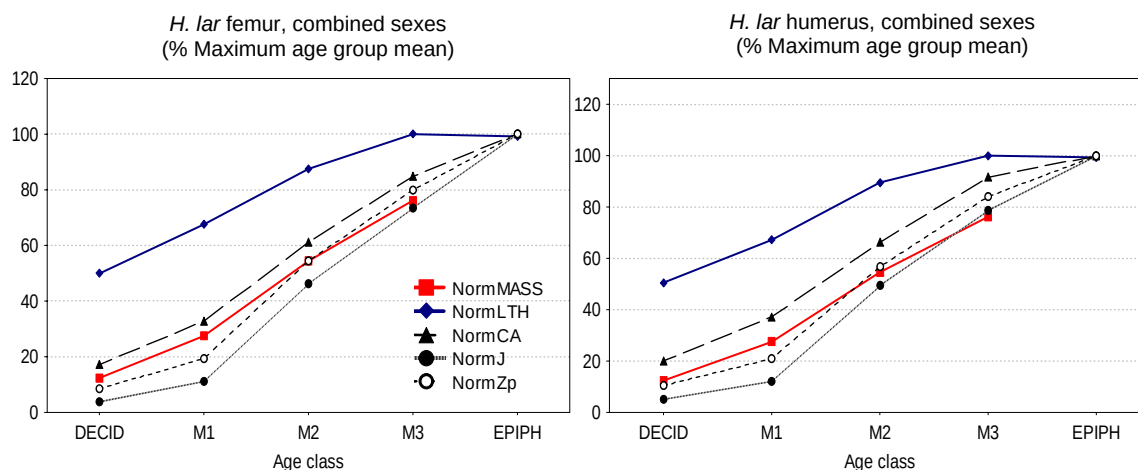


FIGURE 4-8 b. Mean plots of normalized growth of body mass, bone length and geometric properties by age class: *H. lar*. Individual values were normalized against the maximum age class mean.

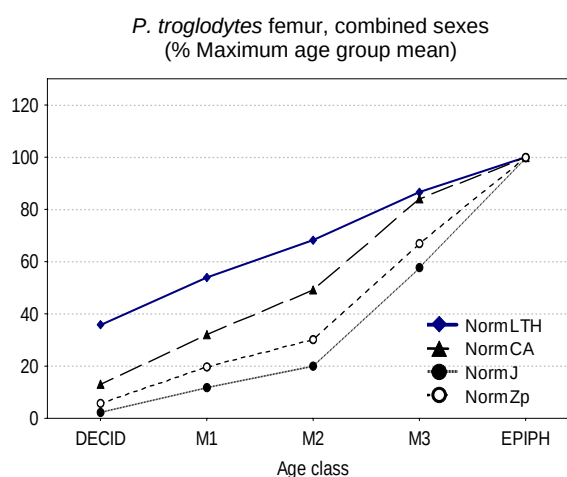


FIGURE 4-8 c. Mean plot of normalized growth of bone length and geometric properties by age class: *P. troglodytes*. Individual values were normalized against the maximum age class mean.

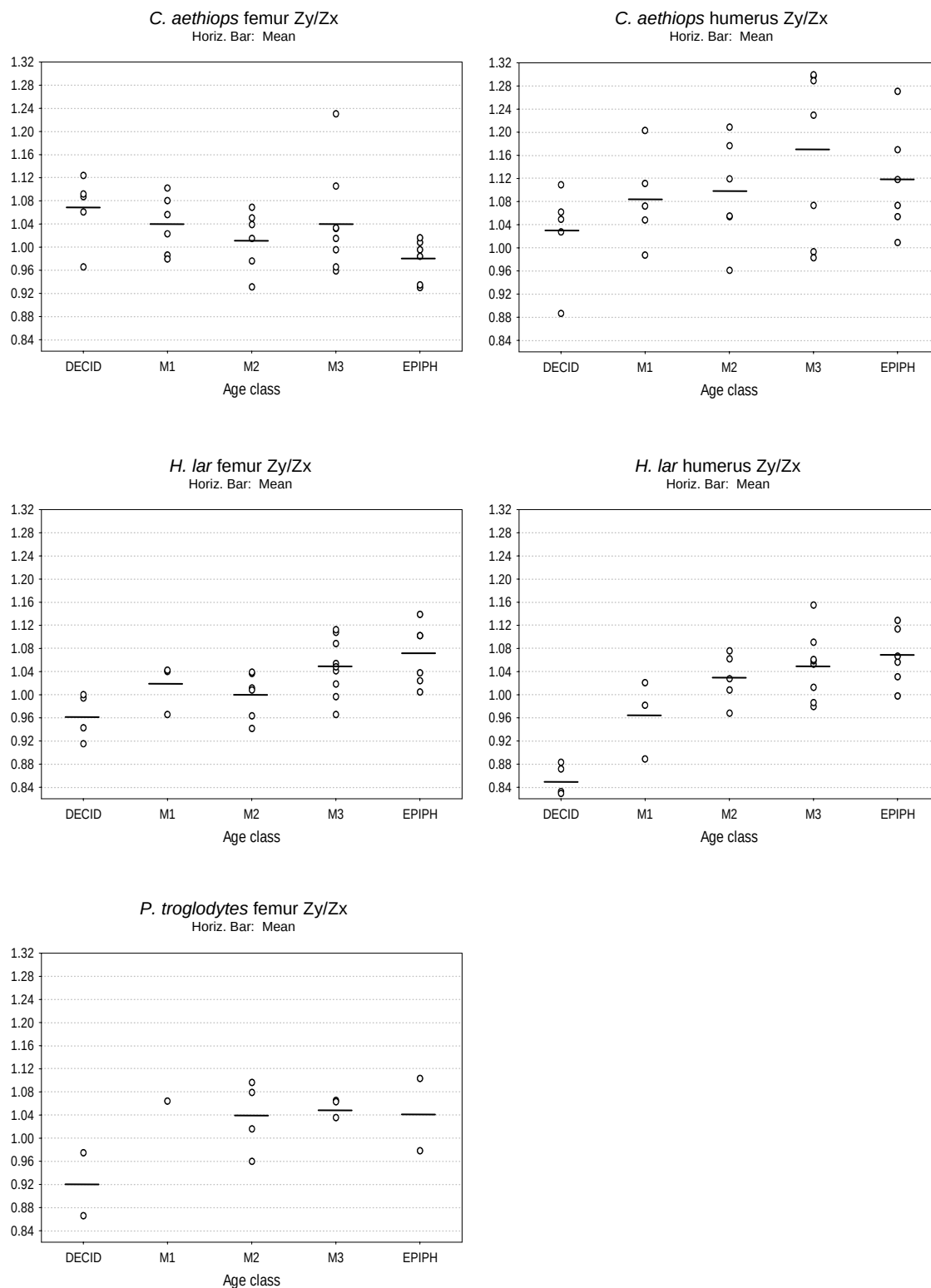


FIGURE 4-9. Plots of Zyl/Zx at the midshaft femur and humerus by age class: raw data and age class means. (Top) *C. aethiops*, (Middle) *H. lar*, and (Bottom) *P. troglodytes*.

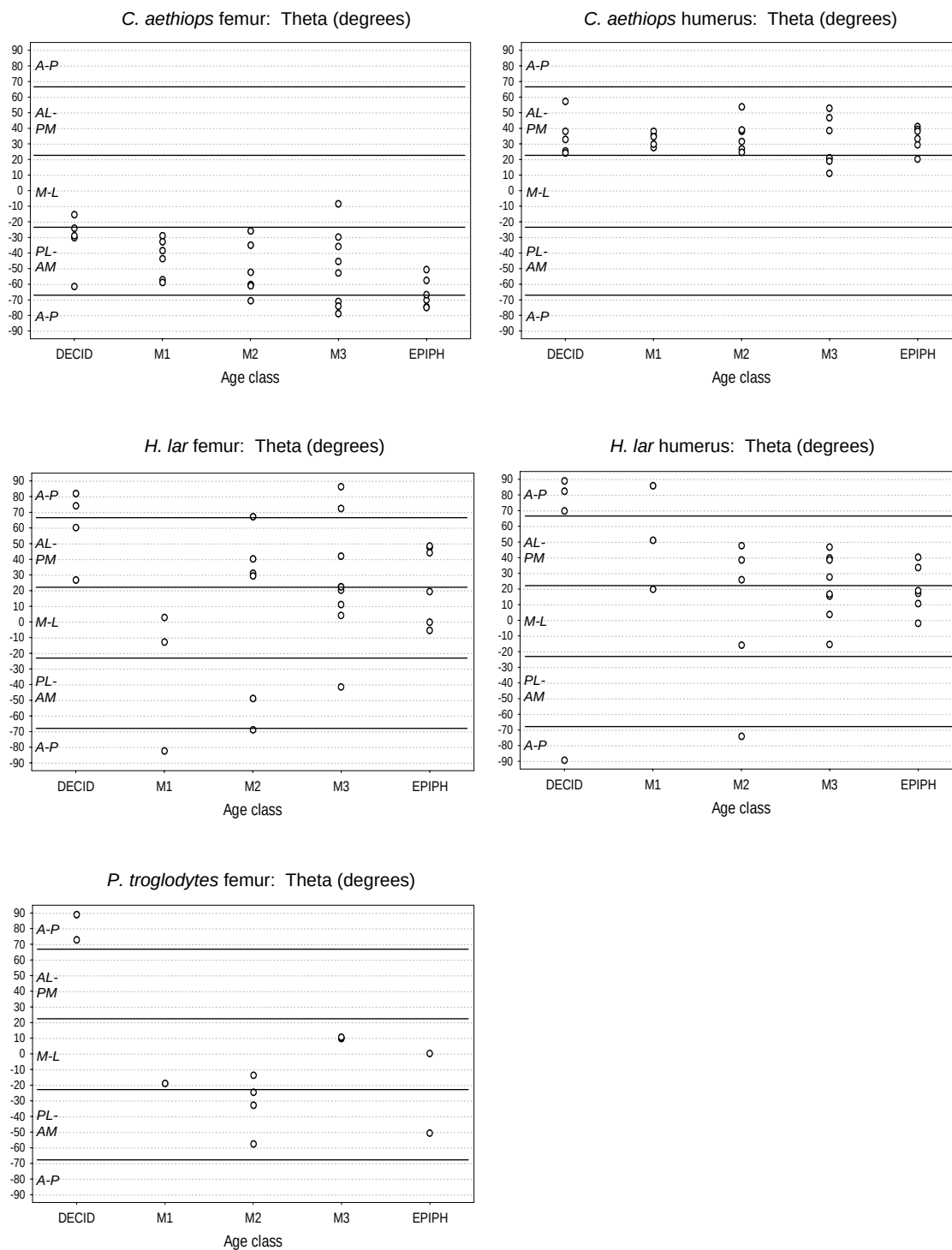


FIGURE 4-10. Plots of the orientation of $I_{\max}$ (theta) at the midshaft femur and humerus by age class: raw data. (Top) *C. aethiops*, (Middle) *H. lar*, and (Bottom) *P. troglodytes*. Anatomical orientation denoted along the Y-axis.

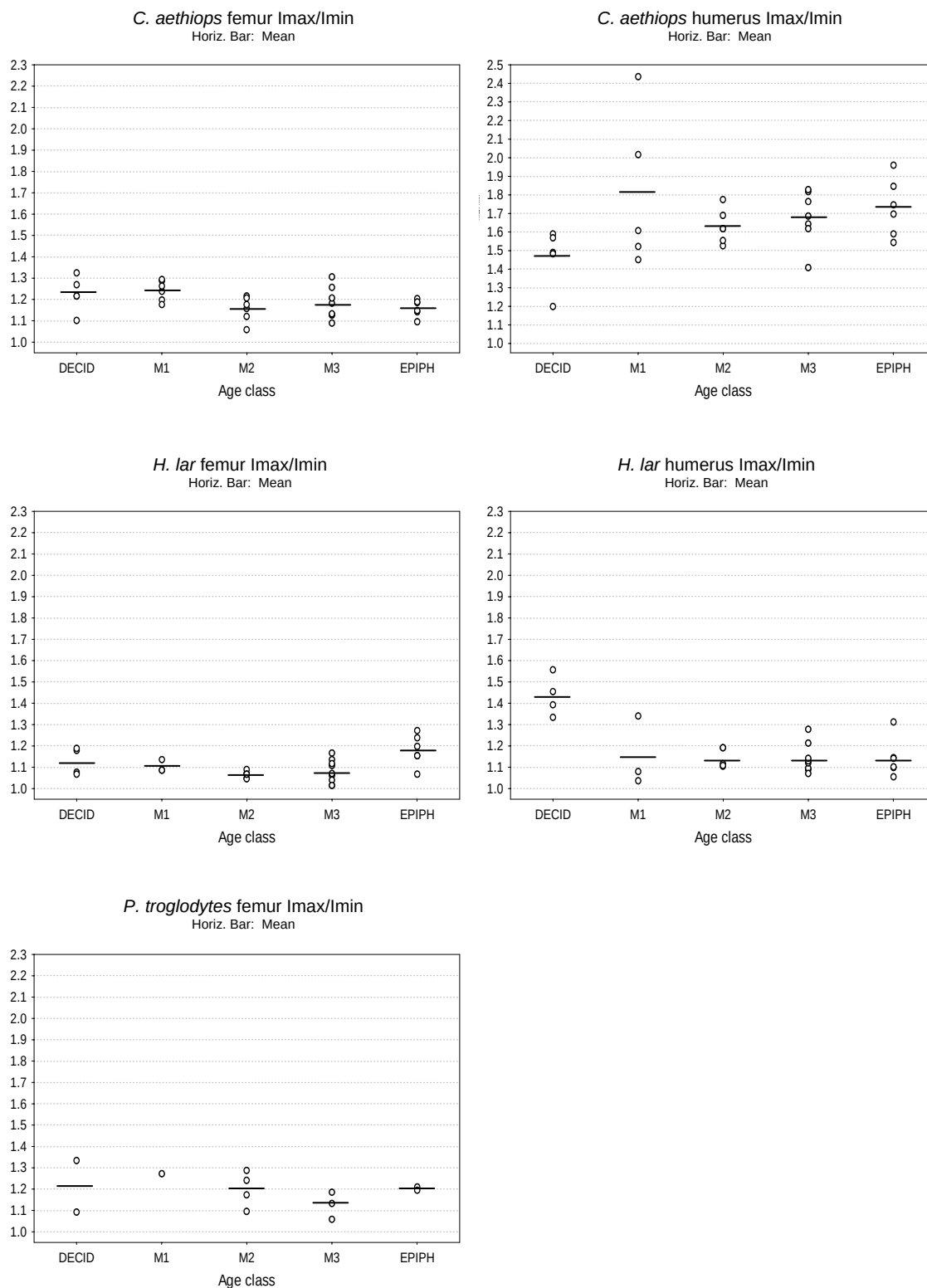


FIGURE 4-11. Plots of Imax/Imin at the midshaft femur and humerus by age class: raw data and age class means. (Top) *C. aethiops*, (Middle) *H. lar*, and (Bottom) *P. troglodytes*.

CHAPTER 5.

PRIMARY BONE MICROSTRUCTURE AND LIFE HISTORY

INTRODUCTION

A series of broadly comparative studies have demonstrated considerable diversity in the microscopic organization of primary bone tissues among vertebrates (Amprino and Godina, 1947; Demeter and Mátyás, 1928; Enlow and Brown, 1956, 1957, 1958; Foote, 1916; Quekett, 1855; Singh et al., 1974). This diversity is observed not only among taxa, but among individuals of different ages and within single skeletons. Quite remarkably, it has recently been shown that significant histological variation can even be observed among individuals that are indistinguishable in their gross skeletal morphology (e.g., postcranial elements of *Diademodon* and *Cynognathus*; Botha and Chinsamy, 2000). This diversity can be largely understood as a reflection of 1) variability in bone depositional rates at different times during ontogeny, 2) local dynamics of bone growth and development, and 3) environmental factors (e.g., Castanet et al., 2001; Castanet et al., 1993; Enlow, 1963; Ricqlès et al., 1991).

Among investigators of other vertebrate groups, there is a tradition of examining primary bone microstructure as a means of deciphering aspects of life history, including growth rates and maturational timing, environmental conditions, and more recently, skeletal function (e.g., Amprino, 1947; Castanet et al., 1993; Chinsamy-Turan, 2005; Erickson, 2005; Margerie, 2002; Peabody, 1961; Ricqlès, 1993). Despite the demonstrated potential of this approach for research in both modern and paleontological contexts, histological investigations of bone microstructure in primate limb diaphyses has

been opportunistic in nature (as part of the comparative studies mentioned above) or has tended to focus on secondary intracortical remodeling (e.g., Bouvier and Hylander, 1981; Burr, 1992; Burr et al., 1989; Paine and Godfrey, 1997; Schaffler and Burr, 1984). With a few notable exceptions (Castanet et al., 2004; Enlow, 1962, 1963; Newell-Morris and Sirianni, 1982), primary bone microstructure has remain largely unexplored in systematic studies of nonhuman primates.

The current study begins to address this shortcoming by examining ontogenetic variation in primary bone microstructure at the midshaft femur and humerus of *Chlorocebus aethiops* and *Hylobates lar*, and at the midshaft femur of *Pan troglodytes*. The major objectives of this study are to describe ontogenetic and taxonomic variability in patterns of primary bone microstructural organization, and to consider the significance of this variability for discriminating primate growth patterns. Results presented here are meant to provide a baseline understanding of ontogenetic variability in primary bone microstructure (particularly as reflected in primary bone tissue types) in a range of catarrhine taxa. Once this foundation is in place, more informed questions may be addressed in future studies concerning the influence of environmental conditions and other aspects of life history on particular features of the microstructural organization of primary bone.

BACKGROUND

This study focuses on patterns of microstructural organization of *primary* cortical bone tissues, namely, those tissues that were deposited during the process of bone growth at periosteal and endosteal surfaces. The following briefly reviews classifications of

cortical bone microstructure, including the various patterns of tissue organization encountered among vertebrates. This is followed by a discussion of the biological significance of primary bone microstructural diversity, particularly as it is relevant for life history studies.

Typological classification of bone microstructural diversity

Major constituents of bone tissue – bone cells (namely, osteocytes), vascular canals, the collagen fiber matrix, and mineral – may combine to produce a variety of patterns of microstructural organization which can be readily viewed using transmitted light microscopy. While bone microstructure varies along a continuum, investigators have historically developed typological classifications to characterize the considerable diversity observed among vertebrates and to facilitate comparisons among groups (Enlow, 1962, 1963; Enlow and Brown, 1956; Foote, 1916; Francillon-Vieillot et al., 1990b; Pritchard, 1972; Quekett, 1855; Ricqlès et al., 1991; Smith, 1961). Descriptions of bone microstructural diversity presented in several early studies relied on classification schemes based on only a single criterion (e.g., Haversian or non-Haversian in Quekett, 1855; collagen fiber patterns in Smith, 1961) or reflect general misunderstandings (Foote, 1916; Quekett, 1855; see Table 5-1) arising from the use of unstandardized terminology and a lack of knowledge concerning the biological basis of bone tissue organization (see discussion of these and other limitations by Enlow, 1966; Francillon-Vieillot et al., 1990b; and Ricqlès, 1993). As a result, many of these studies do not accommodate the range of bone microstructural patterns observed among vertebrates. This inadequacy in our understanding is further exacerbated by the fact that broadly-comparative studies

(e.g., Amprino and Godina, 1947; Enlow and Brown, 1956, 1957, 1958; Foote, 1916; Quekett, 1855; Singh et al., 1974) have tended to be based upon opportunistic samples, no doubt due to the limitations of specimen availability.

More recent classifications (Enlow, 1962, 1963, 1966, 1982; Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991) benefit from an understanding gained from more systematic comparative and experimental approaches, and are based upon multiple criteria having biological significance. (Tables 5-2 a and b). Among the most comprehensive and useful of these is the classification developed by Ricqlès (1975) and summarized recently with colleagues (Francillon-Vieillot et al., 1990b). This latter classification integrates criteria such as developmental origin, collagen fiber matrix organization, and vascularization into a single biologically-meaningful framework. These criteria, and resulting typological classifications of bone microstructural diversity, are discussed below. Their biological significance will be considered in a following section.

Developmental origin. Primary and secondary bone. The distinction between ‘primary’ and ‘secondary’ is commonly used in reference to the vascular canals of bone (e.g., primary versus secondary osteons, see below) and, by extension, bone tissues in general (Castanet and Ricqlès, 1986-7; Enlow, 1963). **Primary bone** tissues are those deposited *de novo*, where bone did not exist previously, although they are commonly deposited on pre-existing bone surfaces. Primary bone tissues are formed during the process of bone growth. In contrast, **secondary bone** is classically defined as a replacement tissue, i.e., deposited at sites where bone tissue has previously been resorbed (Castanet and Ricqlès,

1986-7; Francillon-Vieillot et al., 1990b).¹ Secondary bone tissues are formed during the process of secondary remodeling, and they are delimited from surrounding tissues by the presence of a reversal cement line. If this occurs within the bone cortex, the resulting structure is called a secondary osteon or Haversian system (Martin et al., 1998).

(Secondary osteonal bone is considered in Chapter 6 of this thesis.)

Endosteal versus periosteal origin. A given cross-section of a long bone cortex may be comprised of tissues originating from both the periosteal or endosteal surfaces, depending on its location along the diaphysis and the extent of medullary expansion (Enlow, 1963). Thus, cortical bone tissues can also be broadly categorized on the basis of their **periosteal** or **endosteal** origin, as shown in Table 5-2 and discussed (where relevant) below.

Collagen fiber matrix organization. The collagen fiber matrix and, concomitantly, the distribution of osteocyte lacunae, in bone are organized along a continuum, from being randomly organized to having a highly-ordered arrangement. Three fundamental patterns of collagen fiber organization are recognized in cortical bone tissues. (Figure 5-1).

1) **Woven bone** [also referred to as “fibrous” bone by some authors (Boyde and Jones, 1998; Enlow, 1963, 1966; Francillon-Vieillot et al., 1990b; Frost, 1960; Pritchard,

¹ It is recognized here that there exists some ambiguity in this distinction as it is classically defined (see discussion by Castanet and Ricqlès, 1986-7). This is particularly true in reference to bone deposited at periosteal and endosteal surfaces (following a previous resorptive phase at the surface), as may occur during such processes as medullary contraction, cortical drift and metaphyseal remodeling. In the current study, the term ‘primary bone’ refers to those tissues formed as a result of endosteal or periosteal growth during postnatal development, and it includes all tissues that are not otherwise classified as secondary osteons or Haversian bone.

1972; Ricqlès et al., 1991; Smith, 1961)] is characterized by loosely-packed collagen fibers ranging in diameter from 0.1 – 3 microns (Boyde and Jones, 1998) and coursing in all directions in a more or less random fashion. The random and loosely-arranged collagen fiber organization results in this tissue having reduced birefringence, generally appearing isotropic when viewed in polarized light. Although, the term “woven” originates from the observation that large collagen fibers have been described to resemble the “warp and woof” pattern of a woven fabric when observed in linear polarized light (Boyde and Jones, 1998; Frost, 1960). Osteoblasts are incorporated into the forming matrix of woven bone as plump and irregularly-shaped osteocytes (enclosed in lacunae), which may reflect their formerly high rate of synthesis of protein and other organic matrix constituents as active osteoblasts. Cytoplasmic processes contained in canaliculi are numerous and often very prominent. Osteocyte lacunae of woven bone are distributed randomly with respect to the bone matrix and they are more numerous compared to other primary bone tissues, occupying as much as half of the matrix volume (Boyde and Jones, 1998). Woven bone achieves its full mineralization rapidly and without the prolonged maturation phase observed in lamellar bone (Boyde, 1980; Boyde and Hobdell, 1969b; Boyde and Jones, 1998).

Woven bone is found in rapidly-growing regions of the immature skeleton, where it may be of endochondral or intramembranous (usually periosteal in the postnatal skeleton) origin (Enlow, 1962, 1963; Frost, 1960). In the fetal skeleton, woven tissue is frequently organized as porous *fine cancellous* tissue. When this tissue is deposited on the calcified cartilage framework during endochondral ossification, it can be recognized by the presence of calcified cartilage spicules (Enlow, 1963). Woven bone may also be

found in older individuals, in areas of fracture callous formation or pathologic periosteal response (Ortner and Turner-Walker, 2003).

2) **Parallel-fibered bone** (Enlow, 1966; Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991) is intermediate in organization between woven bone and lamellar bone (discussed below). It is characterized by closely-packed collagen fibers having a preferred orientation, running approximately parallel to one another. Thus, when viewed under polarized light, this tissue typically appears uniformly dark, when the preferred fiber orientation is perpendicular to the plane of section, or bright, when the preferred fiber orientation is parallel to the plane of section. Osteocyte lacunae are slightly flattened in shape, and tend to have a more regular arrangement than observed in woven bone (Jones and Boyde, 1976). Areas of transition between parallel-fibered bone and woven or lamellar bone, as reflected in the spatial arrangement of the collagen fiber matrix and osteocyte lacunae, are common. Vascularization is usually not dense (Francillon-Vieillot et al., 1990b).

3) **Lamellar bone** (Boyde and Jones, 1998; Enlow, 1963, 1966; Francillon-Vieillot et al., 1990b; Frost, 1960; Ricqlès et al., 1991) is characterized by a highly-ordered collagen fiber arrangement, and is comprised of layers of bone, called lamellae, which are frequently 2 -6 microns in width (Currey, 2002). Osteoblasts become incorporated into the forming matrix as spindle-shaped osteocytes, situated with their long axes parallel to the underlying collagen fiber matrix (Jones and Boyde, 1976). They tend to follow a linear course along lamellae. Osteocyte lacunae are less densely distributed than in other primary tissue types described above, and may comprise as little as 1 – 2 % of the matrix volume (Boyde and Jones, 1998). Primary lamellar bone may be

endosteal or periosteal in origin, or it may be deposited secondarily within the substance of already mineralized bone during the process of intracortical (Haversian) remodeling.

The majority of recent investigators agree that the layered appearance of lamellar bone is the result of a regular change (whether continuous or discontinuous) in the orientation of collagen fiber bundles between successive lamellae, which results in their optical distinction when viewed under polarized light [(Ascenzi and Benvenuti, 1986; Giraud-Guille, 1988; Smith, 1960; Weiner et al., 1997; Ziv et al., 1996), but see (Marotti, 1993; Marotti and Muglia, 1988; Rouiller et al., 1952; Ruth, 1947)]. However, there has been disagreement concerning the precise ultrastructure of the collagen matrix (recently reviewed in Currey, 2002). The classic view (e.g., Gebhardt, 1906) is that all collagen fibers within a lamella are characterized by a single preferred orientation, and this orientation changes abruptly (usually by 90 degrees) from one lamella to the next. Recent models of lamellar ultrastructure recognize that individual lamellae are composed of collagen fibers organized into branching bundles, which may have a single preferred orientation only over limited “domains” (Boyde, 1972; Boyde and Hobdell, 1969a). Thus, the preferred collagen fiber orientation may vary between domains within single lamellae, as well as between adjacent lamellae.

The recent model proposed by Giraud-Guille (1988) suggests that two patterns of organization can be observed in samples of lamellar bone, including an “orthogonal plywood” structure, which corresponds to the classic model described above, and a “twisted plywood” structure. Intermediate patterns between these two extremes also exist. According to the latter, collagen fiber bundles within single lamellae lie parallel to the lamellar boundary plane, and the orientation of their long axis rotates by a constant

angle from one plane to the next. A 180 degree rotation of collagen fiber bundles corresponds to the periodicity of a single lamella, and results in the optical distinction of adjacent lamellae observed in polarized light. Other studies have also corroborated the presence of a continuous transition in collagen fiber orientations in lamellar bone (Ascenzi and Benvenuti, 1986; Weiner et al., 1997; Ziv et al., 1996). Weiner and co-workers (1997) have noted the presence of orthogonal “thick” and “thin” major collagen arrays within lamellae, and suggested that the thickness of the transition zone between them may determine the extent to which lamellar ultrastructure appears to conform to an orthogonal or twisted plywood arrangement.

Bone tissues of periosteal origin belonging to the above tissue types are frequently grouped into two broad categories (Francillon-Vieillot et al., 1990b; Ricqlès, 1975):

1) **Lamellar-zonal bone** includes parallel-fibered and lamellar bone tissues.

Evidence of cyclical growth, in the form of resting lines or annuli (see below), is common. Lamellar-zonal tissues may be avascular, or characterized by simple primary vascular canals or scattered primary osteons (described in further detail below). Vascularization usually is not dense.

2) **Fibro-lamellar complex** is classically defined as a composite of woven bone and lamellar bone. This tissue type is deposited as an initial framework of woven bone organized into fine cancellous trabeculae, characterized by numerous spaces containing connective tissue cells and blood vessels. This framework is later in-filled by lamellar tissue, which is deposited along the cancellous surfaces, gradually “compacting” the cancellous spaces and resulting in the formation of primary osteons (see below).

Vascularization is dense and canals may be incorporated into the forming matrix in a variety of orientations. Several fibro-lamellar tissue types are recognized on this basis (see below). This tissue type corresponds to the periosteal *compacted fine cancellous* tissue of Enlow (Enlow, 1962, 1963, 1982)

As with all tissue types discussed here, variation in features described (e.g., collagen fiber orientation, osteocyte lacunar shape and distribution) is continuous, and intermediate bone tissue patterns are frequently observed. Lamellar-zonal and fibro-lamellar tissue patterns are not mutually exclusive and, in many areas, the initial scaffolding characteristic of fibro-lamellar bone may show a continuous transition from woven to parallel-fibered matrix (Francillon-Vieillot et al., 1990b; Castanet, pers. comm.). If other features characteristic of fibro-lamellar bone are also present (e.g., dense and regularly-distributed primary osteons, also see below), this latter tissue organization (having an initial scaffolding of parallel-fibered matrix) may be classified as fibro-lamellar (Currey, 1984, 2002).

Vascular patterns. Vascular canals may be incorporated into growing bone as either simple primary vascular canals or primary osteons (Enlow, 1963; Francillon-Vieillot et al., 1990b). *Simple primary vascular canals* are enclosed within the forming front of bone at the periosteal or endosteal surface, with the adjacent bone matrix coursing above and below the vascular canal. *Primary osteons* are vascular canals that are surrounded by at least one layer of (parallel-fibered or lamellar) bone tissue concentrically deposited around the canal. Their formation does not involve resorption of older bone tissues and, unlike secondary osteons (i.e., Haversian systems), they are not delimited from adjacent

bone tissue by the presence of a cement line. Primary osteons are commonly formed during the compaction of fine-cancellous woven tissue, resulting in the formation of fibro-lamellar bone.

Simple primary vascular canals and primary osteons may be incorporated into the forming matrix of bone in a variety of orientations (Enlow and Brown, 1956; Francillon-Vieillot et al., 1990b): 1) *longitudinal*, or parallel with the long axis of the bone, 2) *circular*, or circumferential, 3) *radial*, or 4) *oblique*. Fibro-lamellar tissues, in particular, are frequently characterized by primary osteons having more than one orientation. In these cases, several additional bone tissue types are recognized: (Figure 5-1).

1) **Laminar bone** (Currey, 1960; Foote, 1916; Francillon-Vieillot et al., 1990b) is a type of fibro-lamellar bone characterized by a two-dimensional network of vascular canals, in which anastomoses are formed between longitudinal and circular primary osteons. This tissue often has the appearance of circular vascular layers (called *laminae*), which are joined by longitudinal primary osteons coursing in and out of the plane of section.

2) **Plexiform bone** (Enlow and Brown, 1956; Francillon-Vieillot et al., 1990b) is a type of fibro-lamellar bone characterized by a three-dimensional arrangement of vascular canals, in which anastomoses are formed between regularly-distributed longitudinal, circular, and radial primary osteons.

3) **Reticular bone** (Enlow and Brown, 1956; Francillon-Vieillot et al., 1990b) is a type of fibro-lamellar bone characterized by an irregular network of anastomosing and obliquely-oriented primary osteons.

4) **Radiating fibro-lamellar bone** (Francillon-Vieillot et al., 1990b) a type of

fibro-lamellar bone characterized by prominent radially-oriented primary osteons, in addition to primary osteons having other orientations.

Other tissue patterns. Compacted coarse cancellous bone (Enlow, 1962, 1963, 1976, 1982; Enlow and Hans, 1996) is a composite tissue of endosteal origin. This tissue pattern results from the filling in of available spaces in endosteal coarse cancellous (trabecular, or “spongy”) bone, itself composed of tissue having a parallel-fibered or lamellar organization. The presence of compacted coarse cancellous bone is indicative of inward endosteal growth, usually associated with metaphyseal remodeling in the proximal or distal regions of the diaphysis during growth. During compaction, lamellar tissue is deposited along the arching contours of coarse cancellous trabeculae, giving this tissue type its distinctively convoluted appearance.

Sharpey fiber bone (Boyde, 1972) occurs in regions where extrinsic coarse collagen fibers (called Sharpey fibers) insert into the periosteal surface of bone, usually at an oblique angle, at sites of soft tissue (i.e., muscle, tendon, or ligament) attachment. The shape of nearby osteocyte lacunae may be influenced by the surrounding extrinsic fibers, and they often appear “stellate” in form (Boyde, 1972).

Significance of primary bone tissue type diversity for life history studies

There is a tradition of examining the histological organization of primary bone tissues as an important source of information about aspects of organismal life history, particularly relating to growth patterns (Amprino, 1947; for recent reviews, see Castanet et al., 1993; Chinsamy-Turan, 2005; Erickson, 2005; Klevezal, 1996; Ricqlès, 1993; and

Ricqlès et al., 1991). This approach has figured prominently in palaeobiological studies of dinosaurs and extinct avians, where it has yielded important insight into such issues as their growth rates and durations, the developmental basis for major changes in body size throughout their evolutionary history, and the degree to which their growth patterns may have been influenced by environmental factors (e.g., see also recent studies by Erickson et al., 2004; Horner et al., 2000; Padian et al., 2004; Padian et al., 2001; Ricqlès et al., 2000; Sander and Klein, 2005). Much of this work relies upon an empirical relationship between the microscopic organization of primary bone tissues, particularly those of periosteal origin, and the rates at which they are deposited during growth. Evidence for this relationship is discussed further below, focusing particularly on the significance of primary bone tissue type diversity.²

Relationship between bone growth rates and tissue type diversity. Amprino (1947) originally proposed that variation in the microscopic organization of primary cortical

² Given what is known about the contribution of collagen and mineral to material properties of bone (Currey, 2002; Martin et al., 1998; Martin and Ishida, 1989), variability in primary bone microstructure is also expected to have mechanical significance. However, while secondary osteonal bone has received a great deal of attention in this respect, little is known about the material properties of primary bone tissue types, particularly in relation to one another. It has been suggested that, as a result of its loosely-organized arrangement, woven-fibered bone may be weaker than lamellar bone (Currey, 2003). Further, bovine fibro-lamellar bone is known to be markedly anisotropic in its material properties (Currey, 2002, 2003; Reilly and Burnstein, 1975). Based on comparative studies, Margerie and colleagues (Margerie, 2002; Margerie et al. 2005) have proposed that the orientation of primary osteons in laminar fibro-lamellar tissues may represent a “strain mode-specific” adaptation to torsional loads generated in the wing skeleton and other horizontal limb elements of avians. However, studies by other researchers and on other vertebrate groups have provided only partial support for this hypothesis (Lee, 2004; Skedros and Hunt, 2004). The mechanical significance of primary bone microstructural diversity warrants further attention, particularly in experimental investigations, and is beyond the scope of the current study.

bone tissues reflects differences in their rates of deposition, which itself varies at different stages of life. This idea was based on the observation that the organization of the collagen fiber matrix of bone varies in a regular manner across different growth stages within taxa. He noted that deposition of loosely-organized woven-fibered bone coincides with periods of rapid growth during early ontogeny, in which large quantities of bone tissue are required to build the skeleton. This is followed by a transition to deposition of parallel-fibered bone, and then more highly-organized lamellar bone, as rates of body growth decrease. Further, Amprino observed that animals characterized by different patterns of body growth (e.g., antelopes vs. dogs, and among the latter, small breeds vs. large breeds) are characterized by differences in the pattern and timing of this succession of primary bone tissue types.

This hypothesis has been confirmed by qualitative and comparative studies of bone histology (e.g., Amprino and Godina, 1947; Enlow, 1962; Enlow and Brown, 1956, 1957, 1958) and, more recently, by quantitative studies employing flouorochrome vital labeling techniques (e.g., Castanet et al., 2004; Castanet et al., 1996; Castanet et al., 2000; Margerie et al., 2002; Margerie et al., 2004; Newell-Morris and Sirianni, 1982; see review of earlier studies by Ricqlès et al., 1991). As a result of this research, it is now well established that the organization of the collagen fiber matrix of cortical bone tissues, as well as other details of their microstructure (e.g., vascularity), varies in accordance with bone growth rates.

Organization of the collagen fiber matrix. Fluorochrome vital-labeling studies, conducted on relatively few vertebrate species to date, have confirmed Amprino's (1947) observations that variation in the organization of the collagen fiber matrix of bone

reflects variation in bone growth rates. According to a review by Ricqlès and colleagues (1991), depositional rates of $0.26 - 5 \mu\text{m} / \text{day}$ have been observed for avascular woven-fibered bone, although it is noted this tissue type often is deposited at rates higher than $0.4 \mu\text{m} / \text{day}$. Depositional rates for avascular parallel-fibered bone deposited at periosteal surfaces are reportedly in the general range of $0.10 - 0.5 \mu\text{m} / \text{day}$, and avascular periosteal lamellar bone depositional rates are $< 0.05 - 0.3 \mu\text{m} / \text{day}$ (Ricqlès et al., 1991; see Table 1, p. 32-33, and references therein). Others have reported depositional rates of $\geq 4 \mu\text{m} / \text{day}$ and $< 1 \mu\text{m} / \text{day}$ for woven and lamellar bone tissues, respectively (Boyde, 1980; Currey, 2002), although it is not clear whether this includes both avascular and vascular tissues, and tissue of endosteal and/or secondary origin.

Systematic and quantitative data concerning depositional rates for primary periosteal bone tissue types are limited in nonhuman primates, likely due in part to the practical and ethical difficulties associated with flouorochrome vital labeling studies. Castanet and colleagues (2004) reported depositional rates of $3 - 8 \mu\text{m} / \text{day}$ for vascular (containing simple vascular canals and scarce longitudinal primary osteons) parallel-fibered bone in the cortex of long bones from juvenile grey mouse lemurs (*Microcebus murinus*). In these same animals, avascular parallel-fibered bone in the outermost cortex was deposited at rates averaging $0.1 \mu\text{m} / \text{day}$.

Newell-Morris and Sirianni (1982) studied development of the midshaft humerus in a sample of 15 prenatal and early postnatal (to 61 weeks) pig-tailed macaques (*Macaca nemestrina*). They reported periosteal depositional rates for vascular woven bone ranging from $3.5 - 20.4 \mu\text{m} / \text{day}$ during the prenatal period, with an average depositional rate of $9.8 \mu\text{m} / \text{day}$ prior to 155 postovulatory days. Deposition of vascular parallel-fibered

bone occurred from 7 – 27 postnatal weeks at an average rate of 4.3 $\mu\text{m} / \text{day}$ (range 0.9 – 10.9), with most observations falling below 5 $\mu\text{m} / \text{day}$. The lowest depositional rates were recorded in individuals older than 1 year of age (58 – 61 postnatal weeks), and ranged from < 1.5 – 4.6 $\mu\text{m} / \text{day}$. These latter measurements represent averaged depositional rates across regions containing both parallel-fibered and lamellar bone.

Primary periosteal bone tissues deposited at the midshaft humerus of pre-term and infant pig-tailed macaques studied by Newell-Morris and Sirianni (1982) were densely vascularized, particularly during the prenatal period when, on average, 7.4 primary osteons were incorporated into the forming matrix each day. This rate dropped during postnatal life, to an average of 2 primary osteons per day. Depositional rates reported for parallel-fibered bone in these animals are generally consistent with rates reported for vascular parallel-fibered bone in grey mouse lemurs, and are higher than rates reported for avascular parallel-fibered bone (Castanet et al., 2004; Ricqlès et al., 1991). This trend reflects a relationship between tissue vascularization and bone depositional rates, discussed further below.

Tissues of endosteal or secondary (i.e., Haversian) origin can be deposited at faster rates than those reported above for periosteal tissues having the same collagen fiber matrix and/or vascular organization (Buffrénil and Pascal, 1984; Margerie et al., 2002; Ricqlès et al., 1991). For example, Margerie and colleagues (2002) reported that the upper range of depositional rates for avascular bone in mallards (*Anas platyrhynchos*) was as much as two times faster for endosteal tissues compared to periosteal tissues. Buffrénil and Pascal (1984) reported depositional rates of endosteal lamellar bone as high as 3.4 $\mu\text{m} / \text{day}$ in the mandible of minks. Endosteal (cortical and cancellous) and

secondary osteonal (presumably parallel-fibered or lamellar) bone depositional rates reported for rhesus macaque long bones and ribs generally range from 1.0 – 1.5 $\mu\text{m} / \text{day}$ (Schock et al., 1975; Schock et al., 1972; Wronski and Morey, 1983), although rates as high as 2 – 3.6 $\mu\text{m} / \text{day}$ for secondary osteonal bone were reported for the femur and mandible of young “monkeys” (Yen and Shaw, 1977). Depositional rates for secondary osteonal bone in humans are similar to those reported for macaques, although this rate appears to decrease with age (reviewed by Martin et al., 1998).

Vascularity. As suggested above, evidence indicates that variation in the vascularity of primary cortical bone tissues also relates to variability in bone growth rates, with more highly vascularized tissues being associated with faster depositional rates compared to less vascularized tissues (recently reviewed by Ricqlès et al., 1991; but see Starck and Chinsamy, 2002 for a critical discussion). This has been most clearly demonstrated recently in a series of systematic studies of extant avians by Castanet and colleagues (Castanet et al., 1996; Castanet et al., 2000; Margerie et al., 2002; Margerie et al., 2004). According to these studies, vascular fibro-lamellar tissues (containing laminar, reticular, radial and, to a lesser extent, longitudinal arrangements of primary osteons) are the most rapidly growing primary bone tissues, compared to less vascular and lamellar-zonal tissues, and they are generally characterized by depositional rates of > 5 – 10 $\mu\text{m} / \text{day}$. Depositional rates as high as 171 $\mu\text{m} / \text{day}$ have been recorded for radial fibro-lamellar bone in king penguin (*Aptenodytes patagonicus*) chicks (Margerie et al., 2004). These findings are in general agreement with studies of mammalian bone, including primates (Buffrénil and Pascal, 1984; Castanet et al., 2004; Newell-Morris and Sirianni, 1982; Ricqlès et al., 1991). The woven bone with primary osteons characterized

by Newell-Morris and Sirianni (1982) as having depositional rates as high as $20.4 \mu\text{m} / \text{day}$ would be categorized as a fibro-lamellar tissue according to current typological classifications (e.g., Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991).

However, there also appears to be considerable variability and overlap in the depositional rates of many fibro-lamellar tissue ‘sub-types’, recognized on the basis of the *orientation* of vascular canals incorporated into their matrices (Castanet et al., 1996; 2000; Margerie et al., 2002; 2004; Starck and Chinsamy, 2002). Despite this variability, certain vascular subtypes of fibro-lamellar bone appear to be associated with faster depositional rates in some taxa. For instance, in king penguin chicks, radial fibro-lamellar bone was associated with significantly faster depositional rates, and laminar fibro-lamellar bone was associated with significantly slower depositional rates, than other fibro-lamellar tissue types (i.e., longitudinal and reticular) (Margerie et al., 2004). However, longitudinal fibro-lamellar tissues were deposited at *slower* rates compared to reticular and laminar fibro-lamellar tissues in extant ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*) (Castanet et al., 2000).

Focusing on several features of the vascular network of primary periosteal bone in vitally-labeled post-hatchling mallards (*Anas platyrhynchos*), Margerie and colleagues (2002) suggested that the *density* at which primary osteons are incorporated into growing bone is a better indicator of variation in bone depositional rate than vascular canal *orientation*. In their study, fibro-lamellar tissue types characterized by more densely-distributed primary osteons (categorized as ‘FLC type’, defined on the basis of adjacent primary osteons being separated by a radial interval less than their diameter) were associated with faster depositional rates (generally greater than $20 \mu\text{m} / \text{day}$) than primary

tissues having less-densely-distributed primary osteons ('LPO type', generally 20 – 5 μm / day). They also noted that 'FLC-type' tissues were characterized by primary osteons having larger diameters than 'LPO' tissues. Starck and Chinsamy (2002) also reported a difference in primary osteon size between control and experimental food-restricted Japanese quail (*Coturnix japonica*), with the latter characterized by a greater number of small-diameter primary osteons and slower rates of deposition.

Other microstructural features indicating bone growth rate variability. Regular variations in bone growth rates (e.g., owing to seasonal growth rhythms) and temporary interruptions in bone growth are also recorded in microscopic features of bone. These features are often referred to more generally as “growth marks” (Castanet et al., 1993; Ricqlès et al., 1991). While observations of histological features in bone suggested to indicate cyclical or environmentally-mediated growth patterns had been reported by earlier investigators (e.g., Mattox, 1935), Peabody (1961) undertook an extensive comparative examination of what were referred to as “annual growth zones” in the bones of both living and extinct vertebrates. As a result of his work and that of other researchers, including recent experimental approaches, three types of growth marks are recognized histologically in the bone tissues of vertebrates (see recent reviews by: Castanet et al., 1993; Francillon-Vieillot et al., 1990b; Klevezal, 1996). These are described briefly below. Although growth marks are not the focus of the current study, they are noted when observed in primates examined here.

(1) **Zones** (Castanet et al., 1993; Peabody, 1961) are increments of bone deposited during periods of relatively rapid growth, and they are typically comprised of more

vascular and / or less ordered primary bone tissue types (e.g., fibro-lamellar or parallel-fibered bone). Zones generally appear opaque in transmitted light microscopy.

(2) **Annuli** (Castanet et al., 1993; Peabody, 1961) represent periods of slow growth, most commonly being comprised of less vascular lamellar bone. Annuli typically appear as narrow bone layers in comparison to zones, and they appear translucent in transmitted light.

(3) **Resting lines** [also commonly referred to as “lines of arrested growth” (Castanet, 1980; Castanet, 1981; Enlow, 1963; Klevezal, 1996)] represent one category of cement line. They are formed as a result of temporal discontinuities between cycles of bone deposition (i.e., due to temporary cessation in bone growth), and they can be distinguished from other types of cement lines (i.e., from reversal lines) by their smooth contour. Cement lines are generally thought to be collagen deficient and rich in ground substance, and most investigators (e.g., Boyde and Jones, 1998; Castanet, 1981; Frasca, 1981; Skedros et al., 2005) consider them more highly mineralized relative to adjacent bone tissues (but see Burr et al., 1988; Schaffler et al., 1987). Cement lines can be readily identified in transmitted light microscopy where they appear as thin (frequently 1-2 microns in width; Frasca, 1981) translucent bands, in polarized light where they are generally brighter than surrounding tissue, and with the aid of histological staining techniques (Castanet, 1981; Castanet et al., 1993; Klevezal, 1996).

The distribution of growth marks among vertebrates, their significance for discriminating environmentally-mediated growth patterns, and their practical significance for skeletochronological studies has been discussed in several recent reviews (Castanet et al., 1993; Chinsamy-Turan, 2005; Erickson, 2005; Klevezal, 1996; Ricqlès et al., 1991).

Reconstructing bone growth history. As discussed above, bone microstructure exhibits considerable age-related and taxonomic diversity. This variation is suggested to reflect variation in bone growth rates at different times during ontogeny within taxa, and between taxa characterized by different patterns of body growth. However, many of the studies cited above have also demonstrated differences in bone microstructure and / or rates of osteogenesis among elements within individuals, and among different cortical regions within elements (e.g., Castanet et al., 2004; Castanet et al., 1996; Margerie et al., 2004; Newell-Morris and Sirianni, 1982). Such observations underscore the importance of considering the influence of local / anatomical factors on bone growth dynamics and microstructure whenever possible.

Long bones not only undergo increases in linear and cross-sectional dimensions during growth; they must also be continuously remodeled as they acquire their characteristic size and shape. Enlow (Enlow, 1962, 1963, 1976, 1982; Enlow and Hans, 1996) clarified the fundamental tissue-level mechanisms of bone growth remodeling, which involve temporally- and spatially - coordinated patterns of bone deposition and resorption at endosteal (including cancellous) and periosteal surfaces. Mechanisms such as cortical drift and metaphyseal remodeling were described in Chapter 2 of this thesis, and were summarized in Figures 2-1 and 2-2. These processes may entail local destruction of tissues deposited during earlier growth stages and differential rates of osteogenesis across cortical regions within elements.

Bone growth remodeling produces a stratified and heterogeneous appearance of cortical bone in cross-section. Each stratum represents a cycle of bone deposition of

tissue that may vary in its microstructural organization, or a reversal in growth direction, from adjacent strata. Microstructural “signatures” of growth remodeling (*e.g.*, cement lines indicating a reversal in growth direction) observed in transmitted light microscopy of histological thin-sections have been clarified and described in detail by Enlow (Enlow, 1962, 1963, 1976, 1982). Their consistency with features associated with formative and resorptive activity on the periosteal surface of long bones observed in scanning electron microscopy has also been demonstrated (Saunders, 1985). Thus, the local growth *history* of a given cross-section of bone can be reconstructed from its histological organization; each stratum represents a different stage in the growth of the organism and, given an understanding of local remodeling patterns, this history can be interpreted from details of its histological organization.

Previous studies of diaphyseal bone histology in nonhuman primates.

Primates have been included in a series of comparative investigations conducted over the past approximately 150 years, which have examined bone microstructure across a wide range of vertebrate groups (Amprino and Godina, 1947; Demeter and Mátyás, 1928; Enlow and Brown, 1958; Foote, 1916; Quekett, 1855; Singh et al., 1974). These studies have demonstrated that vertebrates in general, and primates specifically, exhibit substantial diversity in the microscopic organization of their primary bone tissues. (Table 5-3 a). However, the significance of many of these early studies for our understanding of primary bone microstructure in primates is limited for reasons discussed above (also see Enlow, 1966; and Ricqlès, 1993).

In recent decades, studies of bone histology in long bone diaphyses of nonhuman

primates have focused to a large extent on secondary intracortical remodeling (reviewed in Chapter 6 of this thesis). Aspects of primary bone microstructure have remained surprisingly unexplored, with a few exceptions. Enlow (Enlow, 1962, 1963) undertook the first systematic investigations, which focused on elucidating basic tissue-level growth mechanisms involved in long bone development in rhesus macaques and other representative vertebrates. His studies remain among the most comprehensive examinations of diaphyseal bone histology to date; variation in both primary and secondary bone tissues was examined in individuals of different ages, and across semi-serial sections extracted along the lengths of individual bone elements. More recently, Newell-Morris and Sirianni (1982) and Castanet and colleagues (2004) employed fluorescent vital labeling techniques in examinations of bone growth and microstructure in *Macaca nemestrina* and *Microcebus murinus*, respectively. Pertinent results from the latter studies were reviewed above (see “*Relationship between bone growth rates and tissue type diversity*”), and are summarized in part in Table 5-3 b. These studies demonstrated substantial age and regional variation in the microstructural organization of primary bone tissues within diaphyseal cross-sections, coincident with variability in bone depositional rates. (Also see Warshaw, 2005, in prep.).

RESEARCH OBJECTIVES

Chapter 4 characterized general patterns of growth in cross-sectional dimensions at the midshaft femur and humerus of *Chlorocebus aethiops* and *Hylobates lar*, and at the midshaft femur of *Pan troglodytes*. Investigation of primary bone microstructure is expected to reveal the underlying growth processes at these skeletal sites. Given the

paucity of systematic research on nonhuman primates, the current study was designed to provide a baseline understanding of ontogenetic variability in primary bone microstructure across skeletal sites in these taxa, focusing particularly on ontogenetic variation in proportions of bone tissue ‘types’, and to consider the significance of this variability for interpreting primate growth patterns. Specific aims of the study are as follows:

1) *To describe ontogenetic and regional variation in primary bone microstructure.* Proportions of whole cross-section cortical area comprised of different primary bone tissue types are quantified, focusing on tissue types of periosteal origin and having relevance for discrimination of bone growth rate variability. Endosteal tissue types and regional variation in tissue type proportions within cross-sections (i.e., across circumferential rings and radial sectors) are also examined, to the extent that they reflect local circumstances of growth remodeling. Other details of primary bone microscopic structure, such as vascular patterns, are qualitatively described.

2) *To address the following question: Do primary bone tissue type proportions vary in accordance with expectations based on growth patterns described previously for other body dimensions?* Focusing on bone tissues of periosteal origin, variability in whole cross-section proportions of primary bone tissue types is analyzed with respect to age class within taxa. This variability is interpreted within the context of previously-described patterns of growth in other body dimensions, particularly cross-sectional geometric dimensions (as described in Chapter 4) and body mass (as summarized in

Table 5-4). Variability in primary bone tissue type proportions is also interpreted in light of local growth and secondary remodeling history. Predictions of this study are necessarily general in nature, due to the paucity of comparative data available for primates:

A) Intraspecific patterns. Primary bone tissue type proportions are expected to vary with age within taxa. During times of relatively rapid body mass growth (i.e., during early ontogeny), individuals are expected to show higher proportions of bone tissues types associated with faster depositional rates (e.g., having a woven-fibered matrix component and greater vascularity). Highly-ordered less vascular (e.g. lamellar) bone tissues will occur in higher proportions as body mass growth slows during late ontogeny. In those taxa (*C. aethiops*, *P. troglodytes*) characterized by sexually-dimorphic patterns of growth and/or the presence of a subadult growth spurt, tissue type proportions are expected to vary accordingly.

B) Interspecific variability. Among individuals of similar biological age (e.g., dental emergence status), those species characterized by faster absolute rates of body mass growth (*P. troglodytes*) are expected to show higher proportions of tissue types associated with fast depositional rates, compared to taxa characterized by slower rates of body mass growth (*C. aethiops* and *H. lar*).

MATERIALS AND METHODS

This project utilizes midshaft femur and humerus bone samples collected from a comparative sample of *Chlorocebus aethiops* (vervet monkeys, $N=32$), *Hylobates lar* (white-handed gibbons, $N=28$) and *Pan troglodytes* (common chimpanzees, $N=12$). As

described in Chapter 1, these taxa were selected to provide a focused comparative context for the investigation of ontogenetic changes in bone microstructural features and their relationship to life history. Postnatal growth patterns of these taxa, particularly patterns of body mass growth, have been characterized previously (e.g., Bolter and Zihlman, 2003; Hamada et al., 1996; Hamada and Udono, 2002; Leigh, 1996; Leigh and Shea, 1995; Turner et al., 1997) and are summarized in Table 5-4.

Materials

Selection of appropriate samples for study. As described in detail in Chapter 3 of this thesis, several criteria were prioritized in the selection of collections from which specimens would be taken for the current study: 1) collections comprising wild-caught individuals from restricted geographic areas, to reduce potential variation in growth and development among individuals living under different ecological constraints (e.g., Altmann and Alberts, 1987; Hamada et al., 1996; Phillips-Conroy et al., 2000; Zihlman et al., 2004); 2) collections comprising an equal distribution of individuals among different age classes throughout the postnatal growth period (discussed below), an equal distribution of males and females, and associated body mass data; 3) collections comprising immature individuals and non-geriatric adults. Geriatric adults were excluded on the basis of tooth wear and evidence of osteoarthritis. Individuals showing obvious evidence of skeletal fractures and pathologies (as described by White, 1991) were also avoided.

Both the invasive nature of the sampling protocol required for histological investigation and the importance of the above selection criteria meant that the taxonomic

range and appropriate samples available for this study were limited. While it was generally possible to meet criteria 1-3 for the *C. aethiops* and *H. lar* samples, many criteria were difficult to meet when selecting appropriate material to comprise the *P. troglodytes* sample. However, given what little is known of this latter taxon, the sample available for this study provides invaluable preliminary data on ontogenetic variability in cortical bone microstructure.

Biological age classes. In the absence of chronological age information for individuals examined in the current study, it was necessary to rely on other criteria to estimate biological age. Dental alveolar emergence was chosen as the most appropriate criterion for estimating biological age in the current study for several reasons (e.g., Smith, 1989, 1992). For statistical analysis in the current study, individuals were classified into five biological age classes, presented in Table 5-5. Choice of dental emergence as a proxy for biological age, and determination of biological age of individuals in the current sample is discussed in detail in Chapter 3 of this thesis.

Tooth eruption and long bone epiphyseal union status were assessed according to the methods of Wintheiser et al. (1977) and Bolter and Zihlman (2003). Individuals continue skeletal growth after full eruption of the dentition (e.g. personal observations, and Bolter and Zihlman, 2003; Watts, 1990). Thus, individuals were only considered adult when they had reached both dental and skeletal maturity, defined by complete permanent dentitions and complete fusion of all long bone epiphyses, respectively. Among adults, geriatric individuals were excluded on the basis of extreme tooth wear or evidence of osteoarthritis as described by White (1991).

Description of the sample. Table 5-6 provides details about sample representation across biological age classes in the current study. Further detailed information about individuals comprising each species sample can be found in Chapter 3 and Appendix B.

Two samples (*C. aethiops* SLW 47 femur, *H. lar* MCZ 41475 humerus) were excluded from this analysis due to preparation artifacts, and resulting difficulty in the interpretation of microstructural details necessary for accurate identification of primary bone tissue types. Further, anteroposterior orientation of the *C. aethiops* SLW 21 humerus was lost during processing, and this particular bone sample was therefore excluded from examination of orientation-dependent variables (i.e., radial sectors).

Sample location

Bone samples extracted from the midshaft femur and humerus were utilized in this study. The midshaft location is commonly chosen for investigations of bone structural properties and histocompositional variables, making results more comparable to other published studies (e.g., Burr, 1992; Goldman et al., 2003; Schaffler and Burr, 1984; Schaffler et al., 1985).

More importantly, sites located more proximally or distally along the diaphysis are subject to more destructive growth remodeling processes, namely, metaphyseal remodeling in which the periosteal cortex is largely resorptive (Enlow, 1962, 1963). With respect to this latter point, it is recognized that anatomical midshaft may not coincide with the true developmental midshaft. The proximal metaphysis of the femur and the distal metaphysis of the humerus, respectively, contribute disproportionately to

the overall lengthening of these bones during growth (Scheuer and Black, 2000), and it is therefore possible that the sampling location relative to the developmental midshaft may be relocated in morphological space relative to other bone regions during growth (Enlow, 1962, 1963). (This is illustrated in Figure 2-2). Serial (i.e., lengthwise) sectioning of representative elements across age classes would allow for the accurate identification of developmental midshaft based on histological details. While such a destructive sampling protocol was not possible here given limitations of specimen availability, sampled regions affected by such remodeling processes should be recognizable on the basis of histological features characterized previously (Enlow, 1962, 1963).

Identification of the midshaft of long bones under investigation is usually based on a measurement of bone length, which includes the epiphyses of a long bone (Ruff and Hayes, 1983). However, this traditional measure of bone length prevents the inclusion of specimens for which epiphyses are either unfused, broken or otherwise missing. To maintain consistency in our definition of midshaft between immature (unfused) and adult specimens, a measure of bone length was developed for the current study which excluded the epiphyses. Bone length measurements and orientations were completed as described in Chapter 3 of this thesis.

Image acquisition and image processing

The sample removal, preparation and imaging methods are described in detail in Chapter 3 of this thesis. Bone samples were imaged in both conventional transmitted light microscopy (LM) and circularly-polarized light microscopy (CPL) modalities. This facilitated examination of morphological details necessary for the determination of bone

tissue patterns as described above: the organization of bone cells and vascular canals could be readily viewed in LM, while the organization of the collagen fiber matrix could be readily examined using CPL. Acquired LM and CPL images were then imported into several commercially available software packages for image processing, visualization and data acquisition.

Image acquisition. All images were acquired at low magnification using a Leica DMRX/E Universal microscope (Leica Inc., Bannockburn, IL.) configured with a circularly polarized light filter arrangement and an automated Marzhauser stage (0.1 μm accuracy in 'X' and 'Y'). Images were captured using a JVC KY55BU (5X objective; 1 pixel = 1.4970 μm) or JVC KY55BE (5X objective; 1 pixel = 1.2563 μm) color CCD camera, and were automatically transferred to a Syncroscope Montage Explorer system (Synoptics Ltd., Cambridge, UK.). (The JVC KY55BU camera was upgraded to a JVC KY55BE model in the middle of this study.) The Syncroscope Montage Explorer system relies on pattern-recognition algorithms to automate the montaging of large field-of-view, high-definition images in real-time. This capability overcomes the limitation of a restricted field-of-view in conventional imaging techniques, and produces a single image of an entire cross section of bone (up to 30,000 x 30,000 pixels in size) that retains original microscopic detail afforded by the optical conditions of the microscope.

LM imaging was undertaken with the bottom linear polarizing filter of the CPL filter arrangement removed, creating conventional (unpolarized) transmitted light illumination. To improve LM image quality, individual image fields were corrected in Montage Explorer for illumination and color unevenness using shade correction functions

as the montage was assembled.

CPL imaging of bone samples on the Leica DMRX/E Universal microscope was completed with a circularly polarized light filter arrangement in place, for the strict purpose of visualizing the organization of the collagen fiber matrix in bone to facilitate identification of bone tissue types. While linear polarized light microscopy would have been adequate for the purpose of the current study, visualization was further improved with CPL as this technique eliminates the “Maltese Cross” artifact, in which reduced light intensity occurs at each $45\text{-}225^\circ$ and $135\text{-}315^\circ$ of arc relative to the transmission axes of the polarizer (Bromage et al., 2003). Polarized light microscopy and the CPL filter arrangement are described in further detail in Chapter 3 of this thesis.

CPL images acquired under standardized illumination conditions (described previously by Bromage et al., 2003), while critical for the quantitative gray-level analysis of preferred collagen fiber orientation, were not appropriate for the current study. Pilot images of whole bone cross sections acquired from the study sample according to the method of Bromage et al. (2003) were frequently too dark for adequate visualization and interpretation of bone microstructural features, owing to the minimal brightness of large areas of primary bone tissues in these samples having a preferentially longitudinal collagen fiber orientation. Thus, since CPL imaging in the current study was undertaken for the purpose of visualizing morphological information, illumination conditions for CPL imaging were adjusted depending on section brightness, to permit subtle changes in the organization of the collagen fiber matrix to be visualized.

Following acquisition, all images of left-side elements were horizontally flipped in Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.) to maintain a common

orientation for analysis.

Image processing and creation of bone tissue maps. LM and CPL images were imported into Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.), where they were superimposed for visualization purposes and for the creation of bone tissue maps. Image processing functions are outlined in detail in Appendix D, and are summarized below.

Sequential image processing steps were performed using the ‘Layers’ function. First, a binary overlay was created from the original LM image, on which most non-bone areas (e.g., medullary canals, large resorption spaces, large cracks and other preparation artifacts, but not small vascular canals and Haversian spaces) were assigned to a background color so they could be deleted in later quantitative analysis. Secondly, areas representing distinct primary and secondary (the latter described further in Chapter 6) bone tissue types (discussed further below) were manually traced and color-coded on separate layers. (Figure 5-2). Qualitative observations (e.g., concerning remodeling and vascular patterns) were also annotated on a separate layer, facilitating the reconstruction of the growth history of each individual bone cross-section from its histological organization.

For data acquisition, only those color-coded layers representing different bone tissue types were selected for the creation of composite tissue maps. The binary image overlay was inverted, so that most non-bone areas were assigned to black. A composite tissue map was created using the ‘Flatten’ function. Flattened images were reduced to 25 % or 12.5 % (for *P. troglodytes* samples) of their original pixel dimensions. Finally,

these images were converted to gray-scale, in which each tissue type was associated with a different gray-level value. Non-bone areas were assigned a gray-level value of 0, and thus could be eliminated from quantitative analysis.

Typological classification of bone tissue organization

Patterns of bone microscopic organization were classified into a number of bone tissue ‘types’, following the comprehensive scheme proposed by Ricqlès and colleagues (summarized recently in Francillon-Vieillot et al., 1990b). In some cases, bone tissue types as described by Francillon-Vieillot et al. (1990b) were difficult to operationalize, and required modification (e.g., as in the case of transitional tissue types, FLC-LZPO and PF-LAM, below). Endosteal coarse cancellous tissue, described in detail by Enlow (e.g., 1963), was also recognized in the current study.

Tissue types were identified on the basis of several criteria relating to the collagen fiber matrix, osteocyte lacunar organization and vascularity of bone, as observed in CPL and LM microscopy in the current study. These criteria are described below:

Woven-fibered bone (WOV). Woven-fibered bone (WOV) was identified in the current study on basis of the following criteria. 1) *Loosely-organized collagen fiber organization, in which collagen fibers course in all directions in a more or less random fashion, resulting in a generally isotropic or randomly-organized appearance in polarized light.* 2) *Plump and irregularly-shaped osteocyte lacunae (visualized in LM; often with prominent canaliculi), which are randomly distributed with respect to the matrix.* Osteocyte lacunae are more numerous and densely-distributed in WOVS tissue,

compared to other tissue types. Thus, when WOV occurs in limited patches, osteocyte lacunae frequently appear clumped in their distribution. WOV may be avascular, or vascularized by simple primary vascular canals and / or less densely-distributed primary osteons. WOV (as distinct from FLC, below) was uncommon in bone samples examined here.

Parallel-fibered bone (PF or EPF). (Figure 5-3 a). Parallel-fibered bone of periosteal (PF) and endosteal (EPF) origin is typically described to be intermediate in its microstructural organization between WOV and LAM (below). PF was identified in the current study on the basis of the following criteria. 1) *Closely-packed collagen fibers having a preferred orientation, resulting in an anisotropic appearance in polarized light.* Depending on the orientation of the collagen fiber matrix, PF appears homogeneously dark (if the fiber orientation is perpendicular to the plane of section) to bright (if the fiber orientation is parallel to the plane of section) in polarized light. PF bone was identified as such when approximately 75 % or more of the matrix appeared as described above, lacking any lamellar structure (see below). 2) *Slightly flattened osteocyte lacunae (visualized in LM), which tend to be more regularly distributed compared to WOV.* In practice, a range of intermediate osteocyte lacunar morphologies was observed in PF bone, from being more rounded (though typically not with the prominent canaliculi observed in WOV) to more flattened in shape. E/PF may be avascular, or vascularized by simple primary vascular canals and / or less densely-distributed primary osteons.

Lamellar bone (LAM or ELAM). (Figure 5-3 c). Lamellar bone of periosteal (LAM)

and endosteal (ELAM) origin was identified on the basis of the following criteria. 1) *Highly-ordered collagen fiber matrix having a layered structure, resulting in the optical distinction of individual lamellae in polarized light.* In practice, lamellar bone in regions having a predominantly longitudinal collagen fiber orientation (appearing dark in CPL) was, on occasion, difficult to distinguish from non-lamellar bone on acquired CPL images. (This situation arose most commonly in *H. lar* samples from the DECID, M1, and M2 age classes, where lamellar-zonal tissues more commonly appeared dark in CPL.) Lamellar structure in these cases could more readily be visualized in real-time microscopy by rotating the bottom polarizing filter slightly out of position. When no lamellar structure could be visualized using either technique, tissue was classified accordingly (as PF or PF-LAM). Individual lamellae may be clearly distinct, or they may appear to branch frequently and be less regular in structure. LAM bone was identified as such when lamellar structure was determined to comprise approximately 75 % or more of the matrix. 2) *Flattened or spindle-shaped osteocyte lacunae (visualized in LM), situated with their long axes parallel to the underlying collagen fiber matrix and following a linear course.* In practice, osteocyte lacunae in LAM may have two appearances in LM, depending upon the orientation of their associated collagen fiber matrix. Osteocyte lacunae associated with “bright” lamellae when viewed in CPL (having a predominantly transverse fiber orientation) tend to have a more flattened spindle shape, with their long axis lying in the plane of section and perpendicular to the width of individual lamellae. Osteocyte lacunae associated with “dark” lamellae when viewed in CPL (having a predominantly longitudinal fiber orientation) tend to appear smaller in size and more oval (resembling a peach pit) in shape, as though the section

plane traversed the lacunar axis of smaller diameter. E/LAM may be avascular, or vascularized by simple primary vascular canals and / or less densely-distributed primary osteons.

Parallel-fibered to lamellar transition tissue (PF-LAM or EPF-LAM). (Figure 5-3 b).

Parallel-fibered to lamellar transition tissue of periosteal (PF-LAM) or endosteal (EPF-LAM) origin was identified according to the following criterion. 1) *Where more than 25 % but less than 75 % of the matrix is comprised of lamellar bone.* Osteocyte lacunae may appear as in PF or LAM bone. This tissue may be comprised entirely of PF-LAM, or PF-LAM with alternating cycles of limited PF and / or LAM deposition. E/PF-LAM may be avascular, or vascularized by simple primary vascular canals and / or less densely-distributed primary osteons.

Fibro-lamellar complex and fibro-lamellar transition tissue (FLC and FLC-LZPO).

Fibro-lamellar complex is classically defined as a composite of "...1) a woven or fibrous, cancellous matrix of periosteal origin, and 2) a lamellar matrix, centripetally deposited, forming the primary osteons which ultimately fill the space originally empty in the woven periosteal matrix" (Francillon-Vieillot et al., 1990b; pp. 500-501). However, as also noted by Margerie and colleagues (2002), discrimination of woven-fibered matrix is often practically difficult when limited in its distribution, as is the case in fibro-lamellar bone.

Therefore, fibro-lamellar tissues were distinguished from WOV, PF and LAM based on the following features of the vascular network, which were more easily

observed. 1) *At least 75% of the vascularization is by primary osteons, having a discernable layer of bone deposited centripetally around the vascular canal.* 2) *At least 75% of adjacent primary osteons are separated by an interval approximately equal to or less than twice the primary osteon radial diameter.* 3) *When present in isolated patches, not containing less than 3 primary osteons.* In practice, this tissue was also usually characterized by the presence of plump or irregularly-shaped osteocyte lacunae, having a random or clumped distribution around the entire or partial periphery of primary osteons. Generally, approximately 10-25% or more of all extra-osteonal lacunae in FLC and FLC-LZPO tissue showed such characteristics, and this was interpreted to represent a woven-fibered matrix component.

Within this broader category, two types of fibro-lamellar and fibro-lamellar transition tissues were recognized: *Fibro-lamellar to Lamellar-zonal with primary osteons transition tissue (FLC-LZPO)* and *Fibro-lamellar complex (FLC)*.

1) FLC-LZPO (Figure 5-3 e) was distinguished from WOV, PF, and LAM tissues according to the criteria listed above.

2) FLC (Figure 5-3 d) was distinguished from FLC-LZPO according to the following criteria. 1) *Vascularization exclusively by primary osteons.* 2) *Adjacent primary osteons being separated by an interval less than radial primary osteon diameter.* 3) *More than 50% of extra-osteonal matrix having characteristics of WOV bone, including: a) osteocyte lacunae which are plump in shape, and are dense and randomly-organized, often appearing clumped around the periphery of primary osteons; b) matrix having a generally isotropic or randomly-arranged appearance in CPL.* 4) *Less than 25% of extra-osteonal matrix having characteristics of LAM bone.* In practice, less than

25% of extra-osteonal osteocyte lacunae are flattened to spindle-shaped, and follow a linear course above and below primary osteons when viewed in LM.

Generally speaking, FLC conforms most closely to the classic definition of fibro-lamellar bone quoted above. This tissue is comprised of a predominant woven-fibered extra-osteonal matrix component, and tends to be more densely-vascularized with larger-diameter primary osteons (having multiple concentric lamellae). FLC-LZPO represents a transitional tissue between FLC as classically defined, and densely-vascularized lamellar-zonal tissues (i.e., PF, PF-LAM or LAM) with primary osteons.

Compacted coarse cancellous (ECCC). (Figure 5-3 f). Endosteal compacted coarse cancellous (ECCC) bone was described in detail by Enlow (1963), and was identified in the current study according to the following criterion. 1) *Endosteal matrix of PF, PF-LAM or LAM organization deposited along the contours of coarse cancellous trabeculae, giving this tissue a highly convoluted appearance in LM and CPL (e.g., with local patches appearing to follow an arching or whorled course).* ECCC is often associated with cement lines, which delineate discontinuities in cycles of bone deposition during the process of cancellous compaction.

Sharpey fiber bone (SF). (Figure 5-3 g). When the presence of prominent Sharpey fibers comprised 50% or more of the bone matrix and precluded classification of bone microstructural organization, tissues were classified as Sharpey fiber (SF) bone. Areas of dense Sharpey fibers were identified on the basis of the following characteristics. 1) *Coarse extrinsic collagen fibers that enter the bone cortex usually at an oblique angle*

from the periosteal surface. In practice, SF was always identified in cortical bone tissues of periosteal origin. Prominent Sharpey fibers were distinguished in CPL by their polarization contrast, dependent upon their orientation with respect to the section plane, or in LM by their contrast. 2) *Adjacent osteocyte lacunae often appeared “stellate” in form, and randomly-organized around the extrinsic collagen fibers.*

Interstitial bone (INT). In areas of dense secondary intracortical remodeling (HAV, discussed further in Chapter 6), restricted interstitial areas of primary origin which could not be confidently classified according to criteria reported above were assigned to this category. INT was not considered separately in the current study.

Broad tissue type categories. The above tissue types were, in some analyses, grouped into the following categories:

- 1) Tissues of periosteal origin (PER): FLC, FLC-LZPO, WOV, PF, PF-LAM, LAM, SF.
- 2) Tissues of endosteal origin (END): EPF, EPF-LAM, ELAM, ECCC.
- 3) Tissues of primary origin (PRIM): PER, END, INT.

Methodological considerations in the identification of tissue types

First, tissue types were identified manually in the current study, using same field-of-view images acquired in LM and CPL (as described above), together with real-time viewing in conventional LM and CPL microscopy. While manual identification and

mapping of tissue types is a time-consuming process, automation of this procedure would prove difficult due to the complex 3-dimensional arrangement of collagen fibers, vascular canals and cellular components of bone. Automated pattern-recognition procedures may introduce more error than manual identification by a specialist (Budantsev and Jakovlev, 2000).

Second, it is recognized that bone microstructural organization varies along a continuum, and that intermediate patterns are encountered in regions where tissue types appear to grade into one another. However, investigators have historically used typological classifications, as they permit the standardized characterization of bone microstructural diversity observed among vertebrates and they facilitate comparisons among groups (e.g., Enlow, 1962; 1963; Enlow and Brown, 1956; 1957; 1958; Francillon-Vieillot et al., 1990b; Ricqlès et al., 1991). There is general agreement among current investigators on tissue types examined here, which can be reliably identified on the basis of criteria outlined above.

To provide an estimate of repeatability in tissue type identification and mapping, one bone sample was selected for repeated measurement, the midshaft humerus of *C. aethiops* SLW 27. This specimen exhibited most tissue types examined in the current study, as well as a moderate degree of secondary intracortical remodeling. Three tissue type maps were independently created from this specimen. Results are presented in Table 5-7 and Figure 5-4.

Independent measurements for separate tissue types were similar in whole cross-section proportions (with a maximum range of 4.3 % among measurements) and regional distributions. Some variation was observed in the location of boundaries between fibro-

lamellar and lamellar-zonal tissue types, particularly in regions where these transitions were gradual (e.g., in the posterior cortex between FLC-LZPO and LAM, and in the anterolateral cortex between lamellar-zonal tissue types). However, the most obvious difference from inspection of tissue type maps is in the distribution of FLC tissue (shown in darkest green). This reflects the practical difficulty in discriminating woven-fibered matrix from longitudinally-oriented parallel-fibered bone, particularly when the former is limited in its distribution to the periphery of primary osteons (as also noted by Margerie et al., 2002). Both tissue types appear dark in CPL, and exhibit a continuous range of osteocyte lacunar morphologies. Thus, fibro-lamellar tissues were considered both separately (as FLC and FLC-LZPO, respectively) and in combination (FLC + FLC-LZPO) in analyses to follow.

Image data acquisition

Gray-scale tissue map images were imported into Optimas 6.5 Image Analysis software (Media Cybernetics Inc., Silver Spring, MD.), where data relating to the pixel dimensions of bone areas comprised of primary tissue types and secondary osteonal bone (described further in Chapter 6) across bone regions were collected using a customized image segmentation macro described by Feik et al. (2000) and Goldman (2001) and developed for adult human femoral bone samples. This macro relied on Fourier shape descriptor techniques to automate the determination of periosteal and endosteal surface outlines of each cross-section.³ Following determination of bone outlines, the section

³ On occasion, it was necessary to edit original images in Adobe Photoshop prior to importing into Optimas. Using a small black line of 2 pixel diameter, small cracks open

centroid was then calculated and, based on a user-defined orientation point ('anterior', as described in Chapter 3), the antero-posterior axis of the section was determined. Seven additional lines were then placed, resulting in 8 transects located at equal angles from one another, which divided the cross-section into 16 radial sectors. Following this, periosteal and endosteal outlines were used to define two additional circumferential outlines, equally spaced from one another and from surface outlines, to divide the cross-section into three circumferential rings: an inner ring bordering the endosteal margin, a mid-cortical ring, and an outer ring bordering the periosteal margin. (Figure 5-5). As a result, this macro divided each bone cross-section into a total of 48 segments (16 radial sectors x 3 circumferential rings), from which quantitative gray-level data were extracted. Within each bone segment, the number of pixels representing each gray-level value (or bone tissue type) was calculated, and data were output to a Microsoft Excel spreadsheet. This method permitted the quantitative analysis of regional variation in primary bone tissue within bone cross sections. Additionally, segment data could be collapsed for analysis of whole bone cross-section proportions.⁴

The decision was made to reduce the number of radial sectors analyzed, in order to improve statistical power (for analyses presented in Chapter 6 of this thesis).

Qualitative observations indicated that some regional patterns may be lost by reducing the number of sectors to 4 (corresponding to anterior, posterior, medial and lateral

to the bone surface were closed off and / or large trabeculae which protruded into the medullary space were detached from the endosteal surface.

⁴ This method provided the most useful means available of reliably identifying bone segments for quantitative analysis of regional variability in cortical microstructure. However, it is also noted that bone segments defined on this basis may not be developmentally-homologous. For instance, bone tissues of endosteal and periosteal origin were variably-represented in the inner circumferential ring, dependent upon local growth circumstances associated with specimen age, element and taxon representation.

cortices). (For example, see Figure 5-4). Thus, the number of sectors was reduced to 8, permitting examination of variation in tissue type proportions across anterior, antero-medial, medial, postero-medial, posterior, postero-lateral, lateral, and antero-lateral cortices.

Quantitative description and analysis of tissue type proportions

The proportion of primary bone tissue types (TT) was calculated as a percentage of total cortical area from raw pixel dimensions generated in Optimas according to the following equation:

$$\% TT = (\text{pixel dimensions of TT} / \text{cortical area pixel dimensions}) * 100$$

Statistical analysis (described further below) was restricted to whole cross-section proportions of primary bone tissue types, focusing particularly on tissue types of periosteal origin which are suggested to relate to variation in bone depositional rates. In many cases, assumptions of parametric statistical analysis were not met or could not be verified due to small sample sizes ($n < 5$) within age classes and species groups. Thus, all statistical analyses reported here were performed using nonparametric techniques, in the Statistica 6.0 (Statsoft, Inc.) software package. Except where indicated, results associated with a probability value of $p \leq 0.05$ were considered statistically significant.

Associations between variables. Associations of % area of bone tissue types with age class and cortical area were examined using nonparametric Spearman rank order

correlation. This method assigns ranks to original variates based on their relative magnitudes, and provides a measure of the degree to which the rank order of paired variables is the same. Therefore, this method is appropriate for variables measured on an ordinal scale, and for cases in which bivariate distributions do not meet parametric assumptions of linearity and normality (Sokal and Rohlf, 1995).

Quantitative description of age-related variability in tissue type proportions. For description of age-related variation in tissue type proportions, data were summarized in plots of age class mean proportions (i.e., as % of total cortical area) of individual primary bone tissue types for each taxon. For the description of regional variation in tissue type proportions *within* cross-sections, differences in tissue type distributions among segments within cross-sections can be expected from regional variability in secondary intracortical remodeling alone, making comparisons among circumferential rings and radial sectors (and among taxa) more difficult. Thus, when examining regional variability within cross-sections, data were summarized as a % of primary (i.e., excluding HAV) cortex area.

Statistical comparisons among age classes, sexes and elements within taxa. To examine the influence of age and sex on tissue type proportions, contrasts among group medians were made using nonparametric Kruskal-Wallis (for three or more independent samples) and Mann-Whitney (for two independent samples) tests. Where Kruskal-Wallis tests revealed significant contrasts among age classes, post-hoc pair-wise Mann-Whitney tests were performed to identify significant contrasts. These methods assume that the distributions among groups are similar in shape. Contrasts among groups were also made

using Kolmogorov-Smirnov (for two independent samples) and Median tests (for three or more independent samples), which test for overall differences in the shape and location of distributions among groups. Results of Mann-Whitney and Kruskal-Wallis tests are presented here. For post-hoc Mann-Whitney comparisons, significance values were corrected for repeated tests using the Bonferroni method, in which p is divided by the number of comparisons (Sokal and Rohlf, 1995). Many post-hoc contrasts were no longer significant at $p \leq 0.05$ after Bonferroni correction. Because small sample sizes may partly account for this result, significant contrasts at $p \leq 0.10$ are also reported.

The influence of sex on the age-related succession of primary bone tissue types was difficult to evaluate in taxa examined here, given small and unequal sample sizes for males and females particularly in the youngest age classes. Categorized bivariate plots were visually inspected for consistent differences among sexes in % area of tissue types in *C. aethiops* and *H. lar*. Statistical contrasts were restricted to a subsample comprised of M3 - EPIPH age classes, due to unequal sex representation and small sample sizes in the youngest age classes.

Qualitative observations suggested that variation in microstructural variables among elements within individuals may not be considered independent. Thus, element contrasts were conducted using Wilcoxon matched pairs tests for dependent samples.

Principle components analysis. Principle components analysis (PCA) was undertaken for visualization purposes and to detect broad interspecific patterns in proportions of primary bone tissue types at the midshaft femur. (The midshaft humerus was not represented in *P. troglodytes*.) PCA is a useful technique for summarizing the variance

contained in a multivariate dataset in terms of principal components, calculated from a correlation matrix of the original input variables. The first principal component accounts for the largest proportion of the total variance, and each additional principal component accounts for as much of the remaining variance as possible. Principal components are uncorrelated and orthogonal to one another, and define a multivariate space which can be used for the purpose of data visualization and to detect similarities and differences among groups (Kachigan, 1991).

Active input variables for calculation of principal components included % of total cortical area of END, and the % of *primary periosteal* cortex area of FLC + FLC-LZPO, PF, PF-LAM, and LAM. Age class and cortical area were included as supplemental variables in Statistica 6.0 (Statsoft, Inc.).

RESULTS

Patterns of primary bone microstructural development at the midshaft femur and humerus are examined in each taxon below, focusing particularly on tissues of periosteal origin. General patterns of secondary (Haversian) intracortical remodeling are also noted, although a more detailed examination of this variable is presented in Chapter 6.

Variability in whole cross-section tissue type proportions

Raw data (reported in pixel dimensions) for tissue types are presented in Appendix F (Table F-1), and descriptive statistics for each taxon sample are also provided (Table F-2). Substantial variability was observed in % area of primary tissue types at the midshaft femur and humerus of *C. aethiops* and *H. lar*, and at the midshaft

femur of *P. troglodytes*. Results of Spearman correlation analyses demonstrated that variability in % area of tissue types in each taxon was significantly correlated with age class and cortical area (CA). (Table 5-8). The pattern of association of microstructural variables with age class and CA was the same, and these latter two variables were highly correlated with one another (*C. aethiops* femur: $r = 0.8763$, $p = 0.0000$; humerus: $r = 0.8852$, $p = 0.0000$. *H. lar* femur: $r = 0.8804$, $p = 0.0000$; humerus: $r = 0.8664$, $p = 0.0000$. *P. troglodytes* femur: $r = 0.9176$, $p = 0.0000$). Associations among variables tended to be nonlinear, as can be appreciated in bivariate plots shown in Figure 5-6 a-c.

In all three taxa, results indicated an overall decrease in periosteal % FLC + FLC-LZPO, %PF, and %PF-LAM, and an overall increase in %LAM, with age class and CA. Among endosteal tissues, there was a general tendency for %EPF and %EPF-LAM to show a negative association with age class and CA, but results for %ELAM and %ECCC were more variable. In *C. aethiops*, %EPF and %EPF-LAM were negatively correlated with age class and CA only at the midshaft humerus. %ELAM was positively correlated with age class and CA at both the midshaft femur and humerus, while %ECCC at the midshaft femur also showed a positive association. In *H. lar*, all endosteal tissue types were negatively associated with age and CA, with the exception of %ELAM at the midshaft humerus. %EPF and %EPF-LAM also showed negative associations with age and CA at the midshaft femur of *P. troglodytes*, while %ELAM and %ECCC showed no significant association. These age trends are considered in further detail below for each taxon, with a particular focus on periosteal bone tissue types.

Age-related variation in *C. aethiops*

Complete descriptive statistics for tissue type % areas are summarized by age class in Appendix F (Table F-3 a). Tissue type maps derived from all individuals in the sample are provided in Appendix G-1.

Whole cross-section proportions: total PER, total END, and HAV. Figure 5-7 shows age class means for % area of total END, total PER and HAV cortex at the midshaft femur and humerus, respectively. An overall increase in %HAV was observed in both elements across age classes, although the humerus exhibited a higher overall %HAV compared to the femur. These and other trends in %HAV will be discussed in Chapter 6 of this thesis. The % total END at the midshaft femur showed a gradual increase with age, reaching an EIPPH age class mean of 9.2 %. The % total END showed a more complicated pattern at the midshaft humerus, with substantial overlap among age classes. However, tissues of END origin comprised an overall greater proportion of total cortical area in the humerus compared to the femur; age class mean % areas of END tissue in the former ranged from 5.6% (DECID age class) to 16.3% (M1 age class). Corresponding age- and element – related differences in the proportion of total PER tissue were also evident.

Statistical contrasts among age classes and bone elements. Results of statistical analysis reported in Table 5-9 revealed that the above age trends in % total END, % total PER, and %HAV were significant at the midshaft femur and humerus. Post-hoc contrasts were significant at $p \leq 0.10$ after Bonferroni correction for those comparisons between the DECID and older age classes. Results reported in Table 5-10 revealed that most

element contrasts were statistically significant ($p \leq 0.05$) in the total sample, and when age classes were considered separately.

Whole cross-section proportions: PER tissue types considered separately. Bivariate plots showing individual values for % area of primary periosteal tissue types are presented by age class and sex in Figure 5-8 a and b for the midshaft femur and humerus, respectively. Males in the DECID and M1 age class showed a tendency for higher % FLC and FLC-LZPO compared to females, although this may simply reflect the small and unequal sample sizes among sexes in these age classes. No consistent sex differences across age classes were apparent. This was supported by statistical analyses. (Table 5-11). Analyses presented below were based on combined-sex samples.

Age class mean % areas of primary periosteal tissue types are presented in Figure 5-9. Variability in tissue type proportions in both elements was considerable among DECID individuals, with this age class showing a greater range of values and larger standard deviations for most periosteal tissue types compared to older age classes. % FLC-LZPO was equally variable in the DECID (range = 34.01; SD = 13.0247) and M1 age classes (range = 34.23; SD = 12.2994) at the midshaft humerus. Variation in % LAM in both elements was greatest in the DECID age class, although considerable variability was also observed within older age classes.

Midshaft femur. (Figure 5-9). FLC and FLC-LZPO tissues characterizing the DECID age class were replaced with LAM tissues in older age classes. In the DECID age class, combined fibro-lamellar tissues (FLC mean = 7.47 %; FLC-LZPO mean = 40.94 %) comprised a large proportion of total cortical area, compared to lamellar-zonal

tissue types (PF mean = 2.93 %; PF-LAM mean = 13.96%; LAM mean = 22.06).

However, this pattern changed in the M1 age class, where LAM tissue comprised a majority of the cortex (mean = 67.65 %). In contrast, combined fibro-lamellar tissues comprised 10% or less of the cortex in all but the single male individual in the M1 age class (FLC + FLC-LZPO mean = 5.63%, min = 0.86%, max = 12.04%). PF-LAM comprised a mean of 6.38% of the cortex among M1 individuals, and declined further with age. Among M3 and EIPH age classes, non-LAM primary periosteal tissues (except SF) typically comprised less than 5% of total cortical area.

Midshaft humerus. (Figure 5-9). The pattern of succession of FLC and FLC-LZPO to LAM at the midshaft humerus was similar to that reported for the midshaft femur, but this transition appeared to occur more gradually in the humerus. In the DECID age class, combined fibro-lamellar tissues (FLC mean = 8.25 %; FLC-LZPO mean = 40.74 %) comprised a large proportion of total cortical area compared to lamellar zonal tissue types (PF mean = 8.12 %; PF-LAM mean = 9.05%; LAM mean = 13.03 %). However, the M1 age class appeared to show an intermediate pattern of tissue type proportions, where FLC-LZPO comprised a mean 12.48 % of cortical area and LAM comprised a mean 38.62 % of cortical area. WOV was observed in only a single individual in the M1 age class. In contrast, LAM tissue comprised the majority of total cortical area in most individuals of the M2 and later age classes (M2 age class mean = 56.59), while proportions of FLC-LZPO declined substantially.

Statistical contrasts among age classes. Age-related trends in periosteal bone tissue types reported above were supported by statistical analysis (Table 5-12). Kruskal-Wallis tests revealed statistically-significant contrasts among age classes for all primary

periosteal tissue types at the midshaft femur and humerus, except PF at the midshaft femur. Many post-hoc contrasts were no longer significant at $p \leq 0.05$ after Bonferroni correction. Because small sample sizes may partly account for this result, significant contrasts at $p \leq 0.10$ were also reported. Post-hoc Mann-Whitney comparisons revealed significant contrasts between the DECID and M1 age class, the DECID and most older age classes, and the M1 and oldest (M3 or EPIPH) age classes in combined fibro-lamellar tissues (FLC + FLC-LZPO) and LAM at the midshaft femur. Significant post-hoc contrasts in PF-LAM at the midshaft femur were identified between the youngest (DECID or M1) and oldest (M3 or EPIPH) age classes. At the midshaft humerus, significant post-hoc contrasts were most commonly identified between the youngest (DECID or M1) and oldest (M3 or EPIPH) age classes, although significant contrasts between DECID and M2 age classes were also apparent for most tissue types. Excluding FLC, there were no significant contrasts between the DECID and M1 age classes at the midshaft humerus, which may reflect a more gradual change in tissue type proportions during early ontogeny.

Statistical contrasts among bone elements. Statistical contrasts among elements (Table 5-13) revealed that the humerus was characterized by a significantly greater % FLC + FLC-LZPO compared to the femur in the total sample, and when M2 – EPIPH age classes were considered separately. % LAM was correspondingly greater at the midshaft femur.

Age and regional variability: quantitative and qualitative observations. Qualitative assessment of individuals revealed ontogenetic variability in additional aspects of

primary bone microstructure which could not be appreciated in the quantitative analysis of bone tissue types. These features, as well as patterns of regional variability across circumferential rings and radial sectors at the midshaft femur and humerus, are described in further detail here, focusing on tissues of periosteal origin. Selected examples of these observations are provided in Figures 5-10 through 5-15, and Figure 5-17. Tissue maps derived from all individuals in the sample are provided in Appendix G-1.

General age-related trends in primary bone microstructure during ontogeny.

Variability in tissue type proportions (reported as a % of *primary* cortex area) across circumferential rings is depicted graphically by age class in Figure 5-10 a and b for the midshaft femur and humerus, respectively. Variability among circumferential rings *within* cross-sections reflected the age-related succession of periosteal tissue types described above.

Fibro-lamellar tissue types (FLC and FLC-LZPO) comprised a substantial proportion of primary cortex area in all three rings at the midshaft femur in the DECID age class, although the proportion of fibro-lamellar tissue types was highest in the inner circumferential ring compared to the outer ring. (Figure 5-10 a and b.) However, as described above, considerable variability was observed in tissue type proportions within the DECID age class compared to older age classes. (See Appendix G -1 a). Individuals SLW 62 and SLW 92 were characterized by a higher proportion of fibro-lamellar tissues (FLC + FLC-LZPO) and a lower proportion of LAM compared to other DECID individuals. These two individuals were smaller in size than others in their age class, and they were also characterized by incompletely-erupted deciduous dentitions, indicating that some variability may be attributed to bone size and / or differences in the relative

ages of individuals. However, inter-individual variability was also observed that does not appear to relate to relative age or size. For example, individual SLW 33 was characterized by a high proportion of fibro-lamellar tissues compared to other individuals of similar dental emergence status and cortical area (e.g., SLW 27). Interestingly, these differences among individuals characterized both the midshaft femur and humerus.

In DECID individuals SLW 62 and 92, fibro-lamellar tissues (FLC + FLC-LZPO) were characterized by a predominantly longitudinal vascular arrangement, with irregular anastomoses variably present. (Figure 5-11 c and d). In FLC, primary osteons were characterized by centripetal deposition of bone having a PF and / or LAM matrix. In FLC-LZPO of these and older individuals, primary osteons were often smaller in size (i.e., surrounded by fewer centripetal layers of bone) and separated by an extra-osteonal matrix increasingly parallel-fibered and / or lamellar in its collagen fiber organization. (Also see Figure 5-12). Primary osteons become less densely-distributed in the outermost cortex and in some cortical regions around the circumference of the section where the cortex showed a transition to a less-vascular lamellar-zonal matrix type (PF, PF-LAM or LAM). Lamellar-zonal cortex was comprised mostly of PF or PF-LAM in individuals SLW 62 and 92. This tissue generally appeared dark in CPL, indicating a predominantly longitudinal collagen fiber orientation. In SLW 92, this transition was marked by an annulus or resting line, most pronounced in the anterior cortex of the humerus. (Figure 5-11 e).

In older DECID individuals (e.g., SLW 27), the extra-osteonal matrix of FLC-LZPO was increasingly LAM in its collagen fiber organization. (Figure 5-12). Further, FLC-LZPO tended to be concentrated in the inner cortex (with the exception of SLW 33,

Appendix G-1 a). A larger proportion of the cortex was comprised of LAM, which was typically vascularized by longitudinally-oriented simple primary vascular canals and scattered small primary osteons. The transition from FLC-LZPO to LAM occurred abruptly or more gradually, depending upon the cortical region.

A slightly increased proportion of FLC-LZPO continued to characterize individuals in the M1 age class compared to all older age classes. However, evidence of recent periosteal deposition in the M1 and older age classes (represented in the outer cortical ring) was almost exclusively LAM in organization at the midshaft femur, and LAM formed the largest proportion of primary cortex area in all three circumferential rings. (Figure 5-10). LAM tissue had a brighter signal in CPL relative to surrounding tissues and compared to younger individuals, indicating an oblique – transverse preferred collagen fiber orientation. Visual inspection revealed that its vascularization density varied regionally, with some regions being largely avascular. (Figures 5-13 and 5-14). Vascular canals also appeared to be less densely distributed in older age classes compared to younger age classes, particularly at the midshaft femur. (Compare Figures 5-13 a and 5-14 a).

Mean proportions of FLC-LZPO in the M1 age class were higher at the midshaft humerus compared to the midshaft femur, particularly in the inner and mid-cortical rings. (Figure 5-10.) Slightly increased vascularity and slightly higher proportions of FLC-LZPO at the midshaft humerus, particularly in the posterior and postero-medial cortex, was observed in some individuals of older age classes. (Figure 5-14 and Appendix G-1 c-e). Visual inspection also revealed a tendency for males to show increased vascularity of the posterior and postero-medial humeral cortex compared to females in the M3 and

EPIPH age classes. (Figure 5-15). Several males also showed more subtle variations in vascularity (including changes in the density of primary osteons) across the thickness of the cortex in this region, with zones of more vascular tissue alternating with zones of less vascular tissue. (Figure 5-15 c).

Growth marks, namely annuli and resting lines, were observed in the primary periosteal cortex of the midshaft femur in two individuals of the DECID age class and in all older individuals. Growth marks varied in number and in severity among individuals, and possibly in relation to age. By first approximation, the number of growth marks appeared to increase with age in the DECID, M1 and M2 age classes. (Figures 5-11 c and e, 5-13 c, and 5-14 c and d). However, this needs to be established in future quantitative studies. The distribution of growth marks at the midshaft humerus was influenced by local circumstances of bone growth remodeling, particularly relating to the pronounced cortical drift pattern described below. Resting lines observed in the anterior and adjacent cortices of the humerus were often associated with discontinuities in bone growth due to drift or resorptive periods at the periosteal surface. Despite the latter complications, there were qualitative similarities in growth marks (i.e., in their number and distribution) observed at the midshaft humerus (particularly the posterior and adjacent cortices) and the midshaft femur. (Figures 5-11 c and e, and 5-14 c and d).

Regional variability and growth remodeling: midshaft femur. At the midshaft femur, variability in tissue type proportions across radial sectors was indicative of cortical drift, most apparent in the DECID age and M1 age classes. Regional variation in tissue type proportions across radial sectors is summarized graphically in Figure 5-16 a.

Several observations indicated a pattern of cortical drift in a predominantly

posterior or postero-medial direction. First, the distribution of vascular fibro-lamellar (FLC and FLC-LZPO) tissues compared to lamellar-zonal (PF, PF-LAM, LAM) tissue types indicated differential periosteal growth in a predominantly posterior or postero-medial direction in several individuals of the DECID age class. As can be appreciated in Figure 5-16 a, FLC and FLC-LZPO comprised a larger proportion of primary cortex area in sectors 3 – 7 at the midshaft femur. However, there was variability among individuals in the direction of growth, from posterior (SLW 92 and 33) to posterior and medial (SLW 62). (Appendix G-1 a). Also, the periosteal margin of these cortical regions was convoluted, showing evidence of active incorporation of primary vascular canals into the forming bone matrix. In contrast, the opposing periosteal cortices were characterized by a larger proportion of lamellar-zonal tissue types with reduced vascularity. (Also see Figure 5-11 a). Older DECID individuals (SLW 76 and 27) and individuals of the M1 age class were characterized by a reduced proportion of FLC-LZPO in all cortical regions, and recent periosteal deposition of LAM around the cross-section. However, qualitative inspection revealed increased vascularity of LAM in the postero-medial, posterior and adjacent cortices of some individuals, suggesting continued differential growth in this direction. (Figures 5-12 a and 5-13 a).

Secondly, although END tissues comprised a relatively small proportion of primary cortex area, they showed a preferential distribution in the anterior and adjacent cortices of several individuals of the DECID (SLW 76, 33) and M1 age classes (SLW 21, 96, 73). This distribution indicates inward (i.e., endosteal) growth in the direction of cortical drift. (Appendix G-1 a and b).

Not all individuals of the DECID and M1 age classes showed evidence of

posterior to postero-medial drift. (For example, see SLW 11 and 37 in Appendix G-1 b). Also, there was more variability in cortical drift patterns among individuals in older age classes. (Appendix G-1 c – e). No consistent regional trend in the quantitative distribution of bone tissue types was evident among older age classes in Figure 5-16 a.

Regional variability and growth remodeling: midshaft humerus. At the midshaft humerus, variability in bone tissue type proportions observed across radial sectors was indicative of a complicated pattern of cortical drift, first in the posterior and postero-medial direction, and during later ontogeny, in the posterior and postero-lateral direction. Regional variation in tissue type proportions across radial sectors is summarized graphically in Figure 5-16 b.

Several observations indicated a prominent pattern of cortical drift. The distribution of vascular fibro-lamellar (FLC and FLC-LZPO) tissues compared to lamellar-zonal (PF, PF-LAM, LAM) tissue types indicated differential periosteal growth of the postero-lateral, posterior, postero-medial and medial cortices (corresponding to sectors 4-7 in Figure 5-16 b) in the two youngest individuals in the DECID age class (SLW 62 and 92) and in SLW 33. (Appendix G-1). The periosteal margin in these cortical regions, particularly in the postero-medial cortex, showed evidence of active incorporation of primary vascular canals into the forming bone matrix. (See Figure 5-11). Older DECID individuals were characterized by deposition of LAM bone in many cortical regions, although this tissue was more densely vascularized in the posterior and postero-medial cortex. (Figure 5-12 b). In contrast, the anterior and antero-lateral cortices were characterized by larger proportions of less vascular lamellar-zonal tissue types (PF-LAM and LAM). The distribution of END tissues was also consistent with

posterior and postero-medial drift.

Periosteal tissue type proportions changed substantially in the M1 age class, as the proportion of lamellar-zonal tissues (particularly LAM) increased. (Figure 5-16 b and Appendix G-1 b). This reflects both medullary expansion and endosteal resorption of earlier-deposited fibro-lamellar tissues, and recent periosteal deposition of LAM. Continued cortical drift, particularly in the posterior and postero-medial direction, was indicated by several observations. First, the proportion of endosteal tissue increased substantially in opposing cortices - the anterior, antero-lateral, and lateral cortex (corresponding to sectors 1 - 3), and in some individuals (e.g., SLW 11 and 47), the antero-medial cortex (sector 8). Secondly, in several individuals of the M1 and older age classes, a reversal line was observed in the anterior and adjacent cortices, representing a resorptive phase between an earlier period of periosteal FLC-LZPO deposition and a later period of periosteal LAM deposition. (Figure 5-17). This resorptive phase was likely extensive, influencing tissue type proportions in older age classes as well. END tissues continue to comprise a substantial proportion of primary cortex area in the anterior, anterolateral and adjacent cortices in the M2 – EIPPH age classes. (Figure 5-16 b).

An apparent pattern of posterior and/or poster-medial drift also characterized most individuals in the M2 age class. (Appendix G-1 c). However, the distribution of END tissue shifted slightly in the M3 and EIPPH age classes. Age class mean proportions of END increased in the medial and anteromedial cortex (corresponding to sectors 7 and 8 in Figure 5-16 b), and several individual showed evidence of drift in a postero-lateral or lateral direction (e.g., SLW 14 and 91 in Appendix G-1 d).

Age-related variation in *H. lar*

Complete descriptive statistics for tissue type % areas are summarized by age class in Appendix F (Table F-3 b). Tissue type maps derived from all individuals in the sample are provided in Appendix G-2.

Whole cross-section proportions: total PER, total END, and HAV. Figure 5-18 shows age class means for % area of total END, total PER and HAV cortex at the midshaft femur and humerus, respectively. % HAV showed no apparent age trend. HAV remodeling was extensive at the midshaft femur and humerus of *H. lar*, and is discussed in detail in Chapter 6 of this thesis. The proportion of total END tissue showed a gradual decrease with age at the midshaft femur and humerus during early ontogeny, from DECID age class means of 16.95 % and 31.34 %, respectively. Tissues of END origin in the DECID age class comprised an overall greater proportion of total cortical area at the midshaft humerus compared to the femur, despite their generally similar proportions among EPIPH individuals (femur = 2.74 % ; humerus = 4.09 %). Corresponding differences in the proportion of total PER were also evident.

Statistical contrasts among age classes and bone elements. Results of statistical analysis reported in Table 5-14 revealed that the above age trends in total END and PER at the midshaft femur and in total END at the midshaft humerus were statistically significant ($p \leq 0.05$). However, post-hoc contrasts were largely non-significant ($p \leq 0.10$) after Bonferroni correction. Results reported in Table 5-15 revealed that most element contrasts were statistically significant ($p \leq 0.05$) in the total sample, and when the M2 – EPIPH age classes were considered separately, indicating increased proportions

of HAV and total END in the humerus. Exceptions to this latter pattern included % total END in the M3 and EPIPH age classes, which did not differ significantly among elements.

Whole cross-section proportions: PER tissue types considered separately. Bivariate plots showing individual values for % area of primary periosteal tissue types are presented by age class and sex in Figure 5-19 a and b for the midshaft femur and humerus, respectively. No consistent sex differences in primary tissue type proportions were visually apparent in these plots. However statistical analysis revealed a significant contrast in % FLC-LZPO at the midshaft humerus among sexes in the M3-EPIPH subsample. (Table 5-16). Males were characterized, on average, by a larger proportion of FLC-LZPO compared to females (male subsample mean = 0.21, min = 0.00, max = 0.64, SD= 0.221; female subsample mean = 0.00, min = 0.00, max = 0.00, SD = 0.000). Further, % LAM approached statistical significance ($p=0.064$) at the midshaft femur, with males characterized by slightly lower proportions of LAM on average than females (male subsample mean = 50.06, min = 53.06, max = 61.29, SD= 9.009; female subsample mean = 59.12, min = 46.34, max = 57.40, SD = 9.549). However, these differences were of small magnitude, and considerable overlap among sexes was apparent upon inspection of bivariate plots. (Figure 5-19). Therefore, analyses below were based on combined-sex samples.

Age class mean % areas of primary periosteal tissue types are presented in Figure 5-20. Variability in tissue type proportions at the midshaft femur and humerus was greatest in the DECID and M1 age classes for % FLC-LZPO and PF, and in the M2 age

class for % PF-LAM. Variability in % LAM was considerable across age classes.

Midshaft femur. (Figure 5-20). In the DECID age class, FLC-LZPO tissue comprised a small proportion of cortical area (mean = 2.45 %). Lamellar-zonal tissues, in contrast, comprised the greatest proportion of the primary periosteal cortex, with PF-LAM tissue being the most common (mean = 17.47 %). PF and LAM were also present in lower proportions (PF mean = 8.41 %; LAM mean = 9.88 %). A similar pattern characterized the M1 age class, although % PF decreased and % FLC-LZPO and LAM increased slightly. The most substantial change in tissue type proportions was evident in the M2 age class, which was characterized by a substantial increase in % LAM and a decrease in proportions of other periosteal tissue types. LAM comprised a mean 49.02 % of cortical area at the midshaft femur in the M2 age class, compared to a mean 9.01 % of cortical area represented by PF-LAM. This trend continued through the M3 and EPIPH age classes.

Midshaft humerus. (Figure 5-20). A similar pattern of microstructural development was evident at the midshaft humerus, although the overall proportion of total PER cortex was lower than observed in the femur. As in the femur, FLC-LZPO was present in its greatest proportion in the M1 age class, comprising a mean 6.81 % of cortical area. Among lamellar-zonal tissues, PF-LAM was the most common tissue type observed in the DECID (mean = 9.94 %) and M1 (mean = 20.88 %) age classes. % LAM increased substantially in the M2 age class to a mean 26.87%. Older age classes were characterized by further increases in % LAM, which comprised a mean 39.87 % in the EPIPH age class.

Statistical contrasts among age classes. Age-related trends in periosteal tissue

types reported above were supported by statistical analysis (Table 5-17). Kruskal-Wallis tests revealed significant contrasts ($p \leq 0.05$) among age classes for all periosteal tissue types at the midshaft femur and humerus (except FLC). However, most post-hoc contrasts were no longer significant at $p \leq 0.05$ after Bonferroni correction. Because small sample sizes may partly account for this result, significant contrasts at $p \leq 0.10$ level were also shown. Post-hoc contrasts revealed no significant variation between the DECID and M1, or between the DECID and M2 age classes, for any periosteal tissue type, supporting a gradual transition in tissue type proportions as reported above. Significant contrasts were more commonly identified between the M2 and older (M3 or EPIPH) age classes, particularly for FLC + FLC-LZPO and PF-LAM at the midshaft femur ($p \leq 0.05$). Also, significant contrasts ($p \leq 0.10$) were identified between the DECID and M3 age classes for all tissue types (except FLC) at the midshaft femur, and for lamellar zonal tissue types (PF, PF-LAM, LAM) at the midshaft humerus.

Statistical contrasts among bone elements. (Table 5-18). Statistical contrasts among elements were largely non-significant ($p \leq 0.05$), with a few exceptions. LAM comprised a significantly larger proportion of cortical area at the midshaft femur compared to the midshaft humerus in the total sample, and when the M2 – EPIPH age classes were considered separately. In contrast, % PF-LAM was significantly greater at the midshaft humerus in the M2 and EPIPH age classes when considered separately.

Age and regional variability: quantitative and qualitative observations. Qualitative assessment of individuals revealed ontogenetic variability in additional aspects of primary bone microstructure which could not be appreciated in the quantitative analysis

of bone tissue types. These features, as well as patterns of regional variability across circumferential rings and radial sectors at the midshaft femur and humerus, are described in further detail here, focusing on tissues of periosteal origin. Selected examples of these observations are provided in Figures 5-22 through 5-28. Tissue maps derived from all individuals in the sample are provided in Appendix G-2.

General age-related trends in primary bone microstructure during ontogeny.

Variability in tissue type proportions (reported as a % of *primary* cortex area) across circumferential rings is depicted graphically by age class in Figure 5-21 a and b for the midshaft femur and humerus, respectively. Variability among circumferential rings *within* cross-sections reflects the age-related succession of periosteal tissue types described above.

Qualitative observations reveal that the basic microstructural organization of periosteal bone tissue types was similar at the midshaft femur and humerus. In the DECID age class, tissues of END origin comprised the large majority of the primary cortex in the innermost circumferential ring, and a substantial proportion of the mid-cortical ring in both elements. The latter observation was particularly pronounced at the midshaft humerus. (Figure 5-21 a and b). Qualitative inspection revealed that the majority of the primary periosteal cortex of DECID individuals was comprised of poorly-vascularized lamellar-zonal tissues. (Appendix G-2 a, Figures 5-22 and 5-23). Lamellar-zonal cortex was generally characterized by regions of PF-LAM appearing dark in CPL, interspersed with periods of PF and / or LAM deposition. LAM may have a predominantly longitudinal or transverse collagen fiber orientation, appearing dark or bright in CPL, respectively. Reversal lines and resting lines were also observed, typically

appearing bright in CPL relative to adjacent tissue.

Fibro-lamellar tissue (namely FLC-LZPO) was limited in its distribution in DECID individuals. This tissue was characterized by a predominantly longitudinal vascular canal orientation, with occasional oblique and circular canals and irregular anastomoses. The extra-osteonal matrix appeared dark in CPL. (Figures 5-22 e and 5-24.) FLC-LZPO was frequently distributed in the innermost periosteal cortex (corresponding to the inner cortical or mid-cortical ring), often in or near regions undergoing secondary intracortical remodeling. In such circumstances, FLC-LZPO tissues were recognized on the basis of osteocyte lacunae shape and distribution and/or the distribution of primary osteons in interstitial areas.

The M1 age class was characterized by an intermediate microstructural pattern, with roughly equal proportions of PF-LAM and LAM in the mid-cortical and outer circumferential rings of the midshaft femur. A decrease in the proportion of END was observed in the mid-cortical ring, particularly at the midshaft humerus, although END still comprised a large majority of primary cortex area in the innermost ring of both elements. (Figure 5-21 a and b.) The proportion of FLC-LZPO increased slightly in the mid-cortical and outer rings of the midshaft humerus, and in the outer cortical ring of the femur. At the midshaft femur, FLC-LZPO was found to be interspersed within larger regions of lamellar-zonal tissue. (Figure 5-25). In the humerus, it often was observed to occur over larger areas. (Figure 5-26). In both elements, its regional distribution was associated with other features indicating cortical drift (see below).

Primary cortex of individuals in the M2 age class showed a substantial increase in % LAM across all cortical regions of the midshaft femur and humerus, although

proportions of PF-LAM remained higher in the latter. (Figure 5-21 a and b). Qualitative inspection revealed that LAM was either avascular or vascularized with scattered primary osteons and simple primary vascular canals having a longitudinal orientation. LAM appeared variably dark or bright in CPL, frequently showing an alternating dark and bright appearance over broad expanses of the cortex. In the latter case, regions characterized by numerous dark lamellae alternated with regions of bright lamellae, indicating apparently repetitive changes in the preferred collagen fiber orientation of the matrix. (Figures 5-27 and 5-28.) Regions of bright lamellae were frequently associated with resting lines or annuli, observed in LM. This pattern also characterized older age classes. Primary bone microstructure and tissue type proportions changed little in the M3 and EPIPH age classes. (Figure 5-21 a and b, and Appendix G-2 d and e).

Regional variability and growth remodeling: midshaft femur. At the midshaft femur, variability in tissue type proportions across circumferential rings and radial sectors was indicative of metaphyseal growth remodeling and cortical drift. These patterns were most apparent in the DECID age and M1 age classes. Observations are summarized below.

A history of metaphyseal remodeling at the midshaft section location examined here was indicated by two observations. First, a substantial proportion of the primary cortex in DECID individuals was endosteal in origin. (Summarized graphically in Figure 5-29). END bone comprised 10.1 – 27.8 % of total cortical area in the four individuals of the DECID age class. While ECCC was observed in small proportions, most recently-deposited endosteal tissues were EPF-LAM or ELAM in organization. Secondly, reversal lines were observed in the periosteal cortex of DECID individuals, indicating

that periods of bone deposition were interrupted by periods of resorption at the periosteal surface. In some regions along the circumference of the cortex, these structures became continuous with cement lines having the smooth-contoured appearance of resting lines. (Figures 5-22 c and d). Reversal lines were also observed in older individuals, although their distribution generally appeared to be associated with an overall pattern of cortical drift (see below).

Regional variation in tissue type proportions across radial sectors at the midshaft femur is summarized graphically in Figure 5-30 a. Several observations indicated a pattern of cortical drift in a predominantly posterior to postero-lateral direction in the DECID and M1 age classes. First, age class mean % END showed a patterned distribution, being highest in the anterior and antero-medial cortices (corresponding to sectors 1 and 8) followed by adjacent cortices. As can be appreciated in Appendix G-2, this pattern was most evident in individual MCZ 35456 of the DECID age class, and in individuals (e.g., 41502) of the M1 age class. However, other individuals showed either no clear patterned distribution of END tissues across radial sectors (e.g., MCZ 36380), or a distribution indicating a different drift direction (e.g., MCZ 41462). Secondly, primary periosteal tissues in the M1 age class also showed a distribution consistent with postero-lateral drift; FLC-LZPO exhibited slightly higher mean proportions in the lateral, postero-lateral, and posterior cortices (corresponding to sectors 3 – 5 in Figure 5-30 a).

This pattern of posterior to postero-lateral cortical drift was observed in some individuals of the M2 (e.g., MCZ 41473, MCZ 41498) and M3 (e.g., SLW 37) age classes. (Appendix G-2 c and d.) However, as a rule, evidence of endosteal growth was less extensive in older individuals, with age class mean proportions of END below 10 %

in most sectors. (Figure 5-30 a). Further, evidence of cortical drift appeared to be less pronounced (if evident) in individuals of older age classes.

Regional variability and growth remodeling: midshaft humerus. As in the femur, variability in tissue type proportions across circumferential rings and radial sectors at the midshaft humerus was indicative of metaphyseal remodeling and cortical drift. These patterns were most apparent in the DECID age and M1 age classes. Observations are summarized below.

A history of metaphyseal remodeling at the midshaft section location examined here was indicated by two observations. First, a substantial proportion of the primary cortex in DECID and M1 individuals was endosteal in origin. END bone comprised 23.9 – 43.4 % of total cortical area in DECID individuals and 7.9 – 17.4 % in M1 individuals. Most recently-deposited endosteal tissues were EPF-LAM or ELAM in organization, although ECCC was also commonly observed in older-deposited endosteal regions. (Summarized graphically in Figure 5-29 b. Also see Appendix G-2 a and b). END bone comprised a majority of cortical area in both the inner and mid-cortical rings in the DECID age class. (Figure 5-21 b). Secondly, as in the femur, the presence of reversal lines indicates that periods of periosteal deposition were interrupted by periods of resorption at the periosteal surface of the midshaft humerus. These structures were observed in several individuals of the DECID, M1 and older age classes, where they may reflect metaphyseal remodeling history. (Figure 5-26). In other cases, these structures were regionally distributed in a manner consistent with cortical drift (i.e., in the cortex opposite the direction of most rapid growth).

Generally, the midshaft humerus was characterized by variable drift patterns

among individuals, and among age groups. This can be appreciated in tissue type maps provided in Appendix G-2. Regional variation in tissue type proportions across radial sectors at the midshaft femur is summarized graphically in Figure 5-30 b. DECID age class mean proportions of END were highest in the lateral and postero-lateral cortices (corresponding to sectors 3 and 4), and a pattern of antero-medial drift can be appreciated in individuals MCZ 41462 and MCZ 41466. (Figures 5-22 and 5-24). However, the other two individuals in the DECID age class showed very different tissue type distributions. (Appendix G-2 a.) Similarly, all three individuals in the M1 age class showed evidence of pronounced cortical drift, but the direction of drift varied substantially among all three. (Appendix G-2 b.) Older age classes were characterized by similar variation in the pattern of cortical drift, where evident, and considerable overlap in END proportions among radial sectors. (Figure 5-30 b).

Age-related variation in *P. troglodytes*

Complete descriptive statistics for tissue type % areas are summarized by age class in Appendix F (Table F-3 c). Tissue type maps derived from all individuals in the sample are provided in Appendix G-3.

Whole cross-section proportions: total PER, total END and HAV. Figure 5-31 shows age class means for % area of total END, total PER and HAV cortex at the midshaft femur of *P. troglodytes*. HAV comprised a mean 54.0 % of cortical area in the total sample and showed no apparent age trend. This will be discussed in detail in Chapter 6 of this thesis. The proportion of END tissues did not exceed 8.6 % in any individual in

the sample, and a slight decline was apparent in the proportion of END cortex among M3 – EPIPH age classes. Corresponding differences in the proportion of total PER were also evident.

Whole cross-section proportions: PER tissue types considered separately. Bivariate plots showing individual values for % area of primary periosteal tissue types are presented by age class in Figure 5-32. Age class mean % areas of primary periosteal tissue types are presented in Figure 5-33.

Midshaft femur. (Figure 5-33). In the DECID age class, FLC tissue was rare, and FLC-LZPO tissue comprised a mean 12.3 % of total cortical area. PF-LAM tissue was equally common, comprising a mean of 12.4 % of total cortical area. PF and LAM tissues were observed in lower proportions. In the single individual representing the M1 age class, FLC-LZPO and LAM each comprised a high proportion of total cortical area, while the proportion of PF and PF-LAM was lower. A similar pattern was observed in the M2 age class; FLC-LZPO still comprised a substantial proportion of cortical area (M2 age class mean = 12.20 %), although LAM was the most common primary periosteal tissue type observed (M2 age class mean = 28.19%). A continued gradual decrease in the proportion of FLC-LZPO, and a corresponding increase in the proportion of LAM, characterized the M3 and EPIPH age classes. M3 and EPIPH age class means for % area of LAM were 34.61 % and 39.46 %, respectively.

Age and regional variability: quantitative and qualitative observations. Qualitative assessment of individuals revealed ontogenetic variability in additional aspects of

primary bone microstructure which could not be appreciated in the quantitative analysis of bone tissue types. These features, as well as patterns of regional variability across circumferential rings and radial sectors at the midshaft femur, are described in further detail here, focusing on tissues of periosteal origin. Selected examples of these observations are provided in Figures 5-35 through 5-39. Tissue maps derived from all individuals in the sample are provided in Appendix G-3.

General age-related trends in primary bone microstructure during ontogeny.

Variability in tissue type proportions (reported as a % of *primary* cortex area) across circumferential rings is depicted graphically by age class in Figure 5-34. Variability among circumferential rings *within* cross-sections reflected the age-related succession of periosteal tissue types described above.

The primary cortex of the two individuals in the DECID age class was characterized by (largely cancellous) tissue of endosteal origin, periosteal fibro-lamellar tissues (particularly FLC-LZPO) and lamellar-zonal tissues. (Figure 5-34). FLC-LZPO occurred in its highest proportion in the mid-cortical ring, and it was most prevalent in the smallest and least HAV-remodeled individual (MNH 83618). Visual inspection revealed that this tissue was characterized by densely-distributed large primary osteons (encircled by several lamellae), having a predominantly longitudinal orientation. Scattered oblique and circular canals and irregular anastomoses were also observed. The outer periosteal cortex of these individuals was characterized by lamellar-zonal tissue (PF, PF-LAM or LAM), loosely vascularized by scattered primary osteons and simple primary vascular canals. (Figure 5-35).

The single individual in the M1 age class was characterized by slightly higher %

FLC-LZPO, which showed a regional distribution in the medial cortex. The vascular pattern observed in FLC-LZPO cortex in this individual was similar to that observed in the DECID age class. (Figure 5-36).

In the M2 age class, FLC-LZPO tissues were characterized by a predominantly longitudinal or reticular vascular pattern. However, two individuals showed an increase in the proportion of circularly-oriented primary osteons in the medial and postero-medial cortex. In these individuals, circular canals regularly anastomosed with longitudinally-oriented vascular canals, forming a laminar FLC-LZPO tissue. (Figure 5-37 b, and posterolateral cortex in Figure 5-38). The anterior and lateral cortex of M2 individuals was generally characterized by a less dense arrangement of small longitudinal primary osteons and simple vascular canals, embedded in lamellar-zonal (largely LAM) or, in some areas, FLC-LZPO tissue.

The primary periosteal cortex of M3 and EIPPH individuals was comprised predominantly of LAM, although regions of FLC-LZPO were observed in the inner periosteal cortex. (See Appendix G-3 d and e, and Figure 5-39). LAM was vascularized by primary osteons and simple vascular canals, which were less densely-distributed. Vascular patterns were predominantly longitudinal, although oblique canals and irregular anastomoses were also observed.

Growth marks were observed in the primary periosteal cortex of M1 – EIPPH individuals. (Figures 5-36 c, 5-38 b, and 5-39 b). Annuli and resting lines varied in number and severity among individuals. In the single individual in the M1 age class, a single resting line was observed in the innermost periosteal cortex, which showed no obvious relation to reversals in the anterior and lateral cortices. (See Figure 5-36).

Individuals in the M2 and older age classes were characterized by multiple resting lines, distributed throughout the thickness of the periosteal cortex. In M2 individuals, these lines were typically more closely-spaced in anterior and anterolateral cortices, and they became more widely spaced (often disappearing locally) in the medial cortex. (Figure 38 b).

Several individuals (particularly in the M1 – M2 age classes) were also characterized by alternations of lamellar-zonal (predominantly LAM) and FLC-LZPO tissue across the thickness of the primary periosteal cortex. This pattern was most pronounced in the medial and adjacent cortical regions of M2 individuals. (See Figures 5-36 and 5-38, and Appendix G-3 b and c). In some individuals, alternate tissue type distributions, together with more subtle changes in microstructural details (e.g., the size and distribution of primary osteons, polarization contrast of collagen fiber matrix in lamellar-zonal tissues, and the occurrence of growth marks) produced a distinctly repetitive microstructural pattern. This was most evident in the individual shown in Figure 5-38, where growth layers (i.e., zones) comprised of FLC-LZPO and vascular LAM tissues were interspersed with narrower layers of less vascular LAM and resting lines.

Regional variability and growth remodeling: midshaft femur. Variability in tissue type proportions across circumferential rings and radial sectors at the midshaft femur was indicative of cortical drift and possibly metaphyseal remodeling. These patterns were most apparent in younger age classes.

A possible history of metaphyseal remodeling at the midshaft section location examined here was suggested by the presence of endosteal tissue. The whole cross-

section proportion of END bone was highest in the DECID age class, where it comprised the majority of cortical area in the inner cortical ring. (Figure 5-34). However, other features indicating endosteal compaction and metaphyseal reduction were not prominent in either individual. END was organized predominantly as coarse cancellous tissue, although specimen MNH 83614 was also characterized by ECCC and circumferentially-arranged lamellar-zonal tissue in the anterior and adjacent cortices. (See Appendix G-3 a). The largest individual (MNH 83614) was characterized by a reversal line in the anterior cortex, although this may be interpreted to reflect posterior cortical drift. END tissues were also observed in individuals of older age classes (Figure 5-40), although in many cases, their distribution across radial sectors may reflect an overall pattern of cortical drift (discussed below). (But see, for instance, individual # 35526 in Appendix G-3 c).

Regional variation in tissue type proportions across radial sectors at the midshaft femur is summarized graphically in Figure 5-41. Several observations indicated a pattern of cortical drift in a predominantly medial and / or postero-medial direction, particularly in the M1 and M2 age classes. First, the distribution of vascular FLC-LZPO tissue compared to lamellar-zonal tissue types (PF, PF-LAM, LAM) indicated differential periosteal growth of the medial and adjacent cortical regions in M1 – M2 individuals. FLC-LZPO tissues comprised a larger proportion of primary cortex in the postero-medial, medial and antero-medial cortices (corresponding to radial sectors 6, 7, 8) compared to opposing cortical regions in the M1 and M2 age classes. Secondly, the distribution of END tissue suggested a more posterior or postero-medial drift direction in many individuals. (For instance, see individuals MNH 83604, 83673, and 83643 in Appendix

G-3 b and c). As can be appreciated in Figure 5-41, there was a trend for proportions of END tissue in the M1 and M2 age classes to be greatest in the antero-lateral, anterior, and /or lateral cortex (corresponding to radial sectors 2, 1, and 3), indicating inward growth and drift in the opposing direction. As reported for other taxa, tissue type distributions in older age classes showed no consistent pattern of cortical drift. (See Appendix G-3 d and e).

Principle components analysis of intra- and interspecific variability

PCA was used for data visualization purposes, and to identify broad similarities and differences among taxa in proportions of primary bone tissue types at the midshaft femur. Active variables included were the % of total cortical area of END, and the % of *primary periosteal cortex* area of FLC + FLC-LZPO, PF, PF-LAM, and LAM.

As shown in Table 5-19 a, the first two principal components together accounted for 82.19 % of the total variance in the sample. PC 1 and PC 2 are considered further below. Factor loadings are provided in Table 5-19 b.

PC 1. PC 1 accounted for 56.11 % of the total variance, and it was most strongly correlated with proportions of lamellar-zonal tissue types. Negative values on PC 1 reflected increased proportions of PF and PF-LAM, while positive values reflected increased proportions of LAM. Factor loadings were also moderately high for % FLC + FLC-LZPO and % END, which were negatively correlated. When supplemental variables were included in the analysis, PC 1 was highly correlated with age class but not cortical area. Visual inspection of Figure 5-42 revealed considerable overlap among taxa

in their distribution along PC 1. However, when taxa were examined separately, younger age classes had more negative values than older age classes. In sum, PC 1 appeared to summarize general age-related transitions from PF to LAM tissue types within taxa described above. Several intraspecific trends were visually apparent:

1) In *C. aethiops*, the DECID age class was distributed negatively on PC 1, while individuals of all older age classes were distributed positively. While several M1 individuals loaded less positively than older *C. aethiops* individuals, M1 – EIPPH age classes exhibited considerable overlap.

2) In *H. lar*, the DECID age class occupied the most negative extreme of PC 1, showing some overlap with the M1 age class. The M2 age class showed an intermediate distribution, exhibiting some overlap with M3 and EIPPH classes towards the more positive end of its range.

3) In *P. troglodytes*, the DECID age class loaded negatively along PC 1, overlapping with the DECID and M1 age classes of *H. lar*. The M1 and M2 age classes showed an intermediate distribution, while older age classes loaded positively and were overlapping in their distribution.

PC 2. PC 2 accounted for 26.08 % of the total variance, and it was most strongly correlated with % END and % FLC + FLC-LZPO. Negative values on PC 2 reflected increased proportions of END tissues, while positive values reflected increased proportions of FLC + FLC-LZPO. *H. lar* was most commonly distributed negatively along this axis, particularly in younger age classes, while most *P. troglodytes* individuals were most commonly distributed positively. Taxa were most clearly distinguished from

one another in younger age classes. Other visually apparent trends included:

1) In *C. aethiops*, DECID age class individuals were distributed more positively than all older age classes. Two individuals of the DECID age class (SLW 62 and 33) were outside the range of all other taxa. M1 individuals were also distributed positively along this axis, although they overlapped with older age classes. M2 – EIPPH age classes showed considerable overlap in their distributions, reflecting the variable proportions of END tissue observed among individuals.

2) In *H. lar*, DECID age class individuals were distributed more negatively than older age classes, reflecting their higher proportions of END. M1 individuals were also distributed more negatively along this axis than most individuals in older age classes. M2 – EIPPH age classes overlapped considerably.

3) In *P. troglodytes*, individuals in younger age classes appeared to show more variation in their distribution along PC 2, with single individuals in the DECID, M1 and M2 age classes occupying the upper half of the species' distribution (and overlapping with DECID *C. aethiops*). All individuals in the M3 and EIPPH age classes were distributed in the lower half of the *P. troglodytes* range.

DISCUSSION

Observations of primary bone microstructure in adult *Chlorocebus aethiops*, *Hylobates lar*, and *Pan troglodytes* were generally consistent with earlier reports based largely on isolated adult individuals [various Old World monkey taxa (Amprino and Godina, 1947; Foote, 1916; Singh et al., 1974), including ontogenetic studies of *Macaca* (Enlow, 1982; Newell-Morris and Sirianni, 1982); *Hylobates* and *Symphalangus*

(Amprino and Godina, 1947; Foote, 1916; Singh et al., 1974); and *Pan troglodytes* (Amprino and Godina, 1947; Foote, 1916)]. (Summarized in Table 5-3). However, primary bone tissues exhibited considerable variation in their microscopic structure, both within and among taxa. Observed patterns of primary bone microstructural development are summarized and discussed below, focusing on tissue types of periosteal origin having relevance for life history studies.

Variability in primary bone tissue types with age and life history.

Results indicated that proportions of primary periosteal tissue types varied in an age-related manner within taxa. In general accordance with the predictions of this study, younger age classes were characterized by higher proportions of tissues associated with faster depositional rates (namely, fibro-lamellar tissues and/or parallel-fibered bone), while older age classes were characterized by higher proportions of tissues associated with slower depositional rates (i.e., lamellar bone). Further, taxa differed in the timing of this transition and in the particular types of bone tissues deposited at different stages of ontogeny. When interpreted within the context of local growth and secondary remodeling history (the latter discussed further in Chapter 6), intraspecific patterns of bone microstructural development showed several broad consistencies with body mass growth patterns. However, some characteristics of postnatal growth, such as sex differences in rates of growth and the occurrence of a subadult growth spurt, may be more readily discerned from subtle variability in microstructural features (e.g., vascular patterns) not measured here. Correspondence of interspecific patterns of primary bone microstructural development with predictions of this study was mixed.

Among endosteal tissues, proportions of tissues associated with faster rates of deposition (endosteal parallel-fibered and endosteal parallel-fibered to lamellar transition) showed a significant negative correlation with age class in all taxa (except the femur of *C. aethiops*), as older age classes were characterized by lower mean proportions of these tissue types compared to younger age classes. Results for endosteal lamellar and compacted coarse cancellous tissue types were more variable across taxa and elements, and appeared to reflect the influence of element-specific growth and secondary remodeling patterns.

Chlorocebus aethiops. The midshaft femur and humerus of vervet monkeys was characterized by statistically-significant variation in whole cross-section proportions of primary periosteal tissues types (except PF at the midshaft femur) across age classes. The first postnatal year (represented by the DECID age class) was characterized by a transition from fibro-lamellar tissue types (FLC and FLC-LZPO) to deposition of less-vascularized LAM tissue, the latter having predominantly longitudinal simple vascular canals and small primary osteons. This transition occurred very early in postnatal ontogeny; recent periosteal deposition of tissue having a PF and LAM matrix at the midshaft femur and humerus of the two youngest DECID individuals in the sample suggests that this transition began before the deciduous dentition was fully erupted, estimated to occur by approximately three months of age (Bolter and Zihlman, 2003). Evidence of recent periosteal deposition at the midshaft femur was almost exclusively LAM in the M1 age class, although proportions of FLC-LZPO remained slightly higher at the midshaft humerus in the M1 age class. There was relatively little change in

periosteal tissue type proportions in older age classes. This latter observation was supported by post-hoc statistical contrasts and by results of PCA. However, qualitative observations revealed age-related variation in overall vascularity of primary LAM cortex.

Observations of primary bone microstructural development in *C. aethiops* showed a pattern similar to that described previously for *Macaca* species. Enlow (1962) reported that the primary periosteal cortex of long bone diaphyses in newborn rhesus macaques (*Macaca mulatta*) was comprised of woven (or “fine cancellous”) bone, which underwent lamellar compaction and incorporation of primary osteons. His observations are consistent with a later study of fetal and neonatal development of pig-tailed macaques (*M. nemestrina*) by Newell-Morris and Sirianni (1982), in which periosteal deposition of woven bone with dense primary osteons (corresponding to tissues classified as FLC here) was observed at the midshaft humerus of fetuses and neonates younger than 6 weeks of age. This latter tissue type was associated with the highest bone depositional rates observed in their study, averaging 9.8 μm to 6.7 μm / day during the prenatal period. During the postnatal period, depositional rates were also high during the first 6 weeks. However, periosteal deposition of less-vascular parallel-fibered bone was observed in pig-tailed macaques older than 6 weeks of age, and was associated with a further decline in bone depositional rates to 2 - 4 μm / day. While there were no observations between 28 – 55 postnatal weeks, lamellar bone deposition was observed at the periosteal surface in 55 – 57 week old animals, and was associated with the lowest bone depositional rates (< 1.5 μm / day) recorded during the study period. (Interestingly, deposition of lamellar bone was observed at the endosteal surface across all postnatal ages in pig-tailed macaques.) A similar transition to deposition of lamellar bone during later growth stages

was also reported for the rhesus macaque (Enlow, 1962).

In the current study, the distribution of fibro-lamellar tissues having a predominantly woven-fibered extra-osteonal matrix and densely-distributed primary osteons (i.e., FLC type) in the two youngest individuals in the DECID age class may similarly represent tissues that were deposited during fetal life or the early neonatal period. Prenatal growth patterns have not been well characterized in many primate species (Brizee and Dunlap, 1986), and to my knowledge, vervet monkeys have not been studied in this respect. However, it follows that the rapid transition to lamellar bone deposition observed here may reflect a similar overall slowing of bone growth rates during the first few months of postnatal life, from their maxima during the prenatal period.

Prior research indicates that postnatal growth in vervet monkeys is most rapid during early ontogeny (Horrocks, 1986; Leigh, 1996; Turner et al., 1997). Body mass velocity curves presented by Leigh (1996: Fig. 4, p. 462) based on captive animals indicated that males and females were characterized by maximum growth rates of approximately 1 kg / year, immediately following birth. Growth velocities declined slightly during the first postnatal year, and male and female velocity curves were similar until approximately two years of age. After this time, females generally showed a deceleration in growth (following a weakly-defined increase in growth velocity), while males exhibited a slight growth spurt approaching a velocity of 1 kg / year. Reported mean ages at body weight growth cessation in captive vervets are 4.00 years for females and 5.50 years for males, and this bimaturism was suggested to be largely responsible for the larger adult size attained in males (Leigh, 1992, 1995, 1996). Other published

accounts of postnatal growth in body weight and linear dimensions in wild vervets have reached similar conclusions (Horrocks, 1986; Turner et al., 1997).

Thus, periosteal deposition of lamellar bone following emergence of the first permanent molar at approximately 1 year of age is coincident with body mass growth velocities in captive animals of below 1 kg/year (as reported by Leigh, 1996). While primary bone tissue type proportions observed here remained similar in the M1 and older age classes, substantial growth in diaphyseal cross-sectional dimensions was evident thereafter (see Chapter 4), in a manner consistent with other body dimensions (Bolter and Zihlman, 2003; Horrocks, 1986; Leigh, 1992, 1996; Turner et al., 1997). Further, while growth in cross-sectional dimensions at the midshaft femur and humerus was consistent with bimaturism and slightly elevated growth rates in male vervets, consistent differences among males and females in the proportions of primary bone tissue types were not observed.

Qualitative observations suggest that more subtle age- and sex-related variation in bone growth rates may be reflected in features of the primary vascular network which were not quantified here. The midshaft femur and humerus were characterized by a tendency for an overall decrease in the vascularity (as reflected in the number of vascular canals present) of the primary periosteal cortex in members of older age classes compared to young age classes. Further, males in the M3 and EIPPH age classes tended to show greater vascularity of periosteal lamellar bone in the posterior humeral cortex compared to females. Prior reports have indicated that primary osteon diameter and density varies in accordance with bone depositional rates, and that more highly-vascularized tissues are associated with faster rates of growth compared to less

vascularized tissues having a similar collagen fiber matrix organization (Margerie et al., 2002; Newell-Morris and Sirianni, 1982; Ricqlès et al., 1991; Starck and Chinsamy, 2002). Further, as the majority of primary periosteal tissues in individuals of the M1 – EIPPH age classes of vervet monkeys was lamellar in organization, it is also possible that sex and age-related variation in bone depositional rates may be reflected in details of lamellar structure (Bromage et al., 2002; Bromage et al., 1998) or in the formation of resting lines (discussed below), which were not quantitatively examined.

Hylobates lar. The primary periosteal cortex at the midshaft femur and humerus in white-handed gibbons was comprised largely of poorly-vascularized lamellar-zonal (i.e., PF, PF-LAM, LAM) tissues at all stages of development. However, statistically-significant variation was observed in whole cross-section proportions of most primary periosteal tissues types (except FLC) across age classes in both elements. In the DECID age class, the most commonly-observed lamellar-zonal tissue type was PF-LAM. This tissue type continued to comprise the greatest mean proportion of primary cortex area in the M1 age class at the midshaft humerus. Individuals in the M2 age class showed a substantial increase in the proportion of LAM, and evidence of recent periosteal bone deposition was predominantly LAM in all older age classes. Post-hoc statistical contrasts supported the generally similar tissue type proportions in the DECID and M1 age classes compared to all older age classes, and a gradual transition from PF-LAM to LAM in the M2 and older age classes. This gradual transition was also evident from results of PCA.

Unexpectedly, mean proportions of fibro-lamellar bone (particularly FLC-LZPO) were slightly higher in the M1 age class, compared to earlier and later stages in ontogeny,

and they comprised a relatively small proportion of total cortical area at the midshaft femur and humerus across all age classes. This observation was not in accordance with predictions based on body mass growth patterns. According to investigations of captive animals by Leigh and colleague (Leigh, 1992, 1996; Leigh and Shea, 1995), white-handed gibbons are characterized by a fairly linear growth trajectory, lacking evidence of a subadult growth spurt, and showing a steady decline in growth velocity from a maximum of 1.4 kg / year immediately following birth. Accordingly, one would expect to find higher proportions of fibro-lamellar tissues in the DECID age class (based on the pattern observed in *C. aethiops*), followed by a decline in fibro-lamellar tissue type proportions in older age classes.

FLC-LZPO in *H. lar* examined in the current study showed a regional distribution, encountered in: 1) the innermost periosteal cortex, in or adjacent to regions that have undergone secondary intracortical remodeling; and 2) in localized regions of primary cortex, where it appeared to be associated with a pattern of cortical drift and / or to indicate temporary periods of more rapid bone deposition within a largely lamellar-zonal cortex. Thus, this apparent inconsistency may be explained, in part, by the distribution of secondary intracortical remodeling, which was extensive in the inner cortex at the midshaft femur and humerus and which may preferentially affect earlier-deposited bone tissues. (See Chapter 6). However, this does not account for the predominance of slower-forming lamellar-zonal tissues primary (i.e., not secondarily-remodeled) cortical regions, even in DECID individuals. Rather, tissue type proportions reported here may be best explained within the context of bone growth remodeling processes at these skeletal sites, which may have important consequences for local

patterns of bone microstructural organization within diaphyseal cortices (Enlow, 1962; 1963).

Two observations are relevant for understanding the growth remodeling history as it pertains to tissue type patterns observed at the midshafts of the femur and humerus. First, a substantial proportion of the primary cortex of DECID individuals was endosteal in origin. Despite considerable secondary intracortical remodeling in these elements, primary endosteal bone comprised 10.1 – 27.8 % of total cortical area at the midshaft femur and 23.9 – 43.4 % of total cortical area at the midshaft humerus. Secondly, DECID individuals were characterized by prominent reversal lines in the periosteal cortex of the midshaft femur and humerus, indicating that periods of bone deposition were interrupted by periods of bone resorption at the periosteal surface. These observations are consistent with a pattern of metaphyseal remodeling described by Enlow (1962; 1963). (See Chapter 2, Figure 2-2). These observations indicate that the anatomical midshaft location of the femur and humerus in DECID individuals is not coincident with the true developmental midshaft, and that it was, at an earlier stage of ontogeny, located in or near the vicinity of the metaphysis.

Future sampling of other skeletal sites coincident with developmental midshaft may reveal higher proportions of tissues associated with faster depositional rates (e.g., fibro-lamellar tissues, or lamellar-zonal tissues with increased vascularity). Singh (1974) reportedly observed primary vascular bone with predominantly longitudinal and occasional radial vascular canals and “simple lamellae” in selected skeletal elements of a 2 year-old white-handed gibbon. [It was not clear from his description whether the “simple lamellae” refer to centripetal deposition of lamellae around vascular canals to

form primary osteons, or whether he was referring to primary vascular bone having a lamellar matrix organization (i.e., corresponding to LAM here).] The radial vascularization reported by Singh (1974) was not observed in the cortical bone tissues of white-handed gibbons examined here, although such a pattern may indicate more rapid bone depositional rates (Margerie et al., 2004).

A second unexpected finding was the statistically-significant contrast among sexes in the proportion of FLC-LZPO at the midshaft humerus of *H. lar*, conducted on a subsample comprised of the M3 and EPIPH age classes. Males were characterized, on average, by a larger proportion of FLC-LZPO compared to females. However, this difference was very slight (male subsample mean = 0.21, SD= 0.221; female subsample mean = 0.00, SD = 0.000). Gibbons are generally described as lacking sexual dimorphism in body mass growth patterns (Leigh, 1992, 1996). Although Leigh and Shea (1995) observed slightly higher body mass growth rates in males compared to females during later ontogeny in their sample of captive *H. lar*, this observation was suggested to reflect sampling bias (Leigh and Shea, 1995: 42). The observation of sex differences in the current study are similarly interpreted to be *not* of biological significance. This conclusion is supported by the lack of statistically-significant contrasts among sexes in external cross-sectional dimensions at the midshaft femur and humerus during growth. (See Chapter 4).

These inconsistencies aside, periosteal tissue type distributions in the M1 and older age classes are more easily reconciled with predictions based on body mass growth patterns. FLC-LZPO and lamellar-zonal tissues having a parallel-fibered matrix component (namely, PF-LAM) comprised a larger proportion of cortical area at the

midshaft femur and humerus of gibbons in the M1 age class compared to older age classes, while the latter were characterized by higher proportions of LAM. The M2 age class showed a somewhat intermediate pattern. These observations are broadly consistent with the gradual decline in growth velocity reported for body mass in captive gibbons, as reported by other investigators (Leigh and Shea, 1995, 1996) and discussed above.

Pan troglodytes. Spearman rank correlation analysis demonstrated a statistically-significant decrease in whole cross-section proportions of FLC-LZPO, PF, and PF-LAM, and an overall increase in the proportion of LAM, at the midshaft femur with age in common chimpanzees. In the DECID age class, the majority of the primary periosteal cortex was comprised lamellar-zonal tissues, which together represented a mean 21.3 % of total cortical area. PF-LAM was the most common lamellar-zonal tissue type observed in the DECID age class. However, this tissue type was concentrated in the outermost cortex, while earlier-deposited regions were fibro-lamellar (FLC and FLC-LZPO) in organization. FLC-LZPO comprised a mean 13.1 % of total cortical area in the DECID age class, and was characterized by densely-distributed primary osteons having a predominantly longitudinal orientation with scattered oblique canals and irregular anastomoses. Both individuals in the DECID age class showed a very similar pattern, and the microstructural transition between these two tissue types appeared abrupt around the circumference of both midshaft femur cross-sections. LAM comprised the largest proportion of cortical area in the M1 and older age classes. However, the proportion of FLC-LZPO remained roughly similar across the DECID - M2 age classes, while declining to comprise 2.9 % and 2.2 % of cortical area in the M3 and EIPH age classes,

respectively. These trends were also supported by PCA. Qualitative assessment indicated that reticular and / or laminar vascularization of FLC-LZPO was common in the M2 age class. LAM in the M3 and EPIPH age classes was characterized by primary vascular canals and scattered primary osteons having a predominantly longitudinal orientation.

Interpretation of primary tissue type proportions in *P. troglodytes* within the context of life history is complicated by several factors. As mentioned for *H. lar*, intracortical remodeling at the midshaft femur can be locally extensive, preferentially affecting earlier-deposited cortical regions. (See Chapter 6). Thus, the proportion of FLC-LZPO observed here likely underestimates the extent of fibro-lamellar bone deposition during early (and later) ontogeny at the midshaft femur. Despite this, however, it is also true that intracortical remodeling showed a highly patterned regional distribution within cross-sections. In M1 and older age classes, intracortical remodeling tended to be concentrated in the innermost cortex, and in many individuals, in the lateral half of the cortex at the midshaft femur. (Chapter 6). Thus, broad expanses of primary tissues were left *in tact* regionally, both in the outermost cortex around the circumference of the cross-section, and in the medial cortex across the thickness (i.e., from endosteal to periosteal regions) of the cross-section.

A second possible complication relates to the distribution of endosteal tissues at the midshaft femur, particularly in individuals of the DECID age class, which suggests that tissue type distributions during early ontogeny may reflect the influence of metaphyseal remodeling history at this skeletal site. Endosteal bone was most prominently organized as a coarse cancellous tissue, which is thought to be most

commonly distributed in regions associated with the metaphysis in the proximal and distal thirds of long bones (Enlow, 1963). However, while the largest individual (MNH 83614) was characterized by a small proportion of ECCC and a reversal line which was limited in its distribution to the anterior cortex (both of which may be interpreted to reflect posterior cortical drift, as discussed further below), microstructural features indicating endosteal compaction and metaphyseal reduction (associated with metaphysis-to-diaphysis tissue relocation) were not particularly prominent in either individual.

In light of these observations, the transition from fibro-lamellar (FLC and FLC-LZPO) to less vascular lamellar-zonal (namely, PF-LAM) tissues in the midshaft femur of DECID individuals may be interpreted to indicate a relatively rapid deceleration of periosteal bone depositional rates at this skeletal site, evident in individuals having emerged deciduous dentitions and unemerged first permanent molars [estimated on the basis of dental emergence to be approximately 1 – 4 years of age (Nissen and Riesen, 1964; Smith et al., 1994; estimated age at emergence of M1 based on Zihlman et al., 2004)]. This would be consistent with reports of early postnatal growth in captive *P. troglodytes*. Based on a longitudinal study of colony-reared individuals, Gavan and others (Gavan, 1953; Grether and Yerkes, 1940) reported a rapid decline in body mass growth rates during infancy, from initial mean growth increments of approximately 5 – 6 kg/year following birth. This trend was most pronounced during the first postnatal year. While not all reports on body mass growth have demonstrated such a trend (Hamada et al., 1996; Leigh and Shea, 1995, 1996), a similar deceleration in growth rates during the first 3 – 4 years of life was shown for linear dimensions by Hamada and Udono (2002).

During later postnatal growth, prior research has shown that body mass growth

velocities in captive common chimpanzees reach approximately 3 – 4 kg/year by the beginning of the juvenile period, when an acceleration in growth is initiated at approximately 5.5 years of age. Male and female growth trajectories are similar until approximately 7.5 – 8 years of age, after which time males exhibit a pronounced growth spurt and reach a peak postnatal growth velocity of just under 8 kg / year (Leigh, 1996; Leigh and Shea, 1995; 1996). While females also exhibit a slight increase in body mass growth velocity during this time, they do not exhibit a distinct growth spurt (Grether and Yerkes, 1940; Hamada et al., 1996; Leigh, 1996; Leigh and Shea, 1995; 1996). Similar characteristics have been reported for wild-living common chimpanzees, although growth is delayed relative to their captive counterparts. Pusey (1977) reported that males and females at Gombe showed a similar growth trajectory until approximately 9.6 years of age, and males exhibited a spurt in mass velocity between 9.2 – 13.3 years of age.

Due to the unknown sex of many individuals in the current sample, sexual dimorphism in growth could not be examined. Cross-sectional geometric dimensions showed evidence of substantial growth between the M2 and M3 age classes, estimated at approximately 8.3 - 12.4 years of age (based on the recent chronology for wild-living common chimpanzees provided by Zihlman et al., 2004), compared to earlier ontogeny. (See Chapter 5). However, quantitative tissue type proportions observed during later postnatal periods do not provide clear evidence for a peak in subperiosteal growth rates coincident with the occurrence of a subadult body mass growth spurt, as described above. The significance of a transition from PF-LAM to LAM in lamellar-zonal regions of the cortex of M1 and M2 individuals for growth rate variation is unclear, particularly given that lamellar bone in these age classes tended to be more vascularized over broad

expanses of the cortex compared to PF-LAM observed in DECID individuals. Further, roughly similar proportions of FLC-LZPO among the DECID, M1 and M2 age classes, despite evidence of ongoing secondary intracortical remodeling distributed preferentially in older cortical regions (see Chapter 6), appears to indicate a sustained period of relatively rapid bone growth compared to later ontogeny. (This is not meant to imply that small-scale temporal variations in growth velocity do not exist, and indeed evidence suggests this is the case, as discussed below.) Sector analysis demonstrated that the proportion of FLC-LZPO was highest in the inner and mid-cortical circumferential rings in the single M1 individual, and in the mid-cortical and outer circumferential rings (the latter indicating recent deposition) in the M2 age class. The proportion of FLC-LZPO at the midshaft femur was observed to decline (with a concomitant increase in LAM) and reach adult proportions following M3 emergence (estimated at approximately 12.4 - years of age in the wild; Zihlman et al., 2004).

The lack of clear correspondence between primary bone microstructural development and body mass growth patterns (particularly, the occurrence of a subadult peak in growth velocity) during later ontogeny in this case may instead reflect the reportedly variable occurrence of a subadult growth spurt in linear body dimensions of common chimpanzees (Gavan, 1953; Hamada et al., 1996; Hamada and Udono, 2002; Watts and Gavan, 1982). Studies have failed to demonstrate a consistent and well-defined subadult growth spurt in linear dimensions of either sex, except in cases of catch-up growth in individuals who experience delayed growth during the juvenile period (Hamada and Udono, 2002). Hamada and Udono (2002) reported that average juvenile growth velocities for body length are typically maintained at a rate of approximately 80

mm/year (lacking evidence of significant growth acceleration) until 6.5 years in females and 7.5 years in males, prior to declining during later ontogeny.

Qualitative observations in the current study suggest that variability in subperiosteal bone growth rates may be reflected in features of the primary vascular network which have not been quantified here, such as primary osteon size, density and orientation. Vascular patterns observed in two individuals of the M2 age class showed a more complicated three-dimensional arrangement compared to other age classes; circular primary osteons regularly anastomosed with longitudinally-oriented primary osteons, forming a laminar FLC-LZPO tissue. This latter tissue showed a patterned distribution in the medial and postero-medial cortex of the midshaft femur, an area interpreted to undergo differential periosteal growth associated with cortical drift.

While recent quantitative studies have indicated that the size and density of primary osteons is positively associated with bone depositional rates (Margerie et al., 2002; Newell-Morris and Sirianni, 1982; Starck and Chinsamy, 2002), the significance of vascular canal orientation is less clear. There appears to be considerable variability and overlap in the depositional rates of many fibro-lamellar tissue ‘sub-types’, recognized on the basis of the orientation of vascular canals incorporated into their matrices (Castanet et al., 1996; 2000; Margerie et al., 2002; 2004; Starck and Chinsamy, 2002). While laminar fibro-lamellar bone was associated with significantly slower depositional rates than other fibro-lamellar (including longitudinal and reticular) subtypes in king penguin (*Aptenodytes patagonicus*) chicks (Margerie et al., 2004), it was deposited at faster rates than longitudinal fibro-lamellar bone in extant ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*) (Castanet et al., 2000). The variable results of these studies

may reflect taxon differences, or variation in the organization of other microstructural features of fibro-lamellar bone (e.g., collagen fiber organization of the extra-osteonal matrix, primary osteon density) which were not explicitly measured.

Unfortunately, no comparable quantitative data exist for primates. Whether deposition of laminar bone in common chimpanzees around the time of M2 emergence reflects a local increase in bone growth velocities (as suggested from the results of Castanet et al., 2000) in some individuals, in a manner consistent with other characteristics of somatic growth (Hamada and Udono, 2002; Leigh, 1996; Leigh and Shea, 1995, 1996), or a local decrease in bone growth velocities (as suggested by Margerie et al., 2004), is a question awaiting further study.⁵

Intra- and interspecific variation at the midshaft femur as revealed through PCA. As described above, all taxa examined here were characterized by a transition from periosteal deposition of fast-forming fibro-lamellar (FLC and FLC-LZPO) or lamellar-zonal (PF and PF-LAM) tissues in young age classes to deposition of slow-forming lamellar bone in older age classes. This age-related transition was also evident in the PCA analysis. The first two principal components accounted for 82 % of the variance in the total sample. PC 1 accounted for the majority of this variation (56.11 %), and was

⁵ Laminar bone does not show a distribution consistent with the hypothesis proposed by Margerie and colleagues (Margerie, 2002; Margerie et al., 2005), which states that laminar bone represents an adaptation to torsion-induced strains. This tissue is confined to the medial and postero-medial cortex of the midshaft femur, indicating differential growth of this cortical region, and is associated with structural properties indicating increased relative medio-lateral bending rigidity. (See Chapter 5). While there are no *in vivo* strain data for the midshaft femur of common chimpanzees, other examinations of geometric properties at this skeletal site have reached similar conclusions (Carlson, 2003; Ohman, 1993; Ruff, 1989; Ruff and Runestad, 1992).

highly correlated with age class. This axis predominantly summarized variation in proportions of lamellar-zonal tissue types; more rapidly-forming tissues (PF and PF-LAM) loaded negatively, while lamellar bone loaded positively. PC 2 summarized variation in the proportion of fibro-lamellar tissues (FLC + FLC-LZPO) and endosteal bone; fibro-lamellar tissues loaded positively, while endosteal tissues loaded negatively. While taxa exhibited considerable overlap along PC 1, younger age classes were distributed negatively and older age classes were distributed more positively along this axis when taxa were examined separately. PC 1 and PC 2 also revealed interspecific differences in the timing of this transition between fast- and slow-forming primary tissue types, and in the particular types of primary bone tissues observed among individuals of similar biological age.

In *C. aethiops*, the rapid transition to periosteal deposition of lamellar bone by the time of M1 emergence was reflected in the distribution of this taxon along PC 1 and PC 2. DECID age class individuals were separated from all older age classes, and were distributed positively on both axes. Older age classes, in contrast, exhibited considerable overlap in their distribution along both axes. In *H. lar*, the more gradual nature of the transition between lamellar-zonal tissue types in the DECID – M2 age classes was reflected in the distribution of this taxon along PC 1. Individuals tended to load negatively on PC 2, particularly in the DECID and M1 age classes, reflecting their relatively high proportions of endosteal bone as described above. A gradual transition in primary bone tissue types was also reflected in the distribution of *P. troglodytes* on PC 1 and PC 2. The significance of these intraspecific trends was discussed above.

Taxa were most clearly distinguished from one another along PC 2. *H. lar* most

commonly exhibited negative loadings on PC 2, with DECID individuals occupying the most negative extreme, again reflecting their generally higher proportions of endosteal bone and the sparse representation of fibro-lamellar tissues. In *C. aethiops*, two individuals of the DECID age class were distributed more positively and outside the range of individuals in both other taxa, indicating their higher proportions of fibro-lamellar tissues than *H. lar* and *P. troglodytes* of similar age status. However, the remaining DECID *C. aethiops* individuals were distributed less positively along this axis, while older age classes overlapped considerably with *H. lar* and with older age classes (particularly M3 and EPIPH) of *P. troglodytes*. *P. troglodytes* exhibited positive loadings on PC 2 across age classes, with the exception of two individuals. Although there was some overlap towards the lower end of this taxon's range, individuals of the M1 and M2 age classes tended to be distributed more positively along PC 2 compared to similar age classes in *H. lar* and *C. aethiops*.

In sum, several interspecific trends were recognized. First, although higher proportions of fibro-lamellar bone were observed in the DECID age class of vervets compared to gibbons (an observation which may be attributed to metaphyseal remodeling history in the latter), vervets in the M1 age class were characterized by *lower* proportions of PF-LAM and higher proportions of LAM compared to gibbons of similar biological age. Higher proportions of faster-forming tissues in the M1 age class of gibbons were observed despite their extensive secondary intracortical remodeling, which preferentially affects earlier-deposited cortical regions (see Chapter 6). In both taxa, a transition to periosteal deposition of predominantly lamellar bone is coincident with body mass growth rates of 1 kg / year or less, as reported for captive animals by Leigh and

colleagues (Leigh, 1996; Leigh and Shea, 1995). Slightly increased proportions of PF-LAM tissue in the M1 and, to a lesser extent, M2 age classes in gibbons compared to vervets is in general accordance with the observation that body mass growth velocities are above 1 kg/year (slightly faster than rates of mass growth in vervets) during the first three years of life (Leigh, 1996; Leigh and Shea, 1995).

Observations of tissue type proportions in *P. troglodytes* were less easily reconciled with the considerably faster body mass growth velocities characteristic of this taxon compared to other taxa examined here (e.g., as reported for captive animals by Leigh, 1996). The prediction that *P. troglodytes* would exhibit higher proportions of faster-forming tissue types compared to *C. aethiops* and *H. lar* received only mixed support. Rather, *C. aethiops* individuals in the DECID age class exhibited substantially higher proportions of fibro-lamellar tissue types (mean = 44.4 %, range = 20.7 – 75.8) compared to *P. troglodytes* of similar dental emergence status (mean = 13.1 %, range = 9.4 – 16.8). However, the situation was reversed in the M1 and M2 age classes, where *P. troglodytes* exhibited the highest proportion fibro-lamellar bone (M1 age class individual = 19.3 %; M2 age class mean = 12.3 %, range = 4.8 – 18.5).

Several observations may partly account for this inconsistency. First, *P. troglodytes* was observed to undergo substantially more secondary intracortical remodeling compared to *C. aethiops*. (See Chapter 6). When proportions of fibro-lamellar tissues are calculated relative to *primary periosteal* cortex area in chimpanzees, mean proportions of fibro-lamellar tissues increase to 35.5 % (range = 28.1 – 42.9) in the DECID age class, 35.2 % in the single M1 individual, and 28.2 % (range = 12.7 – 46.8) in the M2 age class. As discussed in Chapter 6, secondary intracortical remodeling

preferentially affects earlier-deposited cortical regions. Thus, the proportion of FLC-LZPO reported here likely underestimates the extent of fibro-lamellar bone deposition during earlier growth stages at the midshaft femur. However, it is also true that lamellar-zonal tissues were more commonly represented in primary cortical regions at the midshaft femur of *P. troglodytes*, even in younger age classes. Thus, secondary intracortical remodeling alone probably does not account for all of this difference observed between taxa. Secondly, the possibility was raised that microstructural characteristics at the midshaft femur may reflect a history of metaphyseal growth remodeling in *P. troglodytes*. Finally, growth rate variability may be reflected in other features of primary bone tissues which were not quantitatively examined in the current study. The tissue type classification scheme followed here did not allow for the discrimination of more subtle variations in the microscopic organization of primary bone tissues, which were qualitatively observed among taxa (e.g., differences in primary osteon diameter, density and orientation). The possible significance of these features for discriminating growth rate variability was discussed above, and they warrant attention in future investigations.

Bone-specific patterns.

While the microscopic organization of primary bone tissues was generally similar between the midshaft femur and humerus within taxa, regional variation was observed in the proportions and distributions of primary bone tissue types both within and among elements. This was especially true in *C. aethiops*. This regional variation, particularly as observed among radial sectors, reflected element-specific growth and remodeling

patterns. Patterns are briefly summarized and discussed below.

Chlorocebus aethiops midshaft femur and humerus. Despite its higher proportion of secondary intracortical remodeling (also see Chapter 6), the midshaft humerus was characterized by statistically-significantly higher proportions of FLC-LZPO compared to the femur in the M2-EIPPH age classes. The proportion of LAM was correspondingly higher at the midshaft femur. The humerus was also characterized by a higher proportion of endosteal bone compared to the femur in many age classes.

Quantitative and qualitative assessment of the midshaft humerus indicated that the distribution of periosteal and endosteal bone tissue types among radial sectors was strongly influenced by postero-medial cortical drift, with periosteal tissue types associated with faster depositional rates being concentrated in the predominant growth direction (i.e., in posterior and medial cortical regions). The opposing side of the cross-section showed increased endosteal tissue proportions and evidence of subperiosteal resorption in many individuals. Thus, although the midshaft femur exhibited slightly larger cross-sectional dimensions than the humerus in some comparisons (see Chapter 4), the increased proportion of FLC-LZPO at the midshaft humerus indicates *locally* faster depositional rates in older age classes, associated with a pattern of pronounced cortical drift in the posterior and medial direction. A similar pattern was also reported for the midshaft humerus of prenatal and early postnatal pig-tailed macaques (*Macaca nemestrina*) (Newell-Morris and Sirianni, 1982).

Postero-medial cortical drift at the midshaft humerus also accounts for observations of cross-sectional geometric shape proportions reported in Chapter 4 of this

thesis. The midshaft humerus of *C. aethiops* showed an increase in mediolateral bending rigidity with age, a predominantly anterolateral – posteromedial orientation of maximum bending strength, and development of a strongly elliptical cross-section when principle moments of area were considered. In contrast, the midshaft femur showed a slight decline in relative mediolateral bending rigidity with age, and development of a more circular cross-sectional geometry. Ontogenetic variation in cross-sectional geometric proportions at the midshaft femur were associated with primary bone tissue type proportions indicating slight posterior and posteromedial cortical drift in the DECID and M1 age classes, and more variable patterns of cortical drift in older age classes.

Hylobates lar midshaft femur and humerus. Statistical contrasts among elements revealed that the midshaft humerus exhibited a significantly lower proportion of LAM compared to the midshaft femur in the M2 – EIPH age classes, an observation which may be accounted for by the greater extent of secondary intracortical remodeling in the former. However, the midshaft humerus also exhibited a significantly larger proportion of PF-LAM. This latter result is difficult to explain, and appears to contradict findings that the midshaft femur was characterized by slightly larger cortical area compared to the humerus in older age classes. (Chapter 4).

Qualitative and quantitative observations of the midshaft femur and humerus indicating a history of metaphyseal growth remodeling at these skeletal sites were discussed above. Distribution of primary tissue types across radial sectors at the midshaft femur generally indicated a pattern of posterior to posterolateral cortical drift in several DECID and M1 individuals. However, less pronounced and more variable patterns of

cortical drift at the midshaft femur characterized older age classes. The midshaft humerus also showed a more variable distribution of primary tissue types across radial sectors. While these observations don't appear to relate in an obvious way to the increase in relative mediolateral bending rigidity observed with age at the midshaft femur and humerus of *H. lar*, they are generally in accord with the more variable orientation of maximum bending rigidity observed in this taxon. (See Chapter 4).

Pan troglodytes midshaft femur. Qualitative and quantitative assessment of regional variation in the distribution of primary tissue types at the midshaft femur indicated a pattern of cortical drift in a predominantly medial and/or postero-medial direction, particularly in M1 and M2 age classes. This was indicated by a tendency for FLC-LZPO to comprise a larger proportion of primary cortex area in the postero-medial, medial and antero-medial cortices. Endosteal bone proportions tended to be highest in anterolateral, anterior, and /or lateral cortices.

As discussed above, secondary intracortical remodeling at the midshaft femur may have an influence on the distribution of primary tissue types observed here, and inferred patterns of cortical drift. In regions undergoing secondary intracortical remodeling, particularly in the innermost and lateral half of the cross-section, interstitial tissues were classified as FLC-LZPO or lamellar-zonal tissue types based on the presence of primary osteons, details of osteocyte lacunar shape and collagen fiber organization. It is therefore possible that proportions of FLC-LZPO tissues in the lateral and adjacent cortices were underestimated based on their appearance in interstitial areas. Alternatively, the distribution of secondary intracortical remodeling may reflect a

consequence of more rapid growth in the medial and adjacent cortices (and cortical drift), and the consequently older depositional history of primary tissues in the lateral and adjacent cortices. (See Chapter 6). In either case, the distribution of primary tissue types and a pattern of cortical drift in a predominantly medial or posteromedial direction was broadly consistent with structural proportions at the midshaft femur, which indicated an increase in relative mediolateral bending rigidity between the DECID and M2 age classes. This pattern was associated with a shift in the orientation of the plane of maximum bending rigidity, from an antero-posterior plane in the DECID age class towards an anteromedial-posterolateral to mediolateral plane in older age classes. (See Chapter 4).

Qualitative observations and future directions: Indications of subtle changes in bone growth rates within individuals.

Quantitative variation among age classes in mean primary bone tissue type proportions revealed broad patterns within taxa, which are suggested to reflect age-related variation in bone depositional rates in partial accordance with species-specific characteristics of somatic growth in other body dimensions. However, given the close relationship between bone microstructural organization and depositional rates discussed above, it follows that small-scale or short-term temporal variability in bone growth rates within individuals should also be commensurate with subtle changes in bone microstructure when observed across individual bone cortices (Ricqlès et al., 1991).

Qualitative observations in the current study identified patterns of variability in primary bone microstructure within individuals, which were not revealed by quantitative

analyses described above. These patterns were reflected in alternating distributions of primary bone tissue types across the thickness of bone cortices, and in several microstructural features not explicitly measured here – features of the vascular network (i.e., vascular canal density and primary osteon diameter), growth marks, and preferred collagen fiber orientation. Variability in these features was observed within individual bone cortices, and is generally interpreted to reflect temporal variability in periosteal bone depositional rates. Their significance for interpreting environmentally-mediated and intraspecific variability in primate growth strategies, as has been reported for body weight and linear dimensions (e.g., DeRousseau, 1990; DeRousseau and Reichs, 1987; Fauchoux et al., 1978; Hamada, 1994; Hamada et al., 1986; Marzke et al., 1996; Strum, 1991; Turner et al., 1997), warrants future attention. These observations include:

- 1) *C. aethiops*. Subtle variations in vascularity of primary periosteal cortex (comprised of FLC-LZPO and LAM) were observed, with zones of more vascular tissue alternating with layers of less vascular tissue. These patterns were most evident in the posterior and postero-lateral cortex of the midshaft humerus in several males during later ontogeny (e.g., Figure 5-15 c). Growth marks were also observed in several individuals of the DECID age class, and in all older individuals. They were most commonly observed in regions of lamellar bone, variably expressed as annuli or resting cement lines. At the midshaft femur, growth marks varied in their number and strength among individuals and possibly in relation to age, showing a tendency to increase in number across early age classes (e.g., Figures 5-13 c and 5-14 c and d.) The distribution of growth marks at the midshaft humerus was influenced by local circumstances of bone growth remodeling, particularly relating to a pronounced pattern of cortical drift.

2) *H. lar*. Growth marks, expressed as annuli and resting lines, were observed in primary lamellar-zonal cortex at the midshaft femur and humerus of individuals in all age classes. However, their interpretation in early age classes was complicated by metaphyseal growth remodeling history and cortical drift, as they were frequently continuous with reversal lines locally. The most notable pattern observed in *H. lar* relates to variation in the polarization contrast of the collagen fiber matrix of lamellar-zonal bone. In particular, lamellar bone frequently showed an alternating dark and bright appearance over broad expanses of the cortex when observed in circularly polarized light; regions characterized by dark lamellae alternated with regions of brighter lamellae, indicating changes in the preferred collagen fiber orientation of the matrix (e.g., Figures 5-27 and 5-28.) Regions of bright lamellae were frequently associated with resting lines.

3) *P. troglodytes*. Growth marks were observed in the primary periosteal cortex of M1 – EIPPH individuals, and they varied in number among individuals and possibly in relation to age. Some individuals, particularly in the M2 age class, were characterized by alternations of LAM and FLC-LZPO tissue across the thickness of the medial and adjacent cortices (i.e., the predominant growth direction, in those individuals showing evidence of cortical drift). This pattern was also associated with more subtle changes in microstructural features of primary bone, including the size and density of primary osteons, polarization contrast of lamellar-zonal tissues, and the occurrence of growth marks. The individual depicted in Figure 5-38 showed a distinctly repetitive microstructural pattern in the anteromedial and medial cortex; growth layers comprised of rapidly-growing FLC-LZPO and richly-vascularized LAM tissues were interrupted by narrower layers of less vascular LAM tissue and resting lines.

Patterns in the distribution of tissue types and vascular pattern of primary bone.

Alternating patterns in the distribution of primary bone tissue types, or in more subtle details of the vascular network of primary bone, were observed in select individuals of all taxa examined here. Similar observations have been reported by previous investigators. For instance, Ricqlès (1983) observed cyclical changes in the microstructure of primary fibro-lamellar tissue in the long bones of a sauropod dinosaur. During periods of apparently slow overall growth, temporary slowing of depositional rates was evidenced by a thin layer of lamellar bone deposition followed by a resting line. During periods of more rapid overall growth, however, temporary slowing of bone deposition was evidenced by reduced vascularization of fibro-lamellar tissue, without the formation of lamellar-zonal bone or resting lines. Such growth cycles were not evident in the most rapidly growing cortical regions. Horner and colleagues (2000) reported the existence of subtle patterns in laminar fibro-lamellar tissues in long bones of a hadrosaurid dinosaur, reflected in changes in such features as the size and density of primary osteons in successive vascular layers.

Such minor variations in adjacent bone vascular layers (e.g., in laminae of fibro-lamellar bone), and potentially in the widths of bone layers in lamellar-zonal tissues, are suggested to indicate subtle variations in bone depositional rates (Ricqlès et al., 1991). These patterns do not necessarily imply cyclical changes in bone depositional rates owing to endogenous or environmental rhythms, but they may reflect temporal variations in bone growth rates over the duration of an individual's growth period (Horner et al., 2000; Ricqlès et al., 1991; but see Sander and Tückmantel, 2003). However, for the most part,

this remains to be determined experimentally. Bromage and colleagues (Bromage et al., 2002; Bromage et al., 1998) demonstrated that in laboratory rats and marine iguanas, there is a certain periodicity to the formation of bone layers in lamellar bone, and that the widths of individual lamellae vary in relation to bone depositional rates. These features warrant further study in other animals.

Variations in the vascular pattern of bone were most evident in rapidly-growing cortical regions in *C. aethiops* and in *P. troglodytes* examined here. They were particularly pronounced in *P. troglodytes* individuals, as summarized above, and this may reflect in part their longer growth durations and faster growth velocities (e.g., Grether and Yerkes, 1940; Hamada and Udono, 2002; Leigh and Shea, 1995, 1996). In slower-growing taxa, *C. aethiops* and *H. lar*, the primary periosteal cortex was largely comprised of lamellar-zonal tissues, where temporary or short-term variations in bone depositional rates were more commonly suggested by the formation of growth marks (i.e., annuli and resting lines) or, in the latter taxon, changes in the collagen fiber orientation of bone. These features are briefly discussed below.

Growth marks: annuli and resting lines. Growth marks were observed in individuals of all taxa examined here. They were variably expressed as thin lamellae, appearing translucent in ordinary LM and sometimes bright in CPL (corresponding to 'annuli', as described by Castanet et al., 1993), or as resting cement lines (also commonly referred to as 'lines of arrested growth'), distinguished by their contrast in ordinary LM and by their bright appearance in CPL. They varied in their number and strength among individuals within taxa, and possibly in relation to age.

Annuli and resting lines have been studied in the cortical bone tissues (particularly in the mandible and long bones) of many vertebrate groups, including mammals, where they are interpreted to represent temporary slow-downs (in the case of annuli) or complete cessations (in the case of resting lines) in bone deposition. Their significance for discriminating life history characteristics has been discussed in several recent reviews (Castanet et al., 1993; Chinsamy-Turan, 2005; Erickson, 2005; for a review of mammal studies, see Klevezal, 1996; Ricqlès et al., 1991). The formation of annuli and resting lines is usually attributed to endogenous growth rhythms, influenced by environmental cues (also see Peabody, 1961), and their annual occurrence in many temperate-dwelling organisms has been established through the implementation of fluorochrome vital labeling and known-aged reference samples. In cases where the periodicity of growth marks is known or can be inferred based on their distribution in modern taxa, they can be used to assess such life history characteristics as age and/or season at death, timing of sexual maturity, growth rates, and growth duration (e.g., also see Castanet et al., 2004; Erickson et al., 2004; Francillon-Vieillot et al., 1990a; Klevezal and Fedyk, 1978).

Annuli and/or resting lines have been noted to occur in primate bone (Enlow, 1963; Klevezal, 1996), although they have received little attention in systematic studies, with a couple of exceptions. In captive grey mouse lemurs (*Microcebus murinus*), the number of growth marks expressed in the tibial diaphysis was shown to be correlated with the number of seasonal photoperiodic cycles experienced, in animals living under both normal control and accelerated photoperiod regimens (Castanet et al., 2004). In an examination of the midshaft femur in an archaeological human population, resting lines

were observed in all individuals older than 4 years of age (Herrmann and Danielmeyer, 1994). The authors also noted a tendency for individuals to exhibit twice as many resting lines as chronological age in years.

In the current study, annuli and resting lines were observed in several DECID individuals and all older individuals in *C. aethiops*, in many individuals of the M1 and older age classes in *H. lar*, and in all individuals of the M1 and older age classes of *P. troglodytes*. In some individuals, there were observable similarities in the number and distribution of growth marks across the width of periosteal cortex at the midshaft femur and humerus (e.g., see Figures 5-11 c and e, and Figures 5-14 c and d). However, their occurrence at the latter skeletal site was affected by cortical drift (in *C. aethiops*) and increased secondary intracortical remodeling compared to the midshaft femur, resulting in local destruction of growth marks formed during earlier periods in ontogeny. Nevertheless, despite increased secondary remodeling in *H. lar* and *P. troglodytes*, broad expanses of primary bone remained *in tact* in the mid- and outer periosteal cortex where growth marks could be discerned. Studies of other vertebrate groups demonstrate the significance of these structures for discriminating organismal growth patterns, and suggest that future investigations in primates hold great promise.

Preferred collagen fiber orientation. At the midshaft femur and humerus of *H. lar*, qualitative observations of individuals in the youngest age classes suggest a tendency for primary lamellar-zonal tissues (particularly PF and PF-LAM) in periosteal cortex to appear dark in CPL relative to surrounding tissues, suggesting a predominantly longitudinal collagen fiber orientation. During later stages of ontogeny, as the primary

cortex is comprised predominantly of lamellar bone, repetitive variations are often observed in the polarization contrast of the collagen fiber matrix. Across the thickness of primary periosteal cortex, regions of dark lamellae alternate with regions of brighter lamellae, indicating apparent changes in the preferred collagen fiber alignment of the matrix between a predominantly longitudinal (appearing dark) to a predominantly transverse (appearing bright) orientation. A similar pattern was reported recently for the alligator femur by Lee (2004).

The preferred orientation of collagen fibers in bone is primarily understood in relation to its significance for the ability of bone to withstand particular modes of loading-induced strains. A number of experimental studies (e.g., Ascenzi and Bonucci, 1967; Ascenzi and Bonucci, 1968, 1972; Ascenzi et al., 1973; Simkin and Robin, 1974; Vincentelli and Evans, 1971) have confirmed that lamellae having a predominantly transverse collagen fiber orientation confer the greatest strength under compressive loading, while lamellae having longitudinally-oriented collagen fibers confer the greatest strength under tensile loading. Bone regions characterized by an “alternate” arrangement of collagen fibers between successive lamellae are best able to withstand torsional loading. Further to this, researchers have demonstrated that the preferred collagen fiber orientation of bone is distributed non-randomly within cross-sections of limb elements (e.g., Boyde and Riggs, 1990; Carando et al., 1989; Portigliatti Barbos et al., 1983; Portigliatti Barbos et al., 1984; Riggs et al., 1993) and the circumorbital region (Bromage, 1992) of a number of mammals, in general accordance with local mechanical loading history.

Much of this research to date has focused on secondarily (Haversian) remodeled

bone, and the significance of preferred collagen fiber orientation in primary bone tissues is less clear (Bromage, 1992; Bromage and Boyde, n.d.; Martin and Ishida, 1989; Mason et al., 1995; McMahon et al., 1995; Riggs et al., 1993; Simkin and Robin, 1974; Skedros and Hunt, 2004; Skedros et al., 2003). Some researchers have reported correspondence between the distribution of preferred collagen fiber orientation in primary bone and the mechanical loading environment (Lee, 2004 reported weak support for such a relationship; Mason et al., 1995; Simkin and Robin, 1974; Skedros et al., 2003). However, Riggs and colleagues (1993) noted that the lamellar or parallel-fibered component of fibro-lamellar bone tissues in the radius of young horses is characterized by a predominantly longitudinal collagen orientation irrespective of strain history, leading to the suggestion that the distribution of preferred collagen fiber orientation in primary bone is determined, at least in part, by patterns of microstructural development.

Others have also noticed a predominance of longitudinally-oriented collagen in primary bone (but Goldman et al., 2003 found the opposite pattern), and have suggested that bone growth dynamics plays a role in the establishment of collagen fiber orientation (Bromage and Boyde, n.d.; Lee, 2004; McMahon et al., 1995). Although observations at the mandibular ramus were in accordance with previously-reported *in vivo* strain data, Bromage and Boyde (n.d.) reported that the mandibular corpus of long-tailed macaques (*Macaca fascicularis*) showed a relatively uniform distribution of preferred collagen fiber orientation, despite its variable loading regime. Tissues of primary periosteal origin in this bone region were characterized by a predominantly longitudinal collagen fiber orientation, while tissues of endosteal origin were predominantly transverse. These observations led them to suggest that preferred collagen fiber orientation in some bone

regions may reflect a compromise between: 1) a strain-mediated adaptive response of osteoblasts, and 2) developmental constraints associated with circumstances of bone growth remodeling, including the particular tissue type within which collagen fiber orientation is established during bone growth.

In this context, qualitative observations of the current study may be interpreted in several ways. First, the presence of regions of lamellae appearing relatively dark in CPL, indicating a predominantly longitudinal collagen fiber orientation, may reflect the importance of tensile loading on the alignment of collagen at the periosteal surface of the midshaft femur and humerus of *H. lar* during growth. While mechanical strain distributions at the midshaft femur have not been characterized, *in vivo* strain analysis of the midshaft humerus of gibbons during brachiation demonstrated a predominantly tensile loading regime, combined with torsion and dorso-ventral bending (Swartz et al., 1989). Gibbons practice other positional behaviors, such as vertical climbing, leaping and bipedalism (Fleagle, 1980), which may impact strain distributions at the midshaft femur and humerus in other ways. In elements experiencing complicated and variable loading regimes, tensile strain particularly is suggested to be an important determinant of collagen fiber orientation (Bromage and Boyde, n.d.; McMahon et al., 1995). However, this explanation would not account for the discontinuous distribution of lamellar regions having a relatively bright appearance in CPL, indicating a predominantly oblique – transverse collagen fiber orientation.

Secondly, variability in the preferred orientation of collagen fibers in primary lamellar-zonal bone may reflect an influence of bone depositional rate on the alignment of osteoblasts at the forming surface, and thus on the establishment of collagen fiber

orientation in underlying tissues. Osteoblasts become incorporated into the forming bone matrix situated with their long axes parallel to the underlying collagen fibers (Jones and Boyde, 1976). If the more randomly-organized collagen fiber matrix of woven bone is associated with faster rates of deposition compared to the highly-ordered collagen matrix of lamellar bone (as reviewed above), it follows that variability in the preferred orientation of collagen fibers in lamellar bone may reflect more subtle changes in bone depositional rates during times of relatively slow overall growth. A similar explanation was proposed by Lee (2004) to account for apparently cyclical alterations in preferred collagen fiber orientation at the midshaft femur of alligators.

A third explanation is that preferred collagen fiber orientation in primary tissues deposited during growth may reflect a compromise between these two factors, as proposed by Bromage and Boyde (n.d.). While the significance of preferred collagen fiber orientation for mechanical properties of bone is better understood, the influence of factors associated with local growth dynamics of bone (e.g., tissue type and depositional rate) in the determination of collagen fiber orientation is largely unknown. Clearly, this is an area that warrants further study.

SUMMARY

The current study aimed to 1) provide a baseline understanding of ontogenetic, regional and taxonomic variability in patterns of primary bone microstructural organization, and 2) consider the potential of this variability (particularly as reflected in primary bone tissue type proportions) for discriminating primate growth patterns. Quantitative results demonstrated that proportions of primary bone tissue types varied

significantly across age classes within all taxa examined. Younger age classes were characterized by higher proportions of tissues associated with faster depositional rates (namely, fibro-lamellar tissue types and/or parallel-fibered bone), while older age classes were characterized by higher proportions of tissues associated with slow depositional rates (i.e., lamellar bone). Interestingly, taxa differed in the timing of this transition, and in the particular tissue types deposited in individuals of similar biological age (e.g., dental emergence status).

When interpreted within the context of local growth and secondary remodeling history, age-related trends in primary tissue type proportions within and among taxa revealed several broad consistencies with expectations based on growth patterns reported previously for other body dimensions. In *C. aethiops* and *H. lar*, the transition to periosteal deposition of predominantly lamellar bone was coincident with body mass growth rates of 1 kg / year or less, as reported for captive animals by previous investigators. Slightly increased proportions of bone tissue types having a parallel-fibered matrix component in the M1 and, to a lesser extent, M2 age classes in gibbons was in general agreement with reports that body mass growth velocities are slightly faster than observed in vervets during the first three years of life. However, observations of *P. troglodytes* were less easily reconciled with their considerably faster body mass growth velocities than other taxa examined here. While the M1 and M2 age classes exhibited higher proportions of fibro-lamellar tissues than observed in individuals of similar biological age in other taxa, the DECID age class of *C. aethiops* exhibited substantially higher proportions of fibro-lamellar tissue types compared to *P. troglodytes*. Several possible explanations were proposed to account for this inconsistency.

Qualitative observations suggest several patterns warranting further investigation. For instance, while sexually-dimorphic growth patterns or the occurrence of a subadult growth spurt were not reflected in quantitative variability in primary bone tissue type proportions in *C. aethiops* and *P. troglodytes*, such variability in bone growth rates may be discerned from subtle changes in microstructural features not measured here, such as vascular canal density and orientation. Visual assessment also revealed variation in other features of primary bone warranting further quantitative study, and which may have significance for interpreting individual variability in bone growth patterns, including features of the vascular network, collagen fiber orientation, and the occurrence of growth marks (namely, annuli and resting lines).

TABLE 5-1 a. Examples of early typological classification schemes for cortical bone tissues: Quekett, 1855*

<i>Haversian systems</i>	] Cortical bone tissues were classified as either "Haversian" or non-"Haversian", based on the presence of vascular canals surrounded by concentric layers of bone. No distinction was made between primary vascular bone and secondary osteonal bone.
<i>Laminae</i> <i>Circumferential</i> <i>Interstitial</i>	

* Cortical bone tissues were classified into several distinct tissue types, recognized on the basis of their vascularity and distribution. Other details of lacunar and canalicular structure were also described.

TABLE 5-1 b. Examples of early typological classification schemes for cortical bone tissues: Foote, 1916*

<i>Undifferentiated bone</i>	] Undifferentiated bone is similar to "woven bone" of current classifications, described in the text.
<i>Differentiated bone</i>	
<i>Lamellae</i> Uniformly lamellated Two-fold division Three-fold division	] "Lamellar" bone appears to correspond to parallel-fibered and lamellar bone of recent classifications, as described in the text. This tissue type was further classified on the basis of its distribution within the cortex - comprising the entire breadth of the cortex, or divided into two or three distinct (internal, middle, external) layers.
<i>Laminae</i>	] Consistent with the laminar bone of recent classification schemes, described in the text.
<i>Haversian systems</i> Ia stage Ib stage Ic stage C stage	] "Haversian" systems included vascular canals surrounded by concentric layers of bone. Four stages of differentiation of Haversian systems were recognized. No distinction was made between primary vascular bone and secondary osteonal bone, as described in the text.

* Cortical bone tissues were classified into several distinct tissue types, recognized on the basis of their distributions, vascularity, and stage of differentiation.

TABLE 5-2. Recent typological classification schemes for cortical bone tissues. A variety of bone tissue patterns are recognized by these authors on the basis of developmental origin, collagen fiber matrix organization, and/or vascularization. Listed here is a summary of common tissue types observed in vertebrate long bones, and which are most relevant for the current study. Secondary osteonal (i.e., Haversian) bone is examined in Chapter 6 of this thesis, and is not considered here.

TABLE 5-2 a. Enlow, 1962, 1963, 1966, 1982; Enlow and Hans, 1996.

<i>Primary vascular bone</i> *	}	Based on presence / absence of vascular canals
<i>Non-vascular bone</i>		
<i>Lamellar bone</i>	}	Based on collagen fiber matrix
<i>Parallel-fibered bone</i>		
<i>Non-lamellar bone</i> (i.e., woven)		
<i>Fine-cancellous bone</i> , of endochondral or periosteal origin **	}	Based on developmental origin and collagen fiber matrix
<i>Compacted fine-cancellous bone</i> , of endosteal or periosteal origin ***		
<i>Inner circumferential lamellar bone</i> , of endosteal origin		
<i>Outer circumferential lamellar bone</i> , of periosteal origin		
<i>Compacted coarse cancellous bone</i> , of endosteal origin		

* May contain simple vascular canals or primary osteons. A number of vascular subtypes (e.g., plexiform) were also recognized by Enlow and Brown (1956).

** Typically comprised of non-lamellar bone matrix.

*** Primary osteons are frequently formed during the compaction of this tissue.

Table 5-2 b. Francillon-Vieillot et al., 1990

Vascular or avascular bone, depending on the presence or absence of primary vascular canals

<i>Woven bone</i> , usually of periosteal origin.*		<i>Fibro-lamellar complex</i> where associated with a lamellar matrix and primary osteons.
<i>Parallel-fibered bone</i> , of endosteal or periosteal origin.*		Categories of <i>lamellar-zonal bone</i> . May be avascular or vascular, with less densely-distributed simple vascular canals and / or primary osteons.
<i>Lamellar bone</i> , of endosteal or periosteal origin.*		
Categories of <i>Fibro-lamellar complex</i> : <i>Fibro-lamellar bone with longitudinal primary osteons</i> <i>Laminar bone</i> <i>Plexiform bone</i> <i>Reticular bone</i> <i>Radiating fibro-lamellar bone</i>		Recognized on the basis of vascular canal orientation.

* May contain simple primary vascular canals or primary osteons, and a number of vascular subtypes may be recognized as explained in the text.

TABLE 5-3 a. Primary bone microstructure in nonhuman primate limb diaphyses: examples of observations of catarrhine primates from early comparative studies.
Where classifications depart from current terminology, tissue types are presented in quotes.

Taxon *	Notes of tissue types observed
Quekett, 1855	
(Each taxon represented by a single specimen, unless noted.)	
<i>Macaca mulatta</i> ("Macacus rhesus", short-tailed monkey) humerus; <i>Semnopithecus entellus</i> (<i>entellus</i> monkey) femur	1) Non-Haversian internal and external circumferential "laminae". 2) "Haversian canals" variably present.
<i>Pongo</i> sp. ("Pithecus satyrus", orangutan) radius and femur; <i>Pan troglodytes</i> ("Troglodytes niger", chimpanzee) radius and tibia	1) Numerous "Haversian systems". 2) Non-Haversian circumferential "laminae"
Foote, 1916	
(Each taxon is represented by the femur of a single individual, unless noted.)	
<i>Colobus guereza</i> ("Colobus abyssinicus caudatus")	1) Largely comprised of external circumferential "lamellae", with "Haversian systems of the Ia and Ib stages of differentiation". 2) A few "Haversian systems of the C stage of differentiation" present, just external to a layer of internal circumferential "lamellae". 3) Internal circumferential "lamellae" present.
<i>Cercopithecus albogularis kolbi</i> ? ("Lasiopyga kolbi", African monkey)	Comprised of circumferential "lamellae", with "Haversian systems of the Ia and Ib stages of differentiation" around the medullary canal.
<i>Papio</i> sp. ("Cynocephalus", baboon)	1) Largely comprised of external circumferential "lamellae" with "Haversian systems of the Ia and Ib stages of differentiation". 2) Internal circumferential and cancellous "lamellae" present.
<i>Mandrillus</i> sp. ("Cynocephalus maimon", mandrill)	1) Largely comprised of external circumferential "lamellae" with "Haversian systems of the Ia and Ib stages of differentiation". 2) Internal cancellous bone present.
<i>Pongo</i> sp. ("Simia satyrus", orangutan)	1) External layer of circumferential "lamellae" of variable thickness, with "Haversian systems of the Ia stage of differentiation". 2) "Haversian systems of the C stage of differentiation" and inter-Haversian "lamellae" present in the middle cortex. 3) Internal layer of circumferential "lamellae" of variable thickness present.
<i>Gorilla</i> sp. (gorilla)	1) Thick external layer of circumferential "lamellae", with "Haversian systems of the Ia stage of differentiation" and vascular canals having various orientations. 2) "Haversian systems of the C stage of differentiation" and inter-Haversian "lamellae" comprise a middle cortical region, and extend externally in the posterior cortex. 3) Thin internal layer of circumferential "lamellae" present.

TABLE 5-3 a continued.

Taxon *	Notes of tissue types observed
<i>Presbytis rubicunda</i> (monkey)	1) Thick external layer of circumferential "lamellae" present, with "Haversian canals of the Ia stage of differentiation". 2) Some "Haversian systems of the C stage of differentiation" present in a middle cortical region, and extend externally in the posterior cortex. 3) Thin layer of internal circumferential "lamellae" present.
<i>Hylobates</i> sp. (gibbon)	1) Thick external layer of circumferential "lamellae" present, with "Haversian systems of the Ia stage of differentiation". 2) "Haversian systems of the C stage of differentiation" comprise a middle cortical region, and extend externally in the posterior cortex. 3) Thin internal layer of circumferential "lamellae" present.
<i>Pan troglodytes</i> ("Anthropopithecus troglodytes", chimpanzee)	1) Thick external layer of circumferential "lamellae" present, with "Haversian systems of the Ia stage of differentiation". 2) "Haversian systems of C stage of differentiation" comprise a middle region of variable thickness. 3) Thin internal layer of circumferential "lamellae" present.
<i>Macaca mulatta</i> ("Macacus rhesus", Indian monkey)	1) Largely comprised of circumferential "lamellae", with "Haversian systems of the Ib stage of differentiation". 2) "Haversian systems of the C stage of differentiation" present internally.
<i>Chlorocebus</i> sp. or <i>Cercopithecus</i> sp. ("Lasiopyga centralis johnstoni", African monkey)	1) Thick external layer of circumferential "lamellae" present, with "Haversian systems of the Ia stage of differentiation". 2) "Haversian systems of the C stage of differentiation" present in a middle cortical region. 3) Thin internal layer of circumferential "lamellae" present.
<i>Chlorocebus</i> sp. or <i>Cercopithecus</i> sp. ("Lasiopyga" sp., African monkey)	1) Thick external layer of circumferential "lamellae" present, with "Haversian systems of the Ia stage of differentiation" and vascular canals of various orientations. 2) "Haversian systems of the C stage of differentiation" present in a middle cortical region. 3) Thin internal layer of circumferential "lamellae" present.

Amprino & Godina, 1947

(Various long bones were studied.)

Macaca sinica ? ("Pithecus sinicus"); *M. mulatta* ("P. rhesus"); *M. nemestrina* ("P. nemestrinus"); *M. sylvanus* ("Innus ecaudatus"); *Chlorocebus sabaeus* ("Cercopithecus sabaeus"); "C. sp."; *Chlorocebus aethiops* ? ("Cercopithecus aethiops"); *Papio hamadryas*; *P. cynocephalus*; *Mandrillus sphinx* ("P. sphinx"); "Colobus ferrugineus"; *Hylobates* (*Symphalangus*) *syndactylus* ("Symphalangus syndactylus")

1) External circumferential lamellae present, with predominantly longitudinal vascular canals and primary osteons. 2) Internal circumferential lamellae variably present. 3) Secondary osteons variably present, depending upon element and species.

TABLE 5-3 a continued.

Taxon *	Notes of tissue types observed
<i>Pongo</i> sp. ("Simia satyrus"), <i>Pan troglodytes</i> ("Antropopithecus troglodytes"), <i>Gorilla gorilla</i>	1) External circumferential lamellae present, with longitudinal vascular canals and primary osteons. (Primary osteons are most prominent in younger, smaller individuals.) Woven primary bone of young individuals transitions to a more parallel – fibered and then lamellar organization in older individuals. 2) Secondary osteons variably present, depending upon age, element and species.
Enlow & Brown, 1958	
(Various bone elements were studied.)	
"modern primates"	1) Primary vascular tissue: typically comprised of lamellar bone with longitudinal vascular canals (ranging from relatively avascular to densely vascularized). 2) Evidence of secondary (Haversian) remodeling is variable, being rare in some taxa and dense in others.
Singh et al, 1974	
(Each taxon represented by a single individual. Rib, tibia and femur were studied.)	
<i>Hylobates lar</i> (female white- handed gibbon, 2 yrs. old); <i>Theropithecus gelada</i> (newborn gelada baboon)	1) Primary vascular bone, with predominantly longitudinal (also radial) vascularization; 2) periosteal lamellar bone; 3) endosteal lamellar and cancellous bone; and 4) rare secondary osteons.
<i>Cercopithecus nictitans</i> (male spot-nosed monkey, adolescent)	1) Primary vascular bone, with predominantly longitudinal and some radial vascularization. Primary osteons were also observed. 2) Secondary osteons?
<i>Macaca mulatta</i> (female rhesus monkey, 4 yrs. old); <i>M. silenus</i> ? (female Wanderoo monkey, adolescent)	1) Primary "non-Haversian" bone. 2) Secondary Haversian bone.

*Taxon designations follow Groves, 2001. Common names as reported in the original text are provided in parentheses. Taxon designations are also provided in quotations where they depart from Groves, 2001.

TABLE 5-3 b. Primary bone microstructure in nonhuman primate limb diaphyses: summary of observations from recent systematic investigations.

Taxon *	Tissue types observed
Enlow, 1962 (Semi-serial sections of the femur, tibia, radius, humerus, and mandible were studied in ontogenetic series.) <i>Macaca mulatta</i> (Rhesus monkeys, and select other vertebrates)	1) Newborn cortex largely comprised of fine cancellous bone. Compacted fine cancellous bone observed postnatally largely results from periosteal deposition, and is frequently vascularized by primary osteons. 2) Outer circumferential lamellar bone also observed, particularly in later growth stages. 3) Inner circumferential lamellar bone and compacted coarse cancellous bone present. The latter is common. 4) Secondary Haversian bone present. 5) Tissue types show a predictable regional distribution within long bones, and this distribution can be understood on the basis of local growth remodeling patterns.
Newell-Morris & Sirianni, 1982 (Vital fluorochrome labeling study of the midshaft humerus in captive animals.) <i>Macaca nemestrina</i> (pig-tailed macaque, prenatal and first year postnatal ontogenetic series; n=15)	1) Periosteal deposition of woven bone with primary osteons during fetal life and the first 6 postnatal weeks. 2) Periosteal deposition of parallel-fibered bone with primary osteons from 6-28 postnatal weeks. 3) Periosteal deposition of lamellar bone with less abundant primary osteons from 55-61 postnatal weeks. 4) Endosteal deposition of lamellar bone in postnatal animals. 5) Secondary osteons observed in postnatal animals.
Castanet et al. 2004 (Vital fluorochrome labeling study of limb diaphyses and the mandible, with experimental manipulation of photoperiod length. Primary bone microstructure and lines of arrested growth were examined.) <i>Microcebus murinus</i> (grey mouse lemur, ontogenetic series aged 0.2 - 11.1 yrs.)	A transition from vascular parallel-fibered bone to avascular parallel-fibered bone was observed to occur by the end of the first growing season. (The latter was also characterized by the formation of lines of arrested growth.)

TABLE 5-4. Summary of relevant life history characteristics of the study taxa.

Adult female body mass (kg)	Adult male body mass (kg)	Neonatal body mass (g) ¹	Maximum neonatal body mass growth velocity ²	Sexual dimorphism in body mass growth ²	Age at adult female body mass: captivity ² (wild)	Age at adult male body mass: captivity ² (wild)	Age at long bone epiphyseal union (wild)	Age at complete dentition (wild)
<i>C. aethiops</i>³								
5.6	7	f: 335.9 m: 334.9	~ 1 kg/yr.	Bimaturism; slight subadult growth spurt, most pronounced in males (~ 1 kg/yr.).	4 yrs. (~ 4 yrs.)	5.5 yrs. (~ 6 yrs.)	> 38 mos.	38-67 mos.
<i>H. lar</i>⁴								
5.3	5.9	f: 407 m: 400	< 1.5 kg/yr.	Limited bimaturism and rate differences. Both sexes grow at a declining rate from birth.	5.75 yrs. (6 - 8 yrs.)	5.75 yrs. (6 - 8 yrs.)		6.1 yrs.
<i>P. troglodytes</i>⁵								
45.8	59.7	f: 1,814 m: 1,877	~ 3 kg/yr.	Minimal bimaturism. Males exhibit a pronounced subadult growth spurt (< 8 kg/yr.).	11.5-12 yrs. (12 yrs. +)	12-13 yrs. (15 yrs. +)	~ 15 yrs. (13.4-16.5 yrs.)	11.36 yrs. (12.4 yrs.)

¹ Source for neonatal body mass data summarized here: (Smith and Leigh, 1998).² While there are some reports based on wild individuals (e.g., Bolter and Zihlman, 2003; Pusey, 1978; Schultz, 1944; Turner et al., 1997), postnatal growth patterns have generally been best characterized for captive animals of known chronological age. Body mass growth data summarized here are from studies of captive animals by Leigh and colleagues (Leigh, 1992, 1996; Leigh and Shea, 1995, 1996).³ Additional sources for *C. aethiops* data summarized here: adult body mass (Fedigan & Fedigan, 1988); age at adult body mass in wild animals (Horrocks, 1986; Fedigan & Fedigan, 1988); age at epiphyseal union and complete dentition (Bolter and Zihlman, 2003).⁴ Additional sources for *H. lar* data summarized here: adult body mass (Carpenter, 1940); age at adult body size in wild animals (Brockelman et al., 1998; Leighton, 1987); age at M3 emergence (Dirks, 2003).⁵ Additional sources for *P. troglodytes* data summarized here: adult body mass (Smith and Jungers, 1997; based on *P. t. troglodytes*); age at adult body size in wild animals (Goodall, 1986; Pusey, 1978); age at epiphyseal union (Kerley, 1966; Zihlman et al. 2004); age at complete dentition (based on maxillary M3 emergence, Smith et al., 1994; Zihlman et al. 2004).

TABLE 5-5. Biological age classes.

Age Class	Alveolar emergence/epiphyseal union criteria
DECID.	alveolar emergence of one or more deciduous teeth
M1	alveolar emergence of one or more permanent 1st molars
M2	alveolar emergence of one or more permanent 2nd molars
M3	alveolar emergence of one or more permanent 3rd molars
EIPPH.	fully erupted permanent dentition, long bone epiphyseal union complete

TABLE 5-6. Sample representation across biological age classes for analysis of primary bone tissue organization (femur/humerus).

Sex	Total N	DECID	M1	M2	M3	EIPPH	Immature (unknown)¹
<i>Chlorocebus aethiops</i>							
F	14/13	1/1	4/5 ²	2/1	4/3	3/3	0/0
M	16/17	4/4	1/1	4/5	3/3	3/3	1/1 ³
<i>Hylobates lar</i>							
F	13/11	2/2	2/2	2/1	2/1	3/3	2/2 ⁴
M	15/14	2/2	1/1	4/4	5/4	3/3	0/0
<i>Pan troglodytes</i>							
F	5	1	0	1	3	0	0
M	2	0	0	1	0	1	0
unk.	5	1	1	2 ⁵	0	1	0

¹ Includes individuals lacking associated dental emergence data.

² SLW 21 Humerus – Orientation lost during processing. This specimen was excluded from analyses of variation among radial sectors.

³ SLW 101 – Assigned to M3 age class for analysis, based on bone length and epiphyseal union.

⁴ SLW 10 & 35 – Assigned to M3 age class for analysis, based on bone length and epiphyseal union.

⁵ MNH 35526 femur – Questionable association with skull having only permanent M1s erupted and unerupted M2s; reassigned to M2 age class based on bone length.

**TABLE 5-7. Intra-observer repeatability in tissue type mapping.
C. aethiops humerus, SLW 27.**

Tissue Type	% of cortical area			Range
	1	2	3	
FLC	5.33	3.10	1.90	3.43
FLC-LZPO	36.15	35.56	39.86	4.30
PF	1.59	1.09	1.80	0.71
PF-LAM	7.89	6.05	6.15	1.84
LAM	22.07	25.66	23.45	3.59
SF	3.15	4.24	3.46	1.09
Total END	10.59	10.54	10.71	0.17
HAV	13.23	13.77	12.66	1.11

TABLE 5-8. Nonparametric Spearman correlation statistics for % area tissue types with age class and cortical area. Significant contrasts at $p \leq 0.05$ are bolded.

Variable	Age Class				Cortical Area			
	N	R	t(N-2)	p	N	R	t(N-2)	p
<i>C. aethiops</i>								
FEMUR								
%FLC	30	-0.6851	-4.9765	0.0000	30	-0.6568	-4.6087	0.0001
%FLC-LZPO	30	-0.8731	-9.477	0.0000	30	-0.7948	-6.9296	0.0000
%FLC + %FLC-LZPO	30	-0.8731	-9.477	0.0000	30	-0.7966	-6.9724	0.0000
%PF	30	-0.3951	-2.2759	0.0307	30	-0.4605	-2.7452	0.0104
%PF-LAM	30	-0.7734	-6.4548	0.0000	30	-0.8085	-7.2708	0.0000
%LAM	30	0.47857	2.88406	0.0075	30	0.46501	2.7794	0.0096
%EPF	30	-0.163	-0.8741	0.3895	30	-0.132	-0.7045	0.4869
%EPF-LAM	30	0.14406	0.77032	0.4476	30	0.08495	0.45114	0.6554
%ELAM	30	0.58021	3.76954	0.0008	30	0.65903	4.63653	0.0001
%ECCC	30	0.61404	4.1167	0.0003	30	0.61524	4.12967	0.0003
%HAV	30	0.61748	4.15385	0.0003	30	0.66674	4.73381	0.0001
HUMERUS								
%FLC	30	-0.4138	-2.4051	0.0230	30	-0.532	-3.3245	0.0025
%FLC-LZPO	30	-0.7751	-6.4903	0.0000	30	-0.62	-4.1816	0.0003
%FLC + %FLC-LZPO	30	-0.7364	-5.7605	0.0000	30	-0.6396	-4.4028	0.0001
%PF	30	-0.6526	-4.5575	0.0001	30	-0.703	-5.23	0.0000
%PF-LAM	30	-0.837	-8.0954	0.0000	30	-0.8118	-7.3561	0.0000
%LAM	30	0.72646	5.59367	0.0000	30	0.73704	5.77061	0.0000
%EPF	30	-0.5555	-3.5349	0.0014	30	-0.583	-3.7974	0.0007
%EPF-LAM	30	-0.4851	-2.9355	0.0066	30	-0.4928	-2.997	0.0057
%ELAM	30	0.54912	3.47672	0.0017	30	0.54685	3.45623	0.0018
%ECCC	30	0.33109	1.85671	0.0739	30	0.30278	1.68108	0.1039
%HAV	30	0.47757	2.87624	0.0076	30	0.42336	2.47274	0.0197
<i>H. lar</i>								
FEMUR								
%FLC	28	-0.2774	-1.4725	0.1529	28	-0.2169	-1.1329	0.2676
%FLC-LZPO	28	-0.8626	-8.6947	0.0000	28	-0.8309	-7.6157	0.0000
%FLC + %FLC-LZPO	28	-0.8646	-8.7744	0.0000	28	-0.8276	-7.5181	0.0000
%PF	28	-0.6786	-4.7109	0.0001	28	-0.6465	-4.3211	0.0002
%PF-LAM	28	-0.8852	-9.7033	0.0000	28	-0.8686	-8.9398	0.0000
%LAM	28	0.67229	4.6307	0.0001	28	0.61576	3.98485	0.0005
%EPF	28	-0.4343	-2.4588	0.0209	28	-0.4491	-2.5629	0.0165
%EPF-LAM	28	-0.7289	-5.4285	0.0000	28	-0.7243	-5.357	0.0000
%ELAM	28	-0.4526	-2.5881	0.0156	28	-0.5369	-3.2454	0.0032
%ECCC	28	-0.6536	-4.4034	0.0002	28	-0.5497	-3.3555	0.0024
%HAV	28	-0.0135	-0.0689	0.9456	28	0.07882	0.40315	0.6901
HUMERUS								
%FLC	25	-0.2889	-1.4475	0.1613	25	-0.2141	-1.0511	0.3041
%FLC-LZPO	25	-0.6495	-4.0967	0.0004	25	-0.622	-3.8095	0.0009
%FLC + %FLC-LZPO	25	-0.6585	-4.196	0.0003	25	-0.6315	-3.9061	0.0007
%PF	25	-0.85	-7.7392	0.0000	25	-0.838	-7.3657	0.0000

TABLE 5-8 continued. Nonparametric Spearman correlation statistics for % area tissue types with age class and cortical area. Significant contrasts at $p \leq 0.05$ are bolded.

Variable	Age Class				Cortical Area			
	N	R	t(N-2)	p	N	R	t(N-2)	p
%PF-LAM	25	-0.7088	-4.8187	0.0001	25	-0.7369	-5.2282	0.0000
%LAM	25	0.79354	6.25405	0.0000	25	0.79769	6.34359	0.0000
%EPF	25	-0.7439	-5.3376	0.0000	25	-0.6929	-4.6087	0.0001
%EPF-LAM	25	-0.8695	-8.4433	0.0000	25	-0.7746	-5.8731	0.0000
%ELAM	25	-0.1277	-0.6176	0.5429	25	-0.1615	-0.785	0.4405
%ECCC	25	-0.768	-5.7506	0.0000	25	-0.7601	-5.6096	0.0000
%HAV	25	0.18192	0.90632	0.3738	25	0.24718	1.24971	0.2235
<i>P. troglodytes</i>								
FEMUR								
%FLC	12	-0.4205	-1.4655	0.1735	12	-0.2849	-0.9399	0.3694
%FLC-LZPO	12	-0.7305	-3.3828	0.0070	12	-0.7063	-3.155	0.0102
%FLC + %FLC-LZPO	12	-0.7737	-3.8618	0.0032	12	-0.7413	-3.4923	0.0058
%PF	12	-0.6639	-2.8074	0.0186	12	-0.6397	-2.6318	0.0251
%PF-LAM	12	-0.5686	-2.1857	0.0537	12	-0.6713	-2.8643	0.0168
%LAM	12	0.59017	2.31183	0.0434	12	0.6993	3.09359	0.0114
%EPF	12	-0.6649	-2.8148	0.0183	12	-0.6834	-2.9601	0.0143
%EPF-LAM	12	-0.8637	-5.4183	0.0003	12	-0.8252	-4.6195	0.0010
%ELAM	12	0.30228	1.00282	0.3396	12	0.22378	0.72605	0.4845
%ECCC	12	-0.0549	-0.174	0.8653	12	0.08542	0.27112	0.7918
%HAV	12	0.0108	0.03414	0.9734	12	-0.1329	-0.4239	0.6806

TABLE 5-9. Kruskal-Wallis and Median tests: Whole section % total PER, total END, and HAV among AGE CLASSES in *C. aethiops*. Significant contrasts in bold ($p \leq 0.05$). Mann-Whitney post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Significant at the p<0.05 and p<0.10 levels after Bonferroni correction.								
% Tissue Type	df	total N	Kruskal-Wallis		Median test		Significant post-hocs (p'=0.005)	Significant post-hocs (p'=0.010)
			H	p	Chi-Square	p		
FEMUR								
Total PER	4	30	13.44634	0.0093	11.4667	0.0218	DE*M3	DE*EP
Total END	4	30	9.678497	0.0462	11.4667	0.0218		DE*M3
HAV	4	30	12.55054	0.0137	7.033333	0.1341	DE*M3	DE*M1, DE*EP
HUMERUS								
Total PER	4	30	12.5157	0.0139	13.90476	0.0076	DE*M3	
Total END	4	30	9.567312	0.0484	6.952381	0.1384		DE*M1
HAV	4	30	10.32670	0.0353	10.60952	0.0313		M2*EP

¹ Age class abbreviations: DECID (DE); M1 (M1); M2 (M2); M3 (M3); EPIPH (EP).

TABLE 5-10. Wilcoxon matched pairs tests: % HAV, total END, and total PER between ELEMENTS in *C. aethiops*. Significant contrasts in bold ($p \leq 0.05$).

Age class	% Tissue Type	Wilcoxon Matched Pairs			
		N	T	Z	p
Total sample	HAV	28	1.00	4.599828	0.00000
Total sample	Total END	28	45	3.5978849	0.00032
Total sample	Total PER	28	0	4.622599	0.00000
DECID	HAV	5	0	2.0225996	0.04312
DECID	Total END	5	0	2.0225996	0.04312
DECID	Total PER	5	0	2.0225996	0.04312
M1	HAV	5	1	1.7529196	0.07962
M1	Total END	5	0	2.0225996	0.04312
M1	Total PER	5	0	2.0225996	0.04312
M2	HAV	5	0	2.0225996	0.04312
M2	Total END	5	1	1.7529196	0.07962
M2	Total PER	5	0	2.0225996	0.04312
M3	HAV	7	0	2.3664319	0.01796
M3	Total END	7	0	2.3664319	0.01796
M3	Total PER	7	0	2.3664319	0.01796
EPIPH	HAV	6	0	2.2013982	0.02771
EPIPH	Total END	6	8	0.5241424	0.60018
EPIPH	Total PER	6	0	2.2013982	0.02771

TABLE 5-11. Mann-Whitney and Kolmogorov-Smirnov tests: % area of PER tissue types between SEXES in *C. aethiops* (M3 - EIPPH subsample). Contrasts were not statistically significant ($p \leq 0.05$).

% Tissue Type	Mann-Whitney							Kolmogorov-Smirnov						
	N _F	N _M	Rank Sum F	Rank Sum M	U	Z	p-level	Max Neg Diff	Max Pos Diff	p-level	Mean F	Mean M	Std. Dev. F	Std. Dev. M
FEMUR														
FLC	7	7	52.50	52.50	24.50	0.0000	1.00000	0.00	0.00	$p > .10$	0.00	0.00	0.000	0.000
FLC-LZPO	7	7	56.00	49.00	21.00	0.4472	0.65472	0.00	0.29	$p > .10$	0.26	0.09	0.367	0.141
FLC +FLC- LZPO	7	7	56.00	49.00	21.00	0.4472	0.65472	0.00	0.29	$p > .10$	0.26	0.09	0.367	0.141
PF	7	7	53.00	52.00	24.00	0.0639	0.94906	-0.14	0.14	$p > .10$	0.07	0.10	0.122	0.195
PF-LAM	7	7	54.00	51.00	23.00	0.1917	0.84801	-0.14	0.29	$p > .10$	0.54	0.88	0.606	1.416
LAM	7	7	41.00	64.00	13.00	-1.4694	0.14172	-0.43	0.00	$p > .10$	70.38	78.73	9.816	10.004
HUMERUS														
FLC	7	6	42.00	49.00	14.00	-1.0000	0.31731	-0.33	0.00	$p > .10$	0.00	0.49	0.000	1.102
FLC-LZPO	7	6	57.00	34.00	13.00	1.1429	0.25310	-0.19	0.52	$p > .10$	3.11	2.88	2.547	2.821
FLC +FLC- LZPO	7	6	51.00	40.00	19.00	0.2857	0.77510	-0.36	0.36	$p > .10$	3.11	3.37	2.547	2.611
PF	7	6	42.00	49.00	14.00	-1.0000	0.31731	-0.33	0.00	$p > .10$	0.00	0.06	0.000	0.117
PF-LAM	7	6	43.00	48.00	15.00	-0.8571	0.39137	-0.43	0.29	$p > .10$	0.97	1.16	0.943	0.588
LAM	7	6	57.00	34.00	13.00	1.1429	0.25310	-0.17	0.50	$p > .10$	56.65	53.68	3.026	6.892

TABLE 5-12. Kruskal-Wallis and Median tests: % area of PER tissue types among AGE CLASSES in *C. aethiops*. Significant contrasts in bold ($p \leq 0.05$). Mann-Whitney post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Bonferroni correction:								
% Tissue Type	df	total N	Kruskal-Wallis		Median test		Significant post-hocs (p'=0.005)	Significant post-hocs (p'=0.010)
			H	p	Chi-Square	p		
FEMUR								
FLC	4	30	24.10105	0.0001	25.0000	0.0001	DE*M3	DE*M2, DE*EP
FLC-LZPO	4	30	23.46386	0.0001	17.1667	0.0018	DE*M3, M1*M3	DE*M1, DE*M2, DE*EP, M1*EP
FLC +FLC- LZPO	4	30	23.46386	0.0001	17.1667	0.0018	DE*M3, M1*M3	DE*M1, DE*M2, DE*EP, M1*EP
PF	4	30	7.829493	0.098	5.2232	0.2652		
PF-LAM	4	30	18.80472	0.0009	17.1667	0.0018	DE*M3, M1*M3	DE*EP
LAM	4	30	13.25574	0.0101	7.0333	0.1341		DE*M1, DE*M3, DE*EP
HUMERUS								
FLC	4	30	23.31658	0.0001	22.54658	0.0002	DE*M3	DE*M1, DE*M2
FLC-LZPO	4	30	18.17469	0.0011	12.28571	0.0153	DE*M3	DE*M2, DE*EP
FLC +FLC- LZPO	4	30	17.16111	0.0018	12.28571	0.0153	DE*M3	DE*M2, DE*EP
PF	4	30	16.79377	0.0021	13.25893	0.0101	DE*M3	DE*EP
PF-LAM	4	30	20.50507	0.0004	14.95238	0.0048	DE*M3	DE*M2, DE*EP, M1*M3, M1*EP
LAM	4	30	20.53425	0.0004	17.61905	0.0015	DE*M3, M1*EP	DE*M2, DE*EP, M1*M2, M1*M3

¹ Age class abbreviations: DECID (DE); M1 (M1); M2 (M2); M3 (M3); EPIPH (EP).

TABLE 5-13. Wilcoxon matched pairs tests: % area of PER tissue types between ELEMENTS in *C. aethiops*. Significant contrasts in bold ($p \leq 0.05$).

		Wilcoxon Matched Pairs			
Age class	% Tissue Type	N	T	Z	p
Total sample	FLC	28	13	0.70014	0.48384
Total sample	FLC-LZPO	28	48	3.52957	0.00042
Total sample	FLC + FLC-LZPO	28	56	3.34740	0.00082
Total sample	PF	28	80	0.60363	0.54609
Total sample	PF-LAM	28	173	0.68314	0.49452
Total sample	LAM	28	12	4.34934	0.00001
DECID	FLC	5	6	0.40452	0.68583
DECID	FLC-LZPO	5	7	0.13484	0.89274
DECID	FLC + FLC-LZPO	5	7	0.13484	0.89274
DECID	PF	5	2	1.48324	0.13801
DECID	PF-LAM	5	2	1.48324	0.13801
DECID	LAM	5	6	0.40452	0.68583
M1	FLC	5	0		
M1	FLC-LZPO	5	1	1.75292	0.07962
M1	FLC + FLC-LZPO	5	2	1.48324	0.13801
M1	PF	5	6	0.40452	0.68583
M1	PF-LAM	5	5	0.67420	0.50018
M1	LAM	5	0	2.02260	0.04312
M2	FLC	5	0		
M2	FLC-LZPO	5	0	2.02260	0.04312
M2	FLC + FLC-LZPO	5	0	2.02260	0.04312
M2	PF	5	3	0.00000	1.00000
M2	PF-LAM	5	5	0.67420	0.50018
M2	LAM	5	0	2.02260	0.04312
M3	FLC	7	0		
M3	FLC-LZPO	7	0	2.36643	0.01796
M3	FLC + FLC-LZPO	7	0	2.36643	0.01796
M3	PF	7	0	1.60357	0.10881
M3	PF-LAM	7	7	1.18322	0.23672
M3	LAM	7	0	2.36643	0.01796
EPIPH	FLC	6	0		
EPIPH	FLC-LZPO	6	0	2.20140	0.02771
EPIPH	FLC + FLC-LZPO	6	0	2.20140	0.02771
EPIPH	PF	6	3	0.00000	1.00000
EPIPH	PF-LAM	6	10	0.10483	0.91651
EPIPH	LAM	6	0	2.20140	0.02771

TABLE 5-14. Kruskal-Wallis and Median tests: % PER, END, and HAV among AGE CLASSES in *H. lar*.

Significant contrasts in bold ($p \leq 0.05$). Mann-Whitney post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

p<0.10 levels after Bonferroni correction.								
% Tissue Type	df	total N	Kruskal-Wallis		Median test		Significant post-hocs (p'=0.005)	Significant post-hocs (p'=0.010)
			H	p	Chi-Square	p		
FEMUR								
Total PER	4	28	11.19007	0.0245	10.6667	0.0306		
Total END	4	28	15.52874	0.0037	10.44444	0.0336		DE*M3
HAV	4	28	3.978243	0.409	1.7777778	0.7765		
HUMERUS								
Total PER	4	25	8.782637	0.0668	5.311355	0.2568		
Total END	4	25	15.00264	0.0047	14.18269	0.0067		DE*M3
HAV	4	26	2.714245	0.6067	4.2	0.3796		

¹ Age class abbreviations: DECID (DE); M1 (M1); M2 (M2); M3 (M3); EPIPH (EP).

TABLE 5-15. Wilcoxon matched pairs tests: % HAV, total END, and total PER between ELEMENTS in *H. lar*. Significant contrasts in bold ($p \leq 0.05$).

Age class	% Tissue Type	Wilcoxon Matched Pairs			
		N	T	Z	p
Total sample	HAV	26	12.00	4.152569	0.00003
Total sample	Total END	25	58	2.8117723	0.00493
Total sample	Total PER	25	0	4.3723732	0.00001
DECID	HAV	4	4	0.3651484	0.71500
DECID	Total END	4	0	1.8257419	0.06789
DECID	Total PER	4	0	1.8257419	0.06789
M1	HAV	3	1	1.069045	0.28505
M1	Total END	3	1	1.069045	0.28505
M1	Total PER	3	0	1.6035675	0.10881
M2	HAV	5	0	2.0225996	0.04312
M2	Total END	5	0	2.0225996	0.04312
M2	Total PER	5	0	2.0225996	0.04312
M3	HAV	8	0	2.5205042	0.01172
M3	Total END	7	13	0.1690309	0.86577
M3	Total PER	7	0	2.3664319	0.01796
EPIPH	HAV	6	0	2.2013982	0.02771
EPIPH	Total END	6	4	1.3627703	0.17296
EPIPH	Total PER	6	0	2.2013982	0.02771

TABLE 5-16. Mann-Whitney and Kolmogorov-Smirnov tests: % area of PER tissue types between SEXES in *H. lar* (M3 - EPIPH subsample). Significant contrasts in bold ($p \leq 0.05$).

% Tissue Type	Mann-Whitney							Kolmogorov-Smirnov						
	N _F	N _M	Rank Sum F	Rank Sum M	U	Z	p-level	Max Neg Diff	Max Pos Diff	p-level	Mean F	Mean M	Std. Dev. F	Std. Dev. M
FEMUR														
FLC	7	8	56.00	64.00	28.00	0.0000	1.00000	0.00	0.00	p > .10	0.00	0.00	0.000	0.000
FLC-LZPO	7	8	57.00	63.00	27.00	0.1157	0.90787	-0.13	0.16	p > .10	0.11	0.10	0.167	0.153
FLC + FLC-LZPO	7	8	57.00	63.00	27.00	0.1157	0.90787	-0.13	0.16	p > .10	0.11	0.10	0.167	0.153
PF	7	8	52.50	67.50	24.50	-0.4050	0.68544	-0.13	0.00	p > .10	0.00	0.00	0.000	0.011
PF-LAM	7	8	57.00	63.00	27.00	0.1157	0.90787	-0.23	0.38	p > .10	1.56	1.55	0.852	1.250
LAM	7	8	72.00	48.00	12.00	1.8516	0.06408	0.00	0.57	p > .10	59.12	50.06	9.549	9.009
HUMERUS														
FLC	6	7	42.00	49.00	21.00	0.0000	1.00000	0.00	0.00	p > .10	0.00	0.00	0.000	0.000
FLC-LZPO	6	7	27.00	64.00	6.00	-2.1429	0.03213	-0.71	0.00	p < .10	0.00	0.21	0.000	0.221
FLC + FLC-LZPO	6	7	27.00	64.00	6.00	-2.1429	0.03213	-0.71	0.00	p < .10	0.00	0.21	0.000	0.221
PF	6	7	42.00	49.00	21.00	0.0000	1.00000	0.00	0.00	p > .10	0.00	0.00	0.000	0.000
PF-LAM	6	7	49.00	42.00	14.00	1.0000	0.31731	-0.29	0.57	p > .10	2.08	2.53	0.246	2.382
LAM	6	7	39.00	52.00	18.00	-0.4286	0.66824	-0.36	0.21	p > .10	38.65	39.98	6.218	8.400

TABLE 5-17. Kruskal-Wallis and Median tests: % area of PER tissue types among AGE CLASSES in *H. lar*. Significant contrasts in bold ($p \leq 0.05$). Mann-Whitney post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Bonferroni correction:								
% Tissue Type	df	total N	Kruskal-Wallis		Median test		Significant post-hocs (p'=0.005)	Significant post-hocs (p'=0.010)
			H	p	Chi-Square	p		
FEMUR								
FLC	4	28	3.896605	0.4202	4.128205	0.3889		
FLC-LZPO	4	28	22.24788	0.0002	18.44444	0.0010	M2*M3, M2*EP	DE*M3
FLC+FLC-LZPO	4	28	22.12436	0.0002	18.44444	0.0010	M2*M3, M2*EP	DE*M3
PF	4	28	24.33660	0.0001	23.91667	0.0001		DE*M3
PF-LAM	4	28	21.97906	0.0002	18.44444	0.0010	M2*M3, M2*EP	DE*M3
LAM	4	28	16.01724	0.0030	9.333333	0.0533		DE*M3
HUMERUS								
FLC	4	25	3.837500	0.4284	3.940217	0.4142		
FLC-LZPO	4	25	12.59430	0.0134	13.06662	0.0110		
FLC+FLC-LZPO	4	25	12.50559	0.0140	13.06662	0.0110		
PF	4	25	20.24784	0.0004	20.00000	0.0005		DE*M3
PF-LAM	4	25	17.83802	0.0013	18.45620	0.0010		DE*M3, M2*M3
LAM	4	25	17.36941	0.0016	11.13019	0.0251		DE*M3

¹ Age class abbreviations: DECID (DE); M1 (M1); M2 (M2); M3 (M3); EPIPH (EP).

TABLE 5-18. Wilcoxon matched pairs tests: % area of PER tissue types between ELEMENTS in *H. lar*. Significant contrasts in bold ($p \leq 0.05$).

Age class	% Tissue Type	Wilcoxon Matched Pairs			
		N	T	Z	p
Total sample	FLC	25	3	0.00000	1.00000
Total sample	FLC-LZPO	25	89	0.92108	0.35701
Total sample	FLC + FLC-LZPO	25	103	0.43447	0.66395
Total sample	PF	25	32	0.08891	0.92915
Total sample	PF-LAM	25	122	1.08973	0.27583
Total sample	LAM	25	1	4.34547	0.00001
DECID	FLC	4	0		
DECID	FLC-LZPO	4	5	0.00000	1.00000
DECID	FLC + FLC-LZPO	4	2	1.09545	0.27332
DECID	PF	4	1	1.46059	0.14413
DECID	PF-LAM	4	0	1.82574	0.06789
DECID	LAM	4	1	1.46059	0.14413
M1	FLC	3	0		
M1	FLC-LZPO	3	3	0.00000	1.00000
M1	FLC + FLC-LZPO	3	3	0.00000	1.00000
M1	PF	3	1	1.06904	0.28505
M1	PF-LAM	3	1	1.06904	0.28505
M1	LAM	3	0	1.60357	0.10881
M2	FLC	5	0		
M2	FLC-LZPO	5	5	0.67420	0.50018
M2	FLC + FLC-LZPO	5	4	0.94388	0.34523
M2	PF	5	0	1.60357	0.10881
M2	PF-LAM	5	0	2.02260	0.04312
M2	LAM	5	0	2.02260	0.04312
M3	FLC	7	0		
M3	FLC-LZPO	7	9	0.31449	0.75315
M3	FLC + FLC-LZPO	7	9	0.31449	0.75315
M3	PF	7	0		
M3	PF-LAM	7	10	0.67612	0.49896
M3	LAM	7	0	2.36643	0.01796
EPIPH	FLC	6	0		
EPIPH	FLC-LZPO	6	1	1.06904	0.28505
EPIPH	FLC + FLC-LZPO	6	1	1.06904	0.28505
EPIPH	PF	6	0		
EPIPH	PF-LAM	6	0	2.20140	0.02771
EPIPH	LAM	6	0	2.20140	0.02771

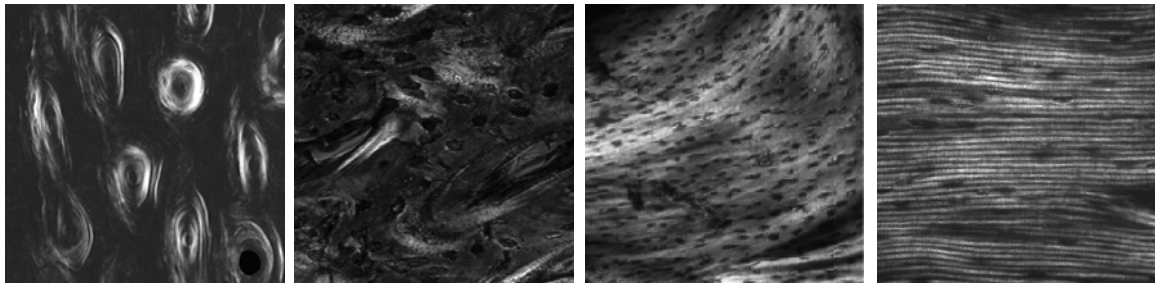
TABLE 5-19 a. Principal components analysis: PC statistics.

PC	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative variance
1	2.805622963	56.11245925	2.805622963	56.11245925
2	1.303883469	26.07766938	4.109506432	82.19012863
3	0.536742268	10.73484535	4.646248699	92.92497399
4	0.344807606	6.896152126	4.991056306	99.82112611
5	0.008943694	0.178873887	5	100

TABLE 5-19 b. Principal components analysis: factor loadings. Age class and cortical area were included as supplemental variables.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
% END	-0.40271	-0.75537	0.511124	0.077372	0.002115
% per FLC+FLCLZPO	-0.57607	0.739935	0.325805	0.111608	0.04515
% per PF	-0.80442	-0.25424	-0.37115	0.387648	0.015634
% per PF-LAM	-0.8672	-0.21037	-0.16926	-0.41689	0.035459
% per LAM	0.95523	-0.27731	-0.05426	0.0479	0.073477
Age Class	0.790446	-0.23031	0.009526	0.124857	0.252434
Cortical area	0.276999	0.177189	-0.01385	0.147165	0.249836

Patterns of collagen fiber organization
(observed in circularly polarized light)



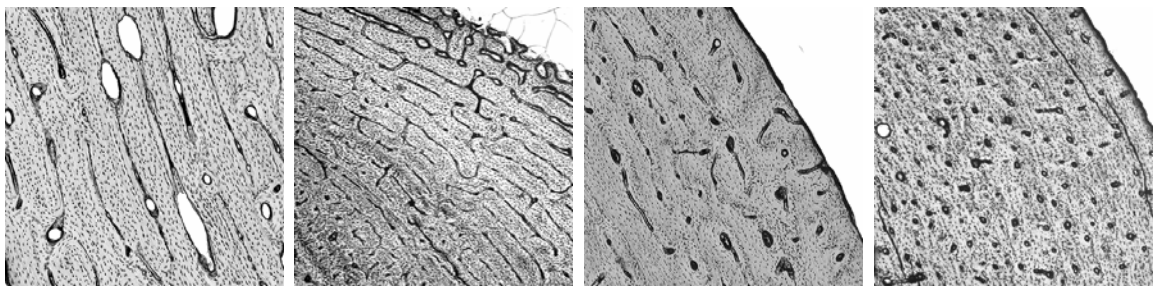
Fibro-lamellar

Woven

Parallel-fibered

Lamellar

Patterns of vascularization
(observed in transmitted unpolarized light)



Laminar

Plexiform

Reticular

Longitudinal

FIGURE 5-1. Patterns of primary bone microstructure. A number of bone tissue types can be recognized on the basis of collagen fiber organization and vascular patterns (e.g., vascular canal orientation). These features are visualized by imaging in circularly polarized transmitted light microscopy (CPL) and conventional transmitted light microscopy (LM), respectively.

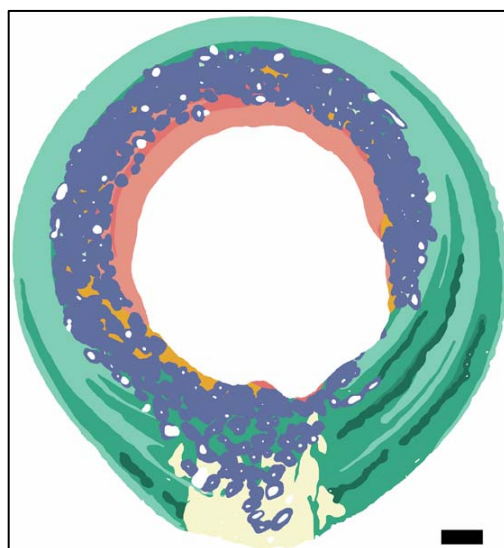


FIGURE 5-2. Tissue type maps. Areas representing different tissue types are color-coded for quantitative analysis.

Left: Tissue type map derived from the midshaft femur of *H. lar* (MCZ 41502).

Below: Tissue type color key. Greens represent tissues of periosteal origin. Warm colors represent tissues of endosteal origin. Within these color families, faster-forming tissue types are represented by darker shades than slower-forming tissue types. Numbers specify gray-scale values (above) and RGB color values (below), as explained in the text.

PERIOSTEAL ORIGIN



WOVEN
(WOV)
(13 45 29)



FIBRO-LAMELLAR
(FLC)
(18 66 45)



FLC-LZPO Transition
(FLC-LZPO)
(19 91 66)



PARALLEL-FIBERED
(PF)
(31 123 92)



PF-LAM Transition
(PF-LAM)
(40 152 113)



LAMELLAR
(LAM)
(109 197 168)



SHARPEY FIBERS
(SF)
(246 244 196)

ENDOSTEAL ORIGIN



PARALLEL-FIBERED
(EPF)
(181 79 82)



PF-LAM Transition
(EPF-LAM)
(221 97 94)



LAMELLAR
(ELAM)
(225 129 116)



COMPACTED COARSE
CANCELLOUS (ECCC)
(225 154 34)

OTHER BONE TISSUES



SECONDARY / HAVERSIAN
(HAV)
(78 92 145)



INTERSTITIAL of
unknown origin (INT)
(181 226 184)

FIGURE 5-3. Primary bone tissue types examined in the current study. *Left:* CPL images, showing collagen fiber matrix of bone. *Right:* LM images, showing osteocyte lacunae (OL) and vascular canals (VC).

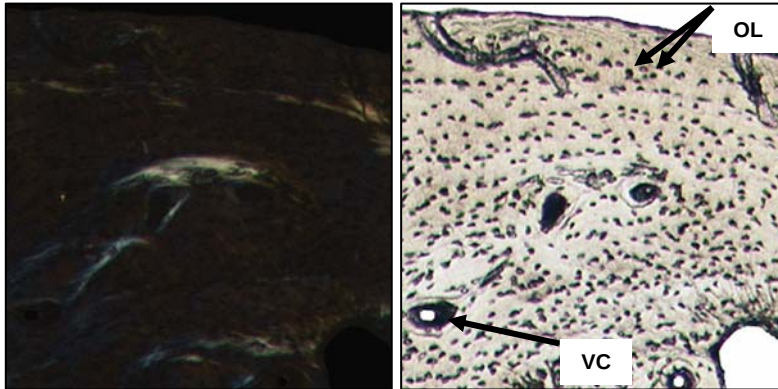


FIGURE 5-3 a. Parallel-fibered (PF). (SLW 92 Humerus; with scattered vascular canals).

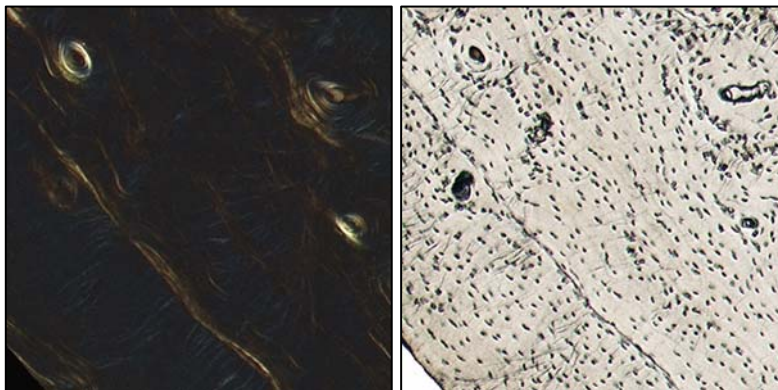


FIGURE 5-3 b. Parallel-fibered to Lamellar transition (PF-LAM). (MCZ 41502 Humerus)

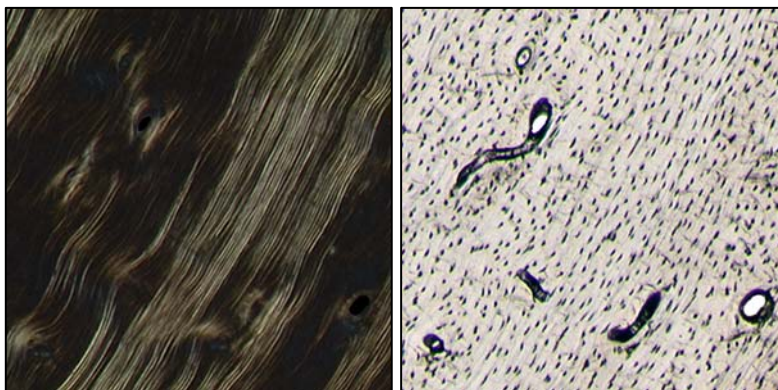


FIGURE 5-3 c. Lamellar (LAM). (MCZ 41429 Femur)

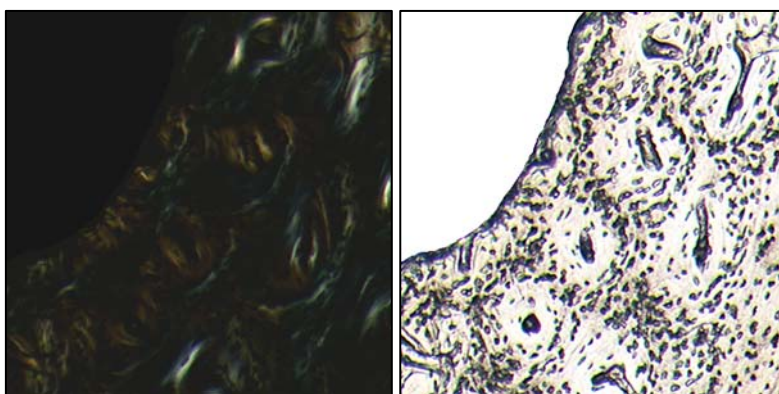


FIGURE 5-3 d. Fibro-lamellar complex (FLC). (SLW 92 Femur)

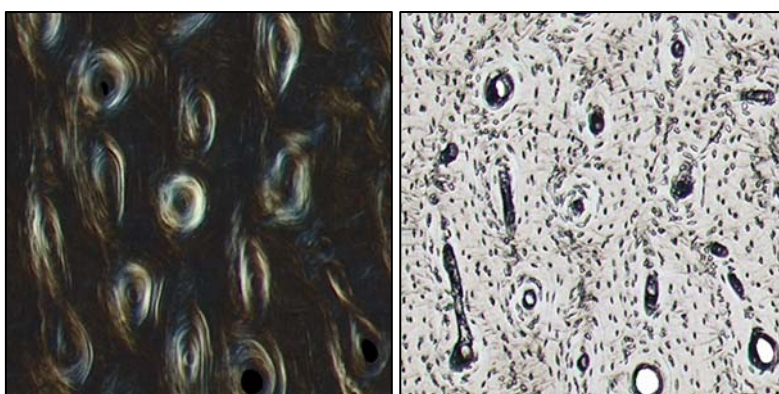


FIGURE 5-3 e. Fibro-lamellar to Lamellar-zonal transition (FLC-LZPO). (MNH 83604 Femur)

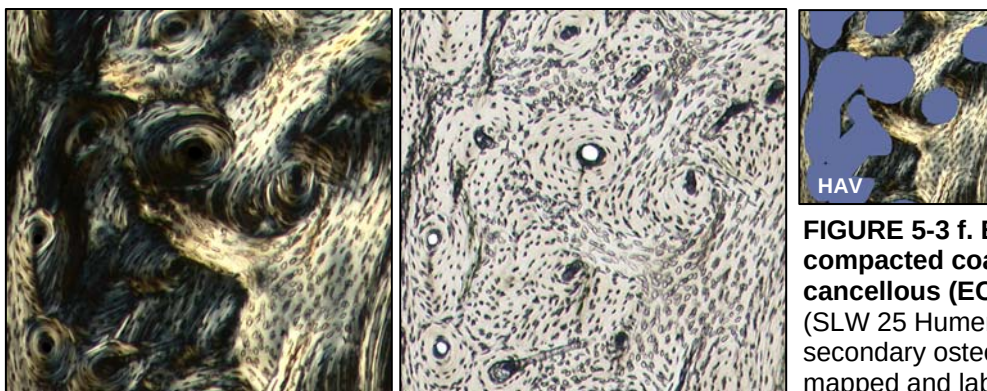


FIGURE 5-3 f. Endosteal compacted coarse cancellous (ECCC). (SLW 25 Humerus; with secondary osteons mapped and labeled HAV.)

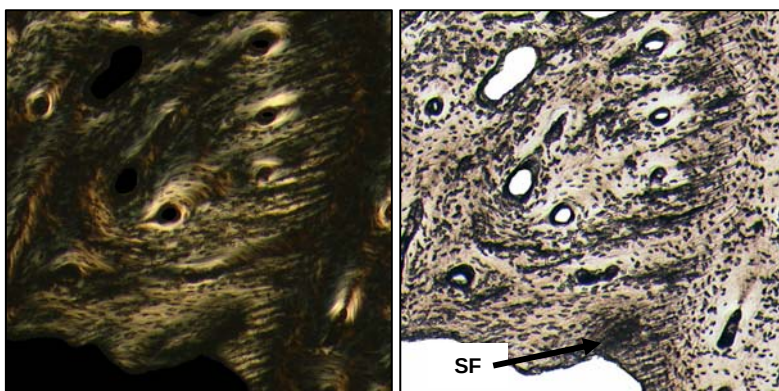


FIGURE 5-3 g. Sharpey's fiber bone (SF). (SLW 73 Femur)

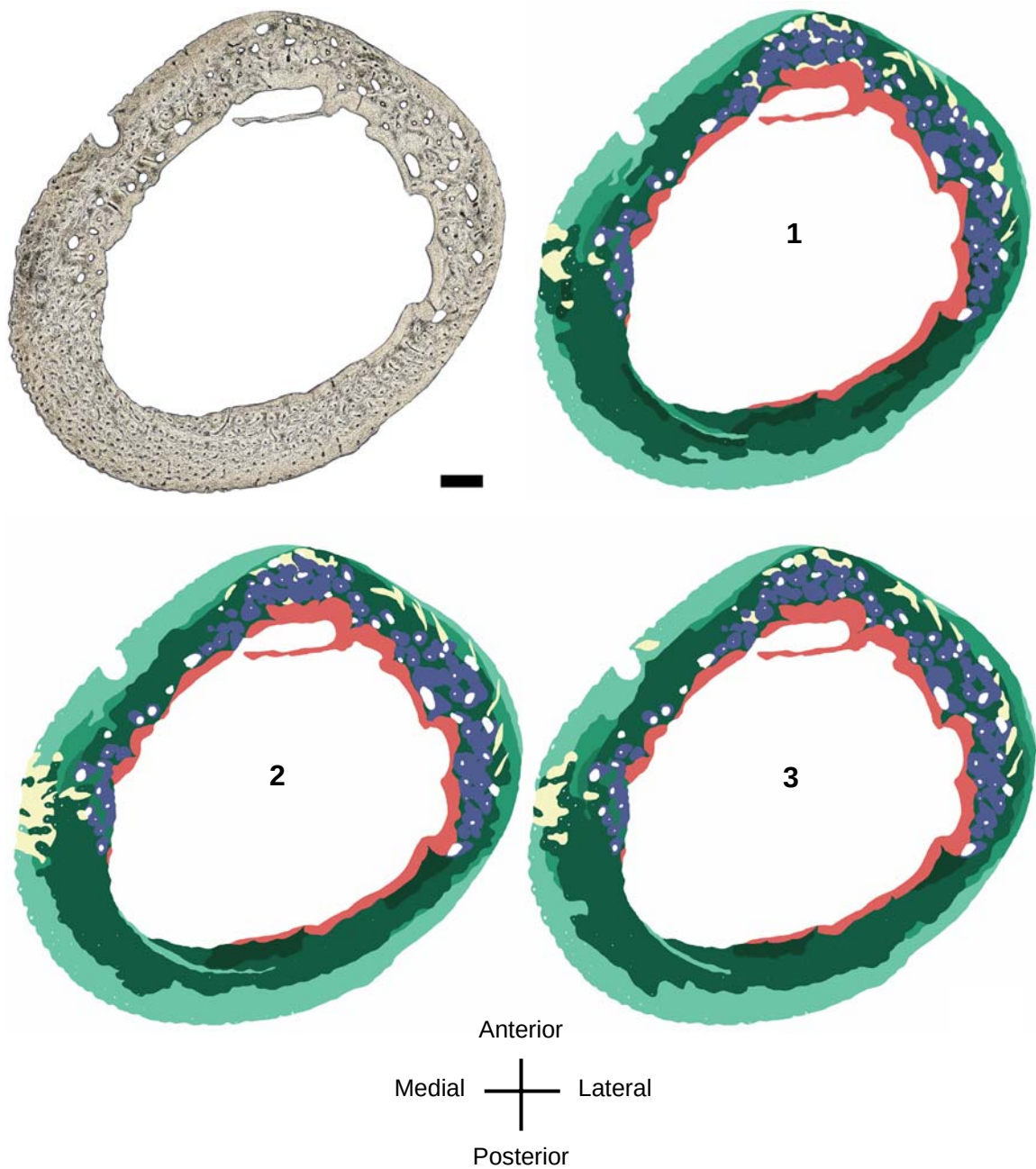


FIGURE 5-4 a. Repeatability of tissue type mapping: tissue type maps of the midshaft humerus of *C. aethiops* SLW 27 resulting from three independent measurements. *Upper left:* LM image. *Upper right and bottom:* Tissue type maps. Colors denote different tissue types, as depicted in Figure 5-2. Scale bar = 0.5 mm.

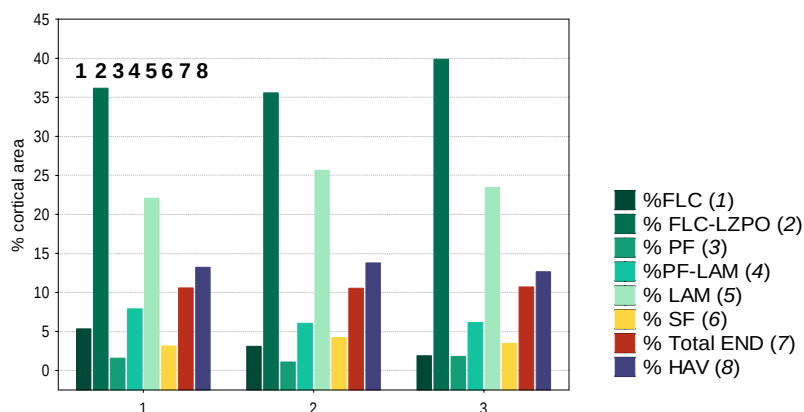


FIGURE 5-4 b. Repeatability of tissue type mapping: tissue type proportions at the midshaft humerus of *C. aethiops* SLW 27 resulting from three independent measurements. Bars representing different tissue types are color coded to correspond with tissue type maps.

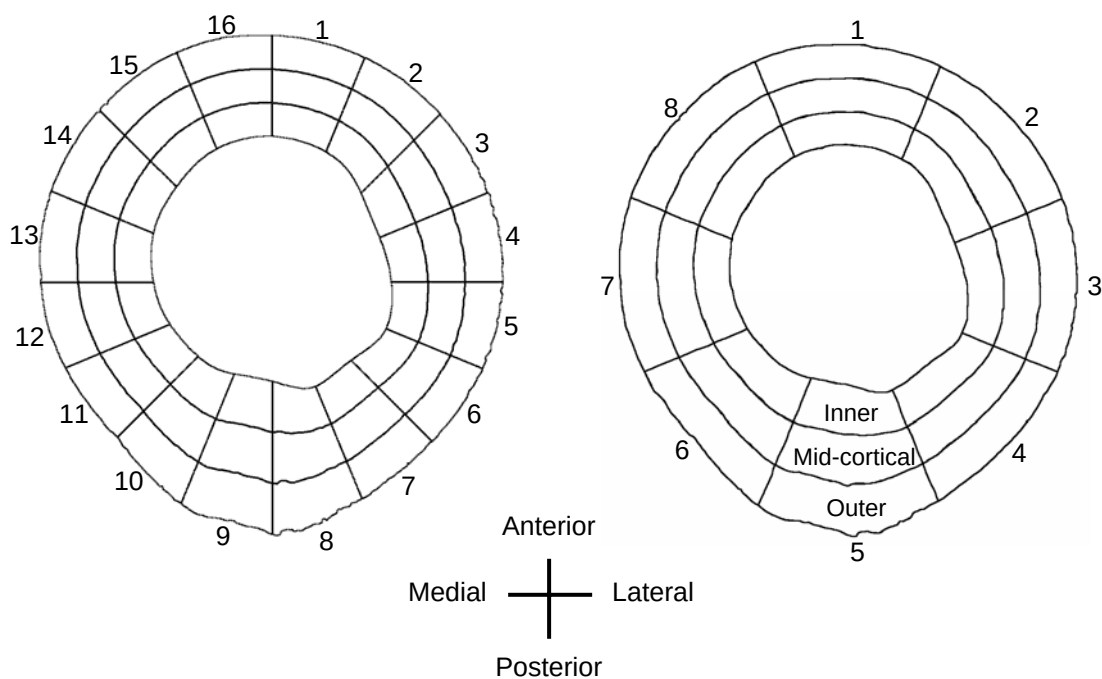


FIGURE 5-5. Bone segmentation analysis. *Left:* A customized macro divides each bone cross-section into 3 circumferential rings (labeled 'Inner', 'Mid-cortical', and 'Outer') and 16 radial sectors, resulting in 48 bone segments. Pixel dimensions for tissue types corresponding to different gray-level values are extracted for each bone segment in Optimas Image Analysis software. *Right:* In the current study, data for 16 radial sectors are collapsed into 8 radial sectors (multiplied by 3 rings), as shown here.

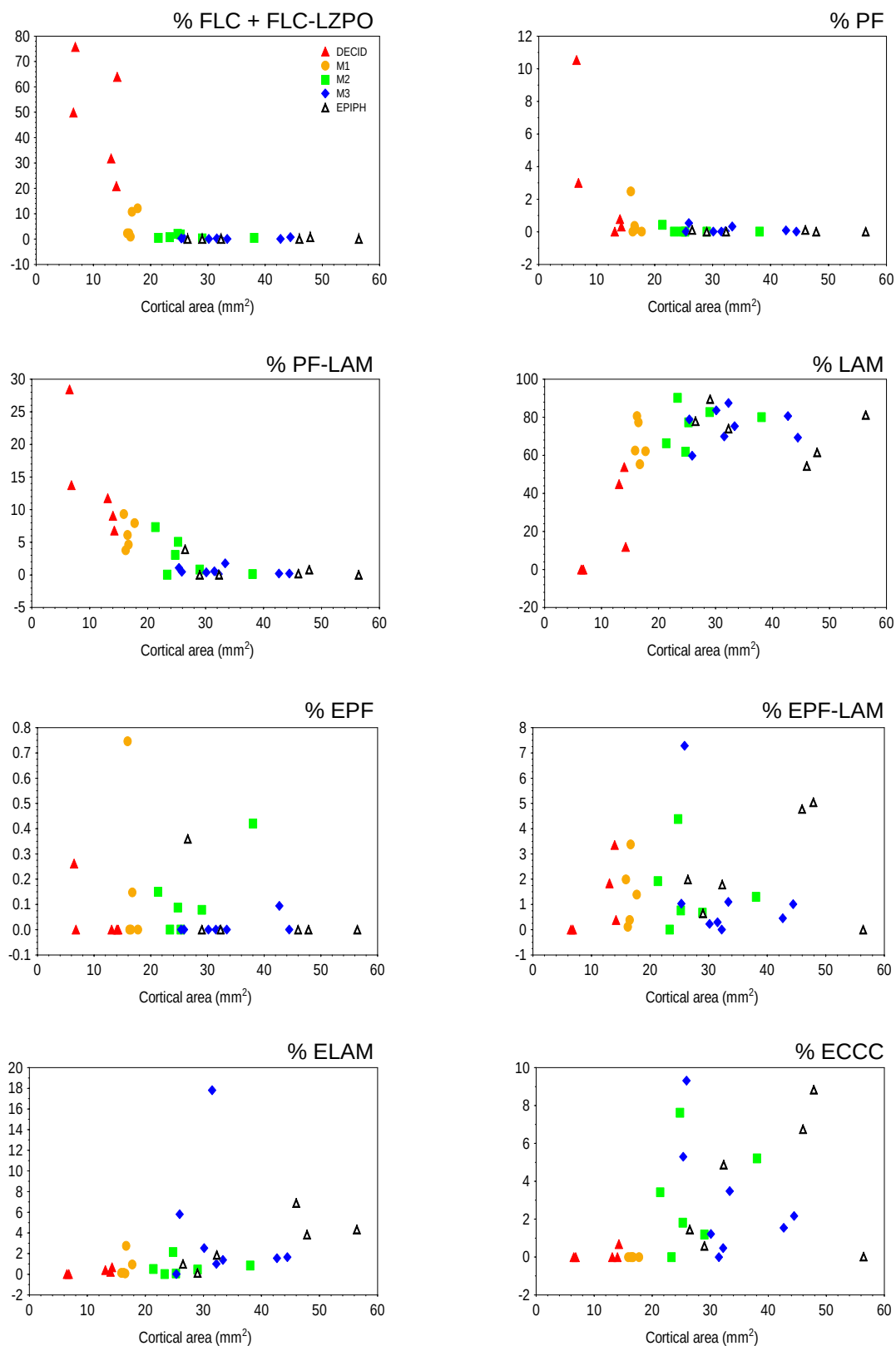


FIGURE 5-6 a. Scatterplots of tissue type proportions by cortical area, categorized by age class: *C. aethiops* midshaft femur.

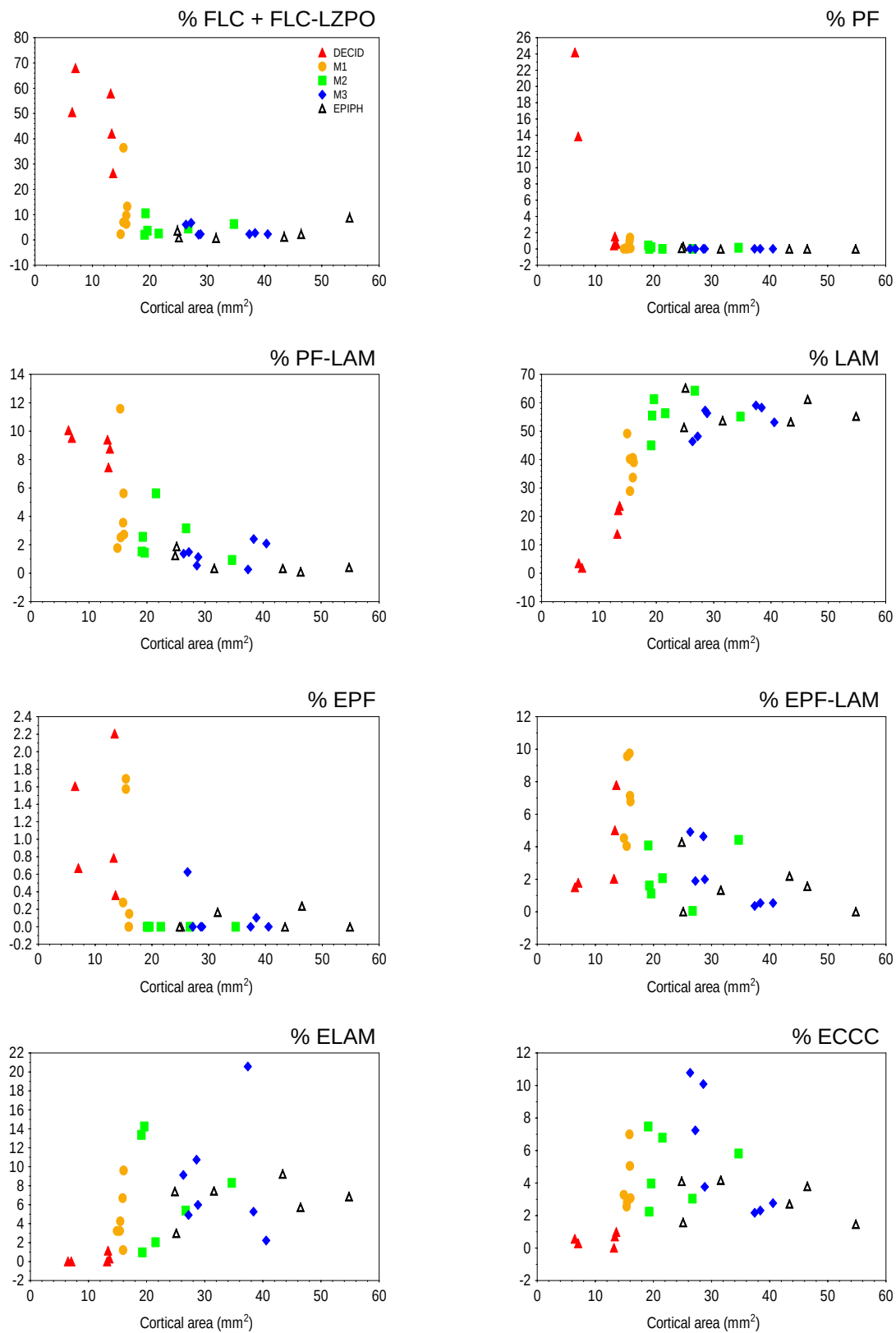


FIGURE 5-6 a continued. Scatterplots of tissue type proportions by cortical area, categorized by age class: *C. aethiops* midshaft humerus.

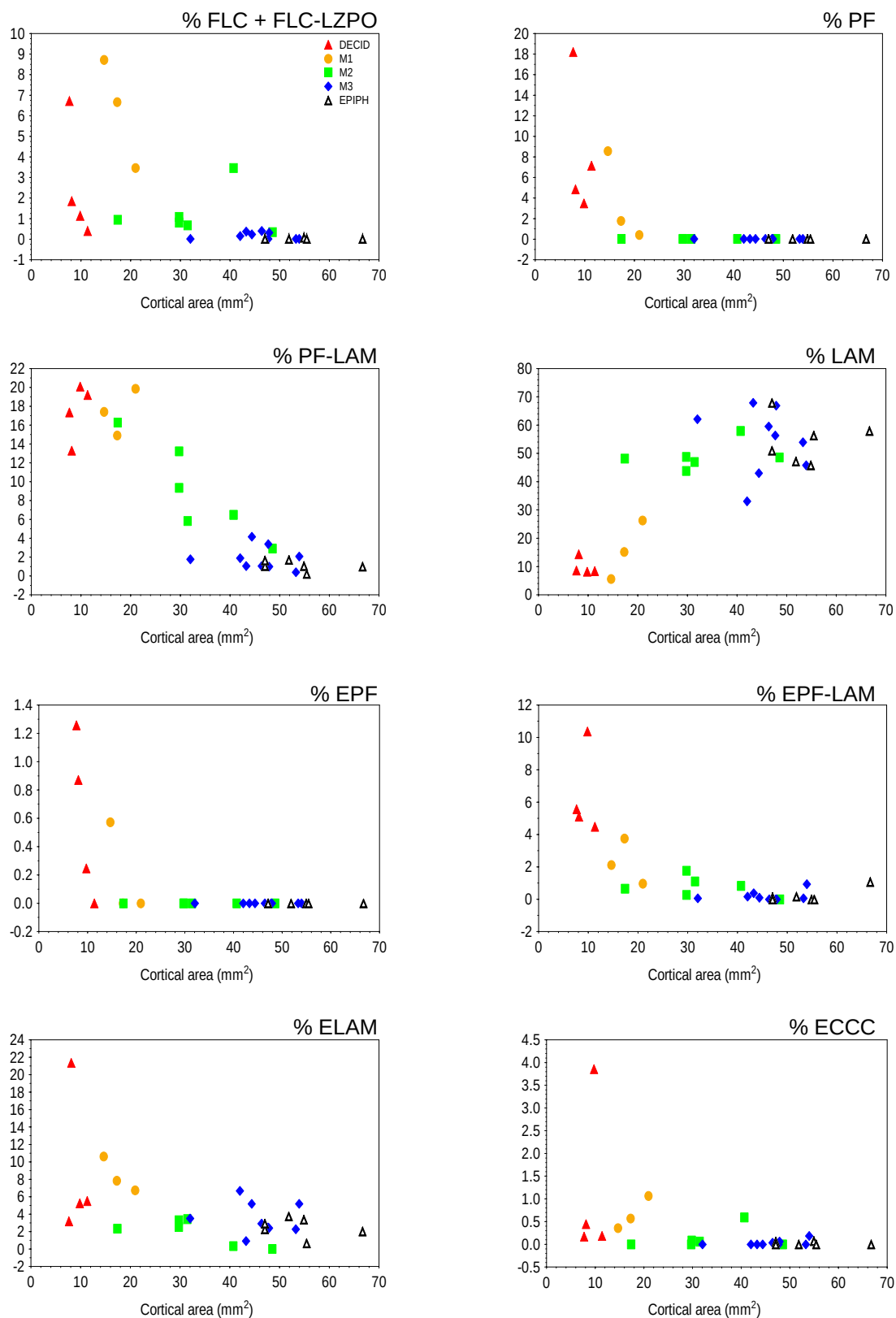


FIGURE 5-6 b. Scatterplots of tissue type proportions by cortical area, categorized by age class: *H. lar* midshaft femur.

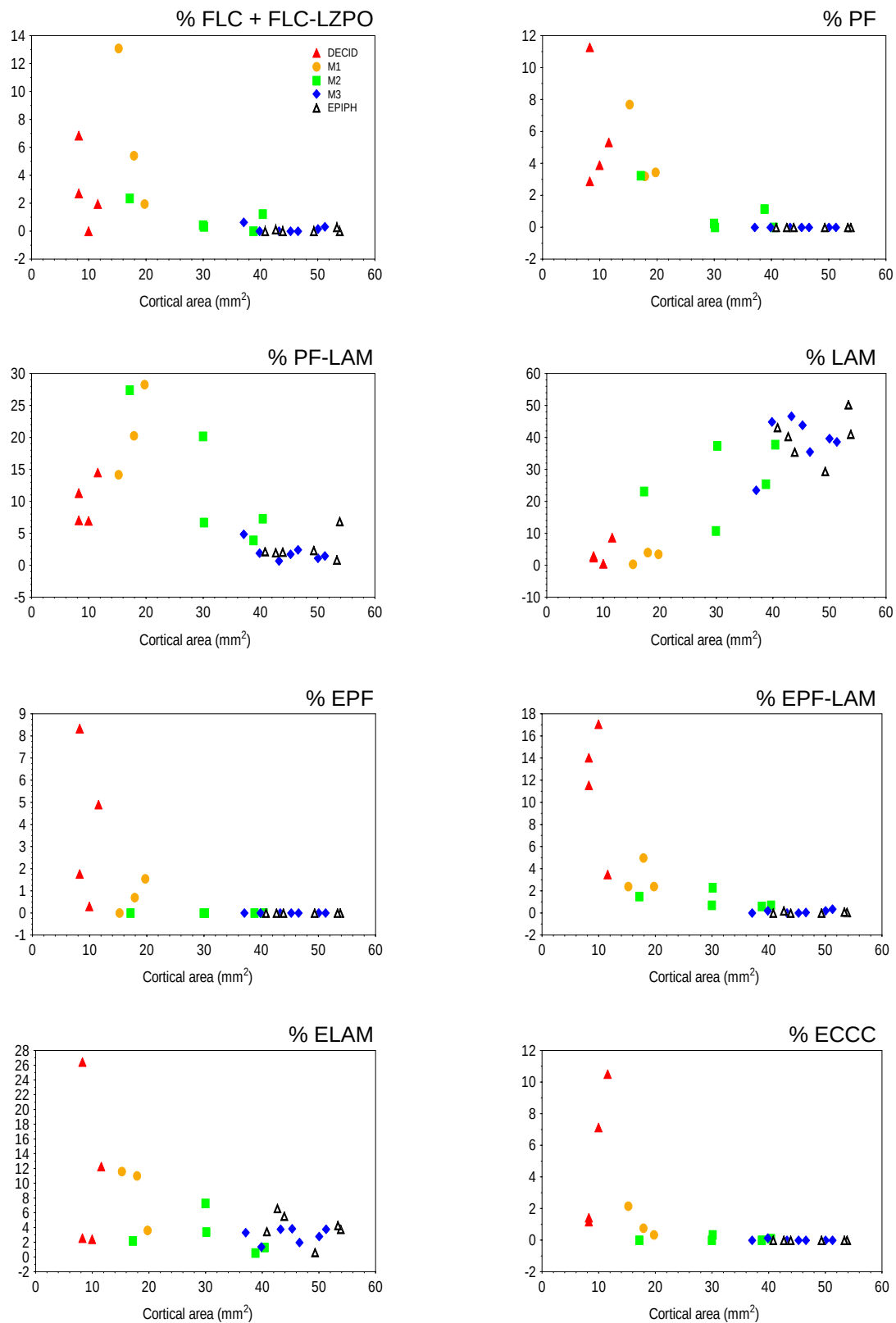


FIGURE 5-6 b continued. Scatterplots of tissue type proportions by cortical area, categorized by age class: *H. lar* midshaft humerus.

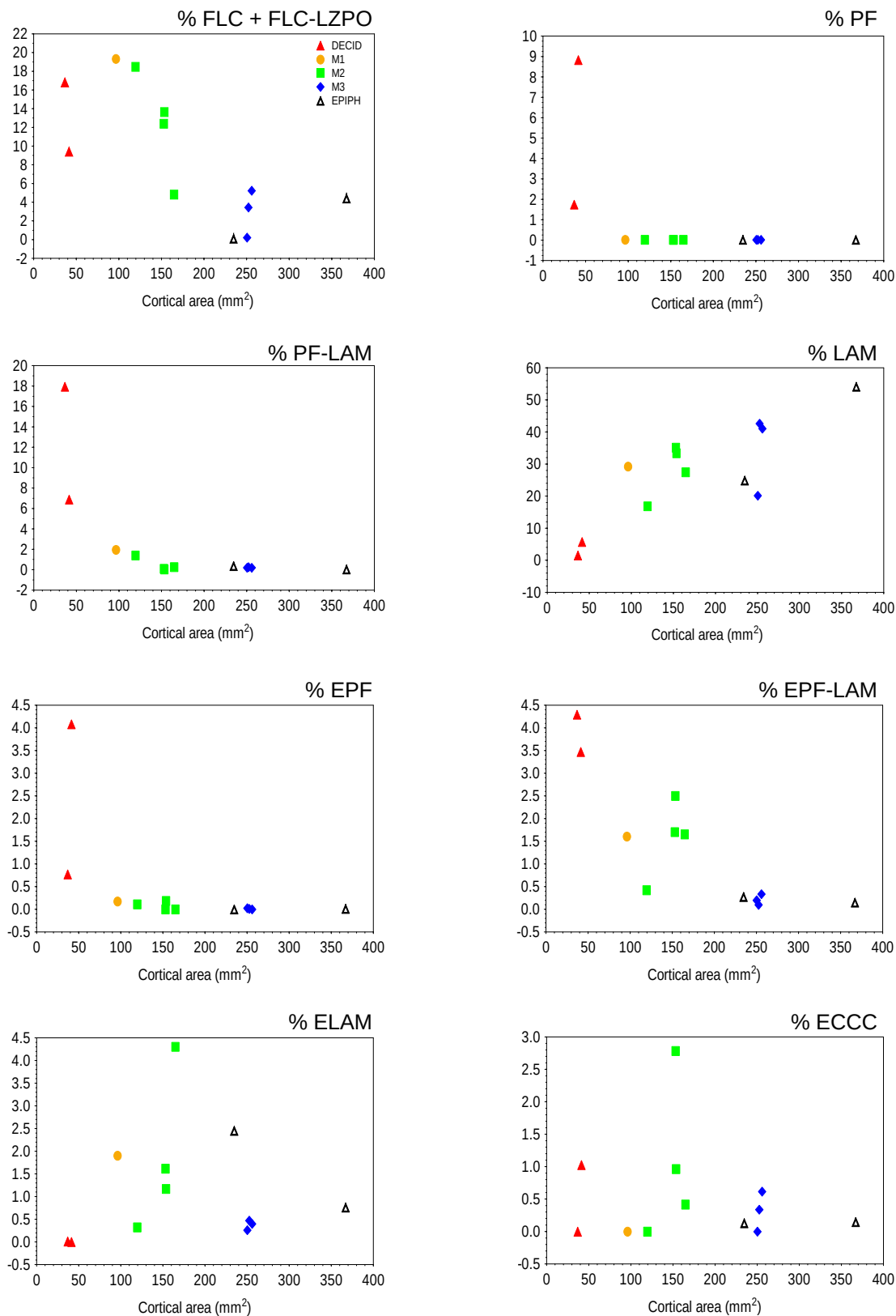


FIGURE 5-6 c. Scatterplots of tissue type proportions by cortical area, categorized by age class: *P. troglodytes* midshaft femur.

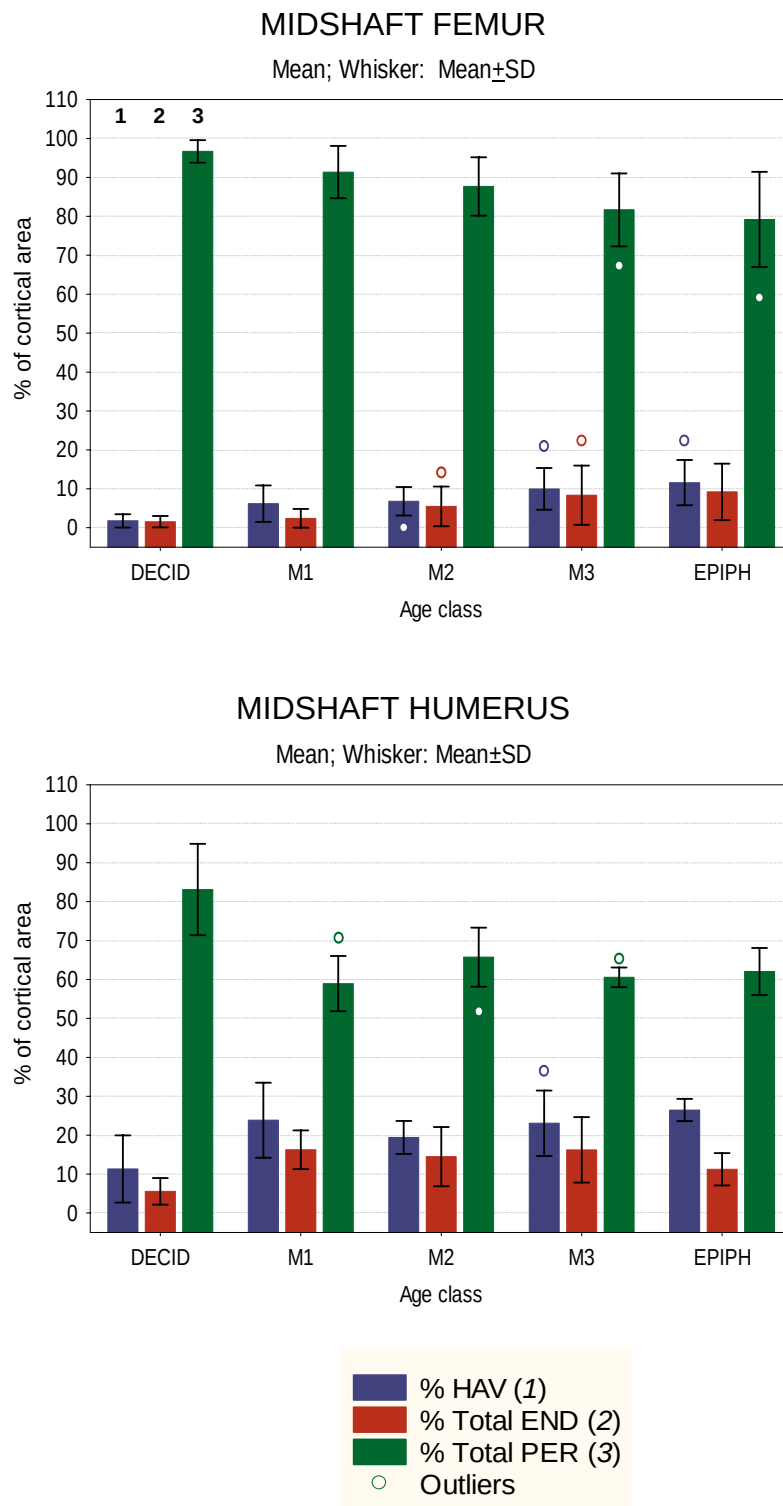


FIGURE 5-7. Plots of age class mean proportions of total cortical area represented by endosteal (END), periosteal (PER) and secondary osteonal (HAV) tissue: *C. aethiops* midshaft femur and humerus. Bars representing different tissue types are color coded to correspond with tissue type maps.

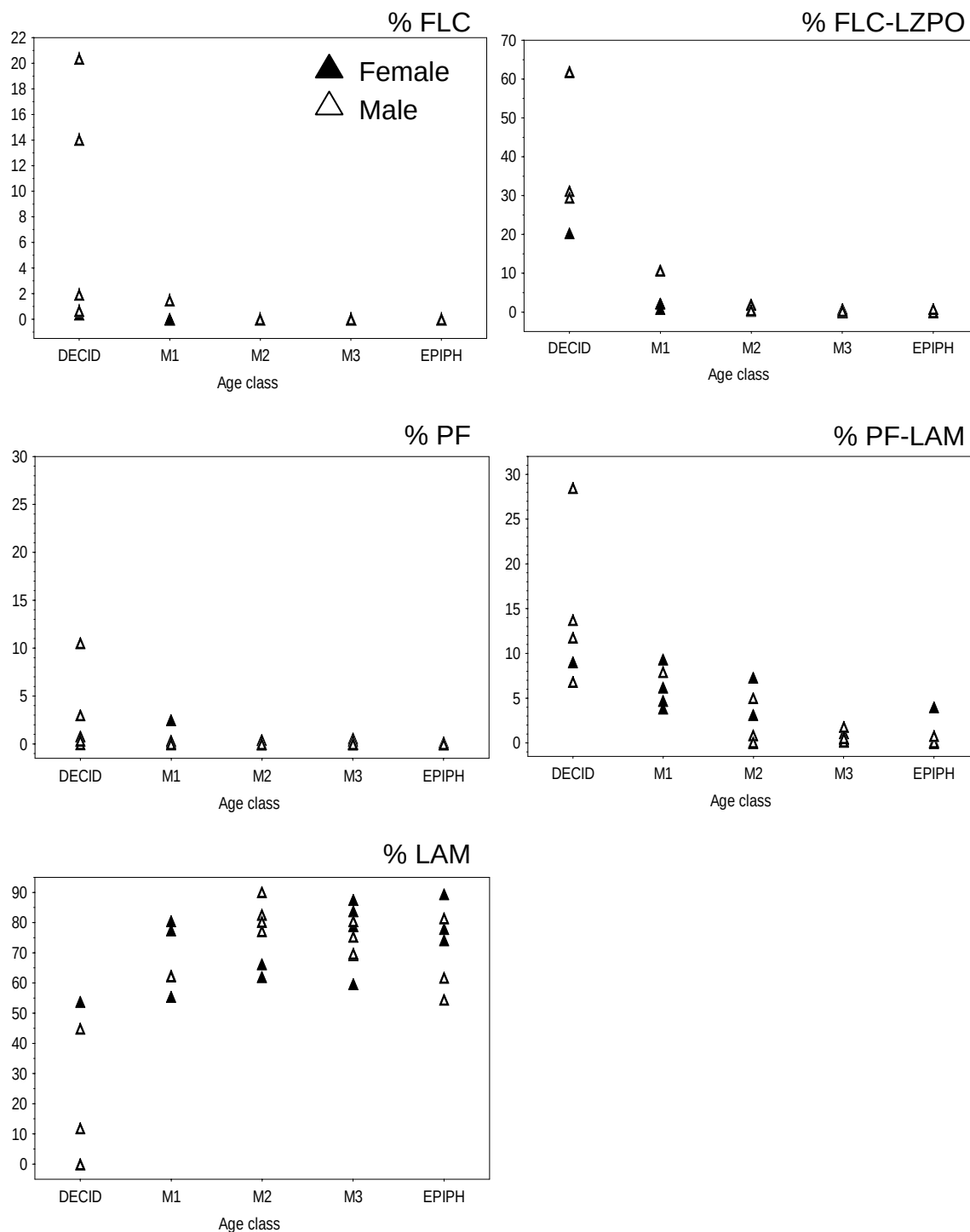


FIGURE 5-8 a. Scatterplots of periosteal tissue type proportions by age class, categorized by sex: *C. aethiops* midshaft femur.

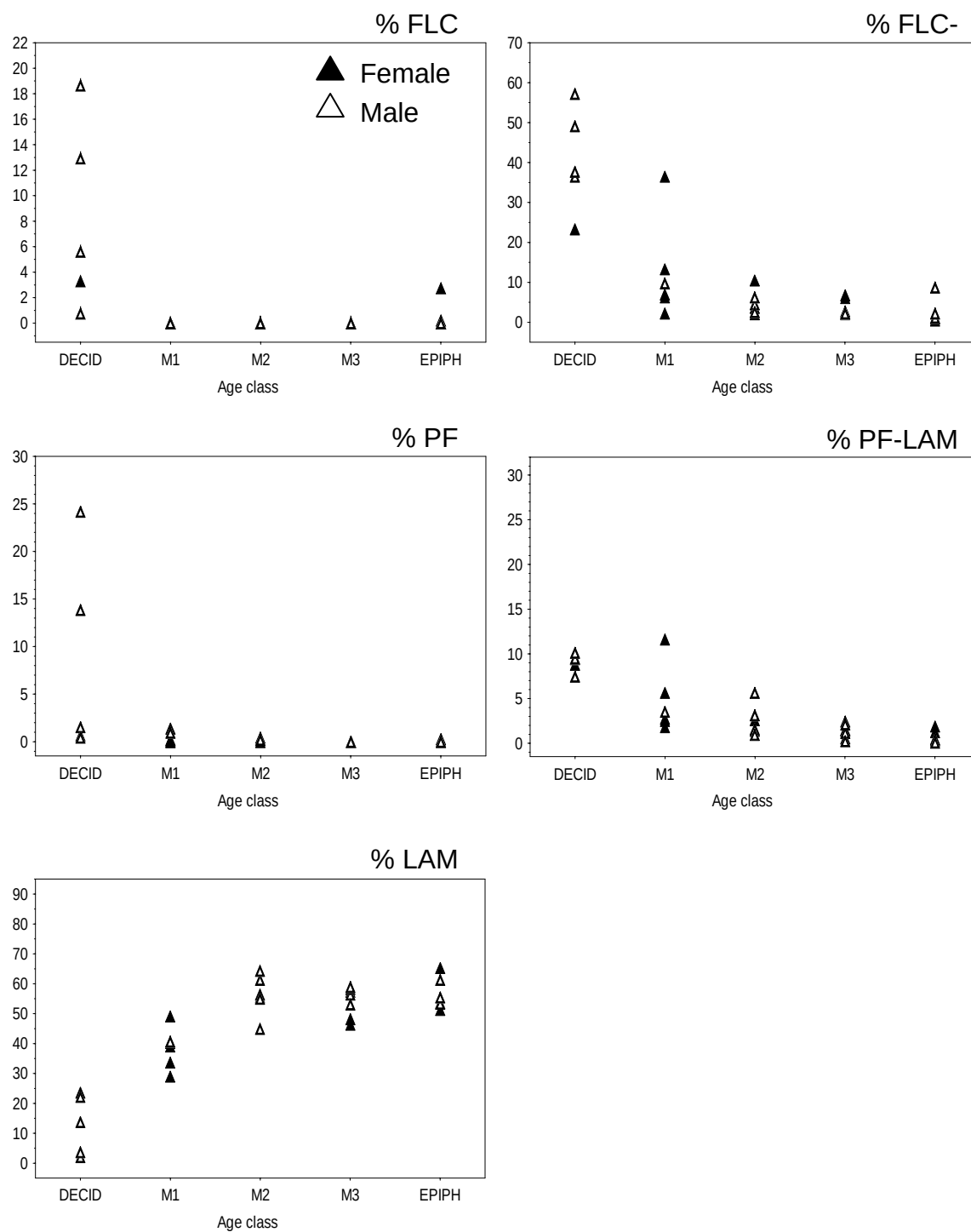
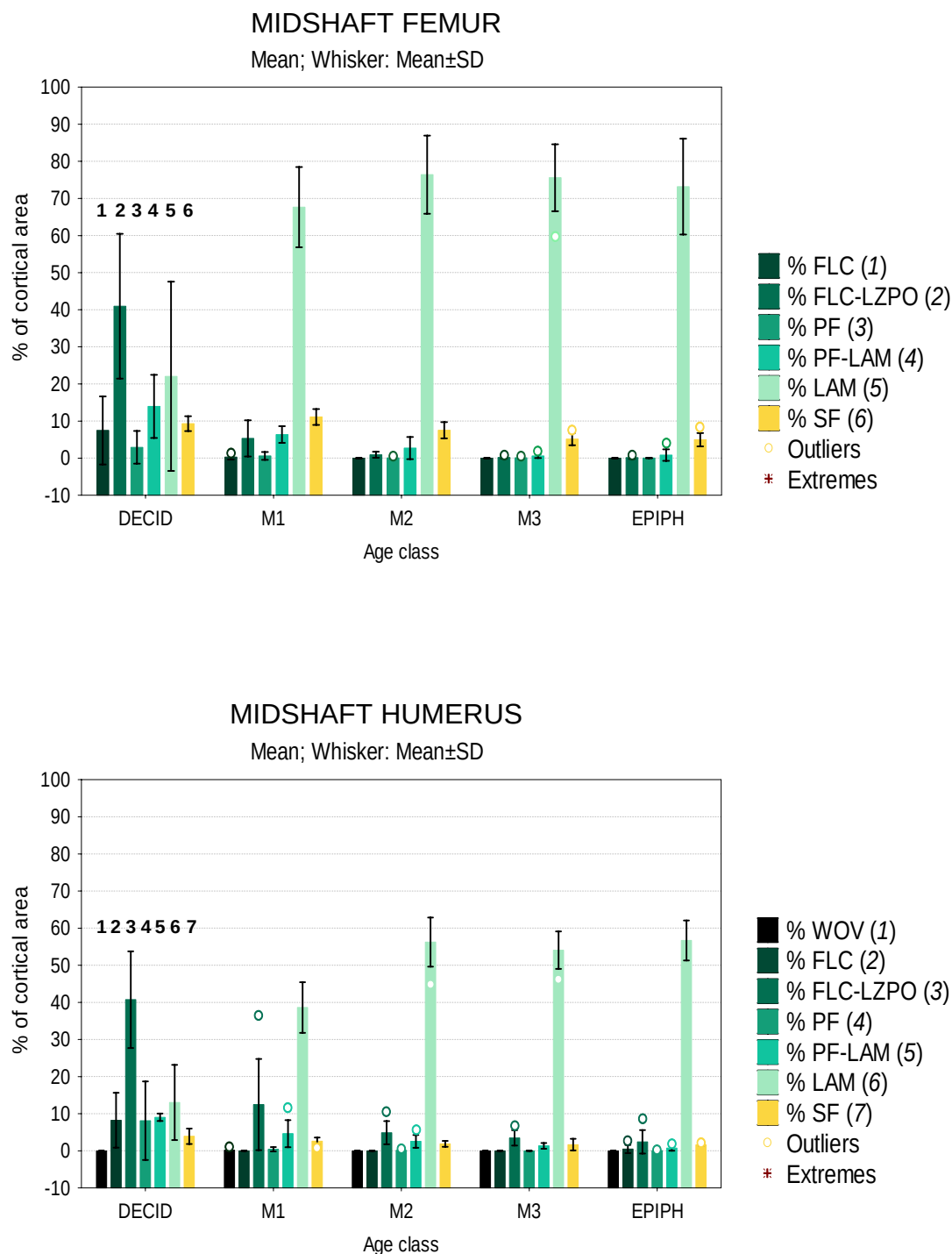


FIGURE 5-8 b. Scatterplots of periosteal tissue type proportions by age class, categorized by sex: *C. aethiops* midshaft humerus.



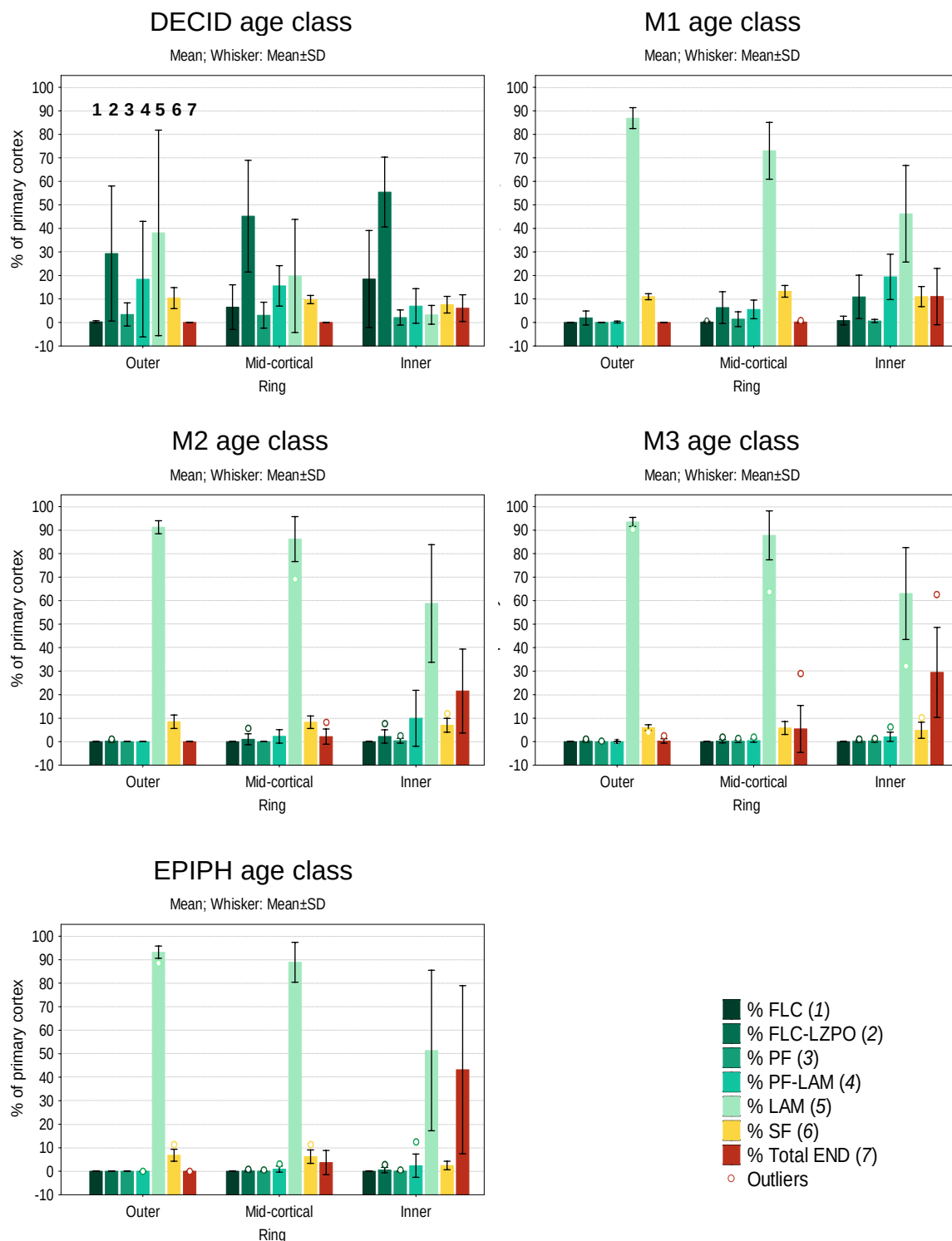


FIGURE 5-10 a. Plots of age class mean proportions of primary cortex area represented by different tissue types, across circumferential rings: *C. aethiops* midshaft femur. Earlier-deposited periosteal tissues (and, to a variable extent, endosteal tissues) are represented in the inner cortical ring, while recently-deposited periosteal tissues are represented in the outer cortical ring. Bars representing different tissue types are color coded to correspond with tissue type maps.

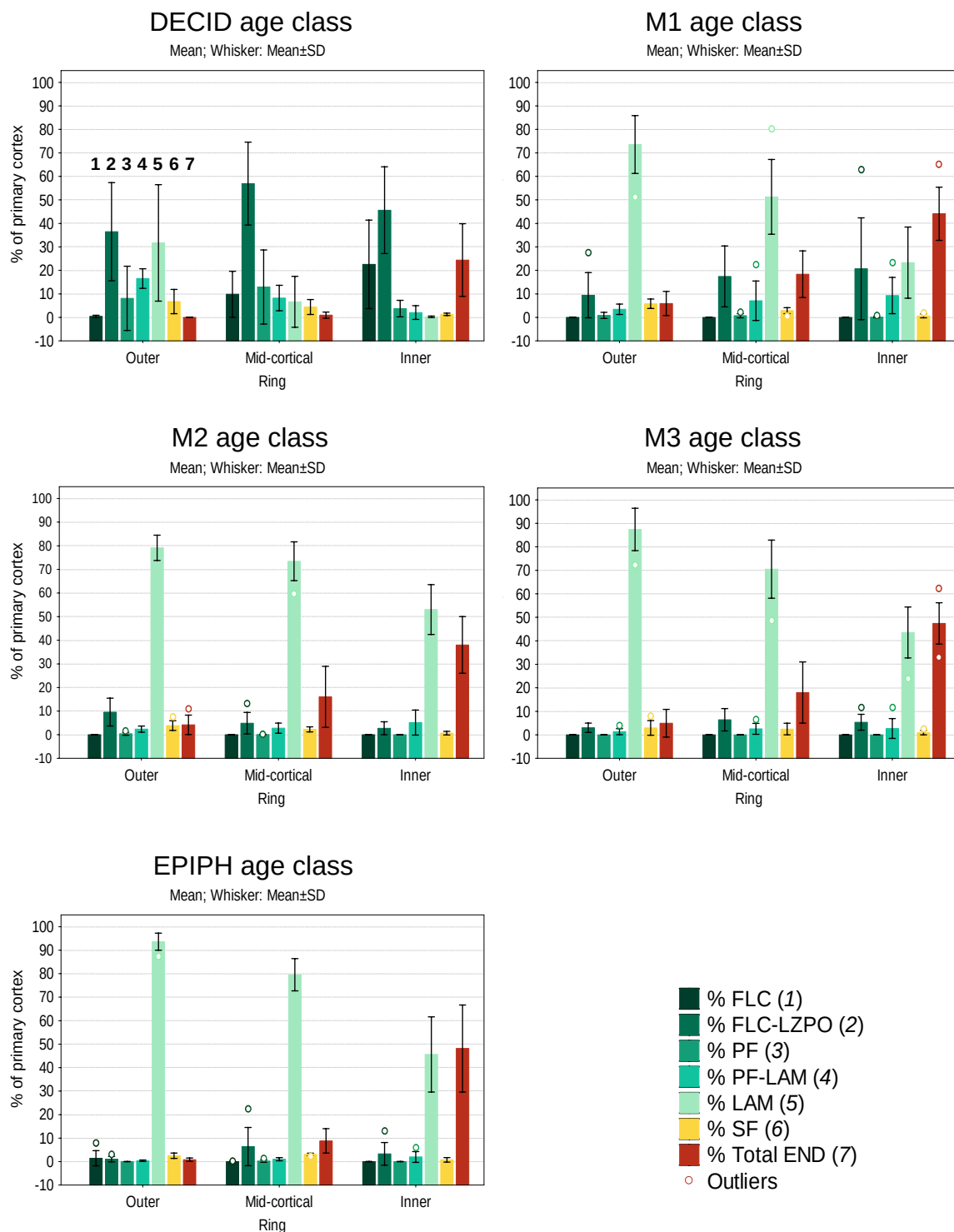


FIGURE 5-10 b. Plots of age class mean proportions of primary cortex area represented by different tissue types, across circumferential rings: *C. aethiops* midshaft humerus. Earlier-deposited periosteal tissues (and, to a variable extent, endosteal tissues) are represented in the inner cortical ring, while recently-deposited periosteal tissues are represented in the outer cortical ring. Bar plots representing different tissue types are color coded to correspond with tissue type maps.

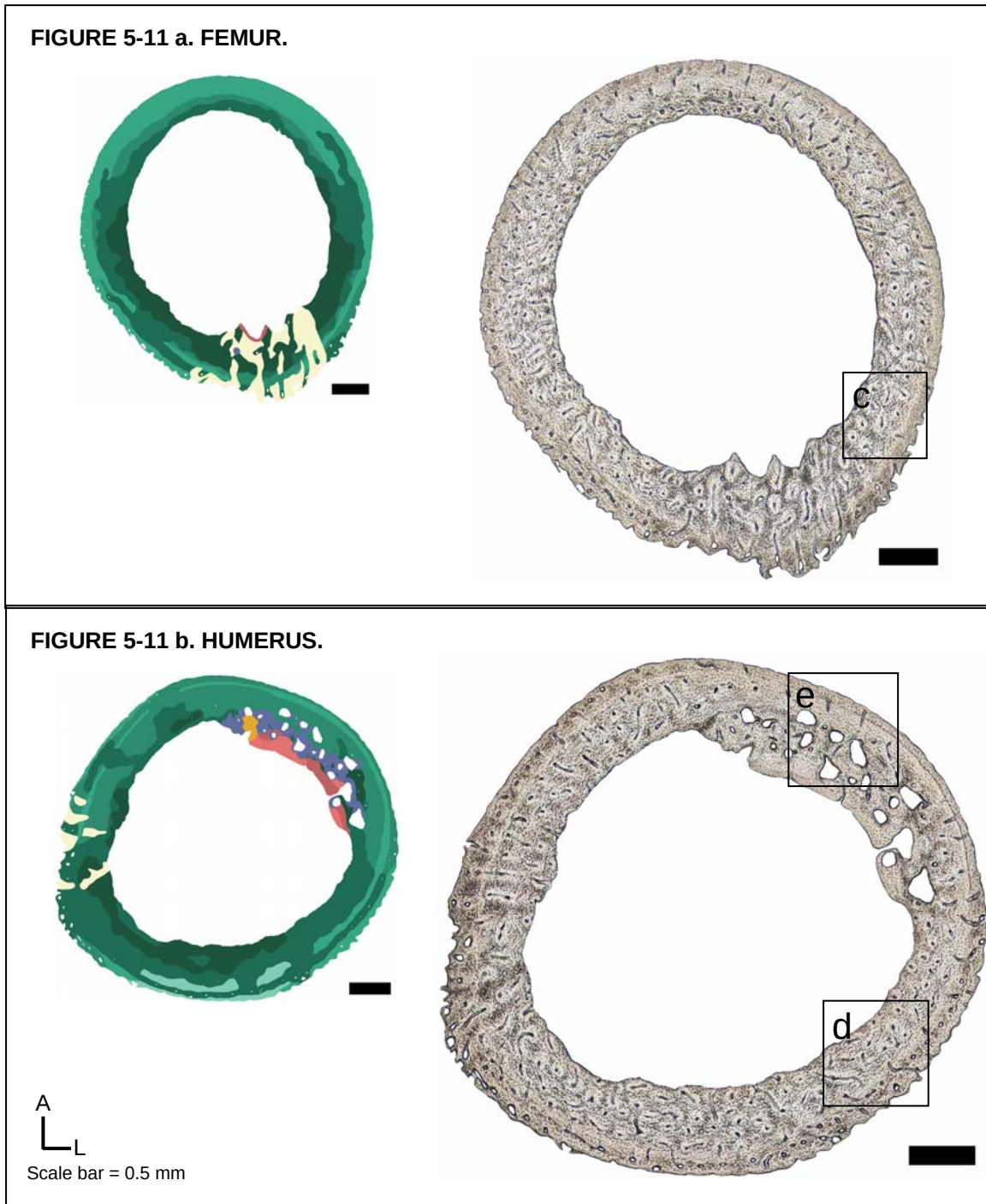


FIGURE 5-11 a and b. *C. aethiops* midshaft femur and humerus images: DECID Male (SLW 92). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as depicted in Figure 5-2. *Right:* LM images. Note the predominantly longitudinal vascular pattern with occasional radial and oblique canals and irregular anastomoses. Insets show location of FIGURES 5-11 c-e.

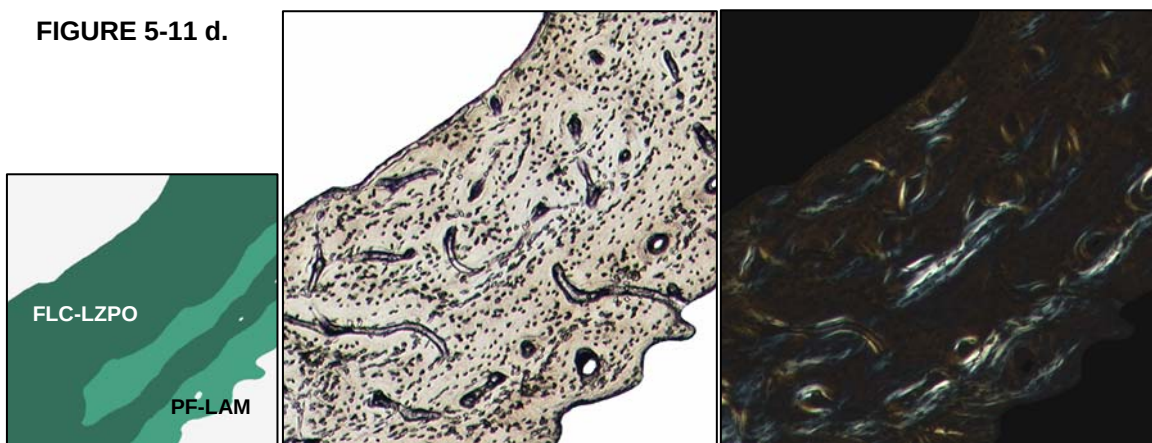
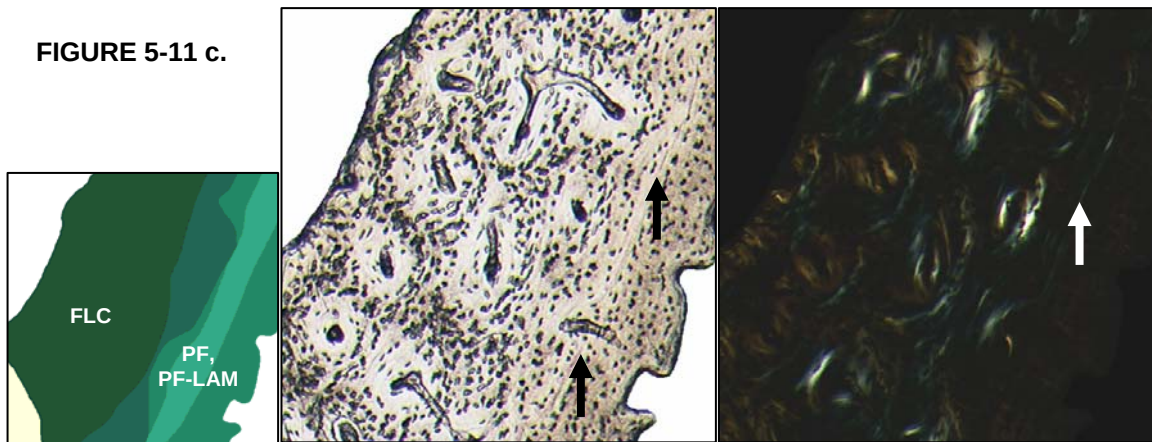


FIGURE 5-11 c. *C. aethiops* SLW 92 midshaft femur images: Fibro-lamellar to lamellar-zonal transition in the posterolateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing vascular canals and osteocyte lacunae. FLC tissue is characterized by plump and irregularly-shaped osteocyte lacunae, which are densely-distributed in a disorganized manner around the periphery of primary osteons. Arrows indicate location of an annulus, indicating a brief period of slowed deposition. *Right:* CPL image. Arrows indicate location of the annulus, which has a slightly brighter CPL signal compared to surrounding tissue.

FIGURE 5-11 d. *C. aethiops* SLW 92 midshaft humerus images: Fibro-lamellar and lamellar-zonal tissue in the posterolateral cortex. *Left:* LM image. Fibro-lamellar (FLC-LZPO) and lamellar-zonal (PF-LAM) tissue, with predominantly longitudinal vascularization and scattered oblique canals. *Right:* CPL image.

FIGURE 5-11 e.

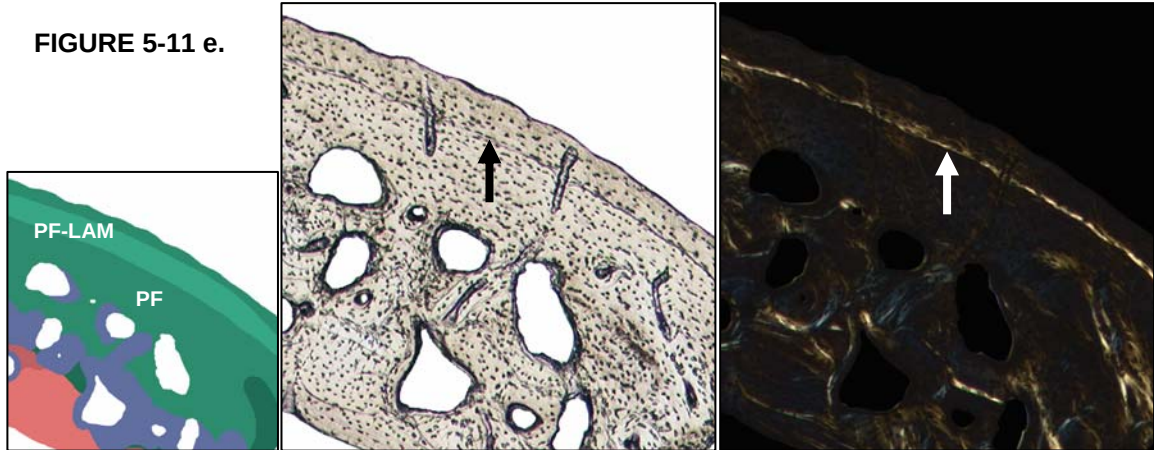


FIGURE 5-11 e. *C. aethiops* SLW 92 midshaft humerus images: Lamellar-zonal tissue in the antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing resting line in scarcely-vascularized lamellar-zonal (PF-LAM and PF) cortex, indicating a brief cessation of bone deposition. Arrows indicate location of resting line. *Right:* CPL image. Arrows indicate bright CPL signal of the resting line, relative to the dark appearance of adjacent PF and PF-LAM matrix.

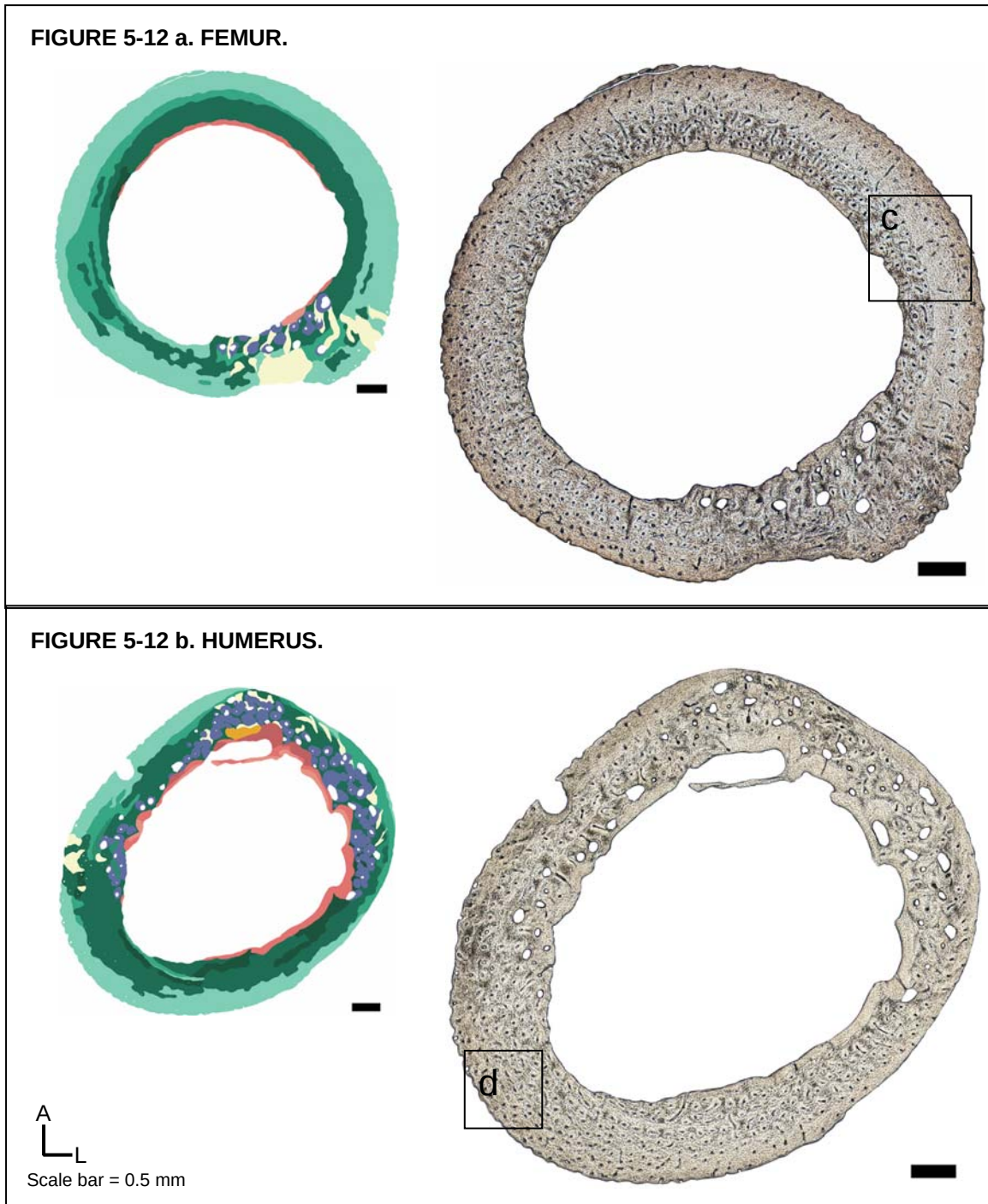


FIGURE 5-12 a and b. *C. aethiops* midshaft femur and humerus images: DECID male (SLW 27). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as depicted in Figure 5-2. *Right:* LM images. Note the predominantly longitudinal vascular pattern. There is an abrupt change in vascular canal density in the antero-lateral cortex of the midshaft femur, whereas this transition occurs more gradually in the posterior cortex. Insets show location of Figures 5-12 c and d.

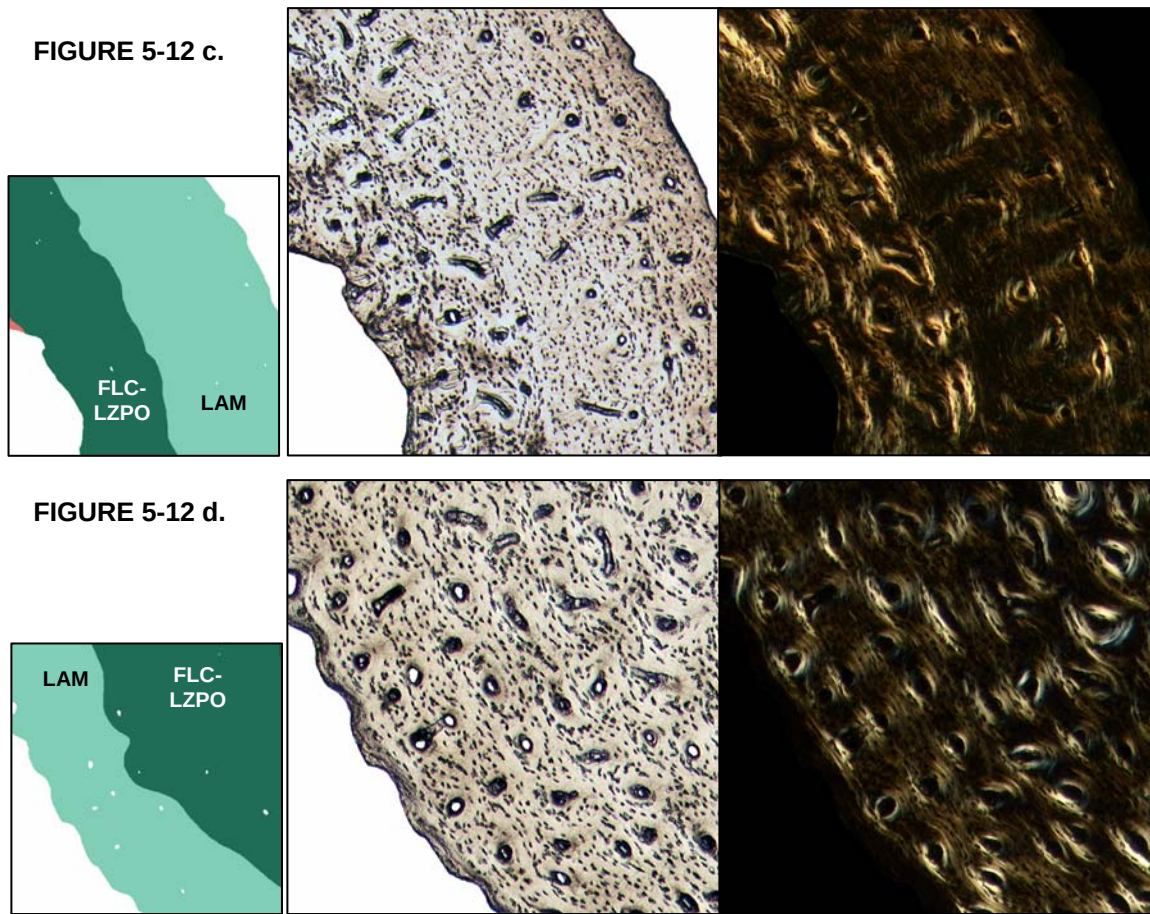


FIGURE 5-12 c. *C. aethiops* SLW 27 midshaft femur images: FLC-LZPO to LAM transition in the antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing abrupt transition from FLC-LZPO to LAM, the latter vascularized with longitudinal simple primary vascular canals and a few scattered primary osteons. *Right:* CPL image.

FIGURE 5-12 d. *C. aethiops* SLW 27 midshaft humerus images: FLC-LZPO to LAM transition in the postero-medial cortex. *Left:* Tissue type map. *Middle:* LM image, showing gradual transition from FLC-LZPO to LAM. *Right:* CPL image. This transition is marked by a gradual decrease in the size and density of primary osteons, eventually characterized by simple primary vascular canals and scattered primary osteons having only a single centripetal lamella. The extra-osteonal matrix of FLC-LZPO appears increasingly LAM in its organization in this transitional region.

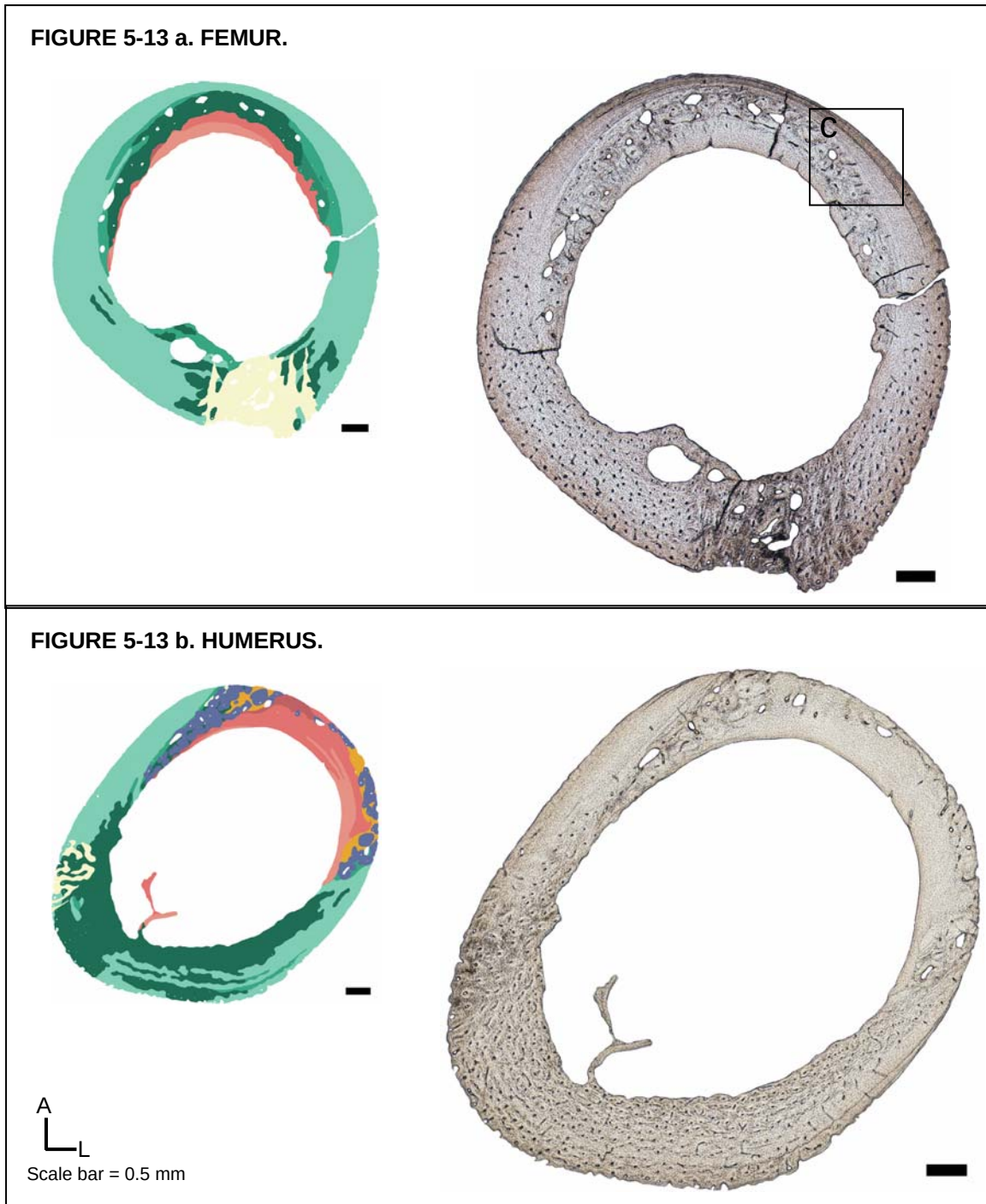


FIGURE 5-13 a and b. *C. aethiops* midshaft femur and humerus images: M1 female (SLW 21). *Left:* Whole cross-section tissue type maps. Colors depict different tissue types, as shown in Figure 5-2. *Right:* LM images. Note the regional variability in overall vascularity of periosteal cortex at the midshaft femur and humerus. Inset shows location of FIGURE 5-13 c.

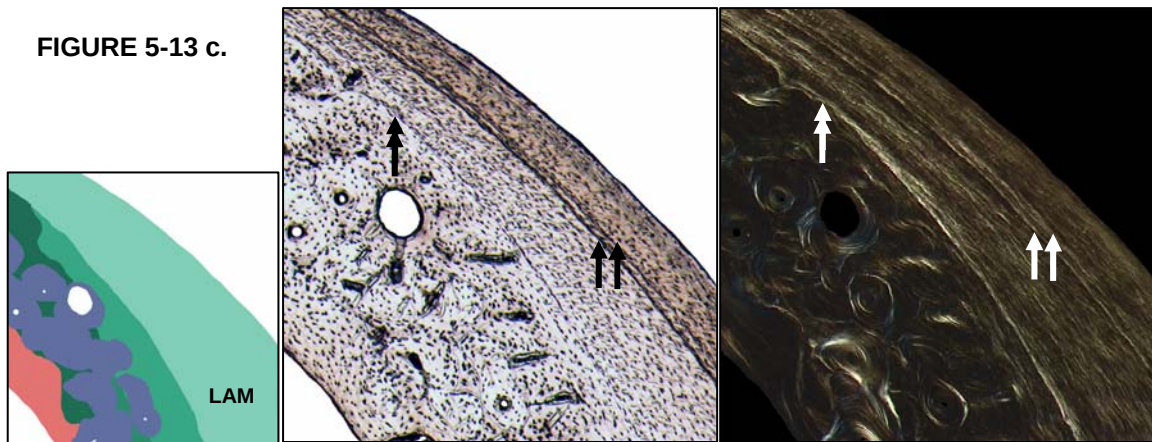


FIGURE 5-13 c. *C. aethiops* SLW 21 midshaft femur images: Growth marks in the antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image, with single arrows indicating the location of 2 (doubled) resting lines in periosteal avascular LAM cortex. The more faint cement line (located deeper in the cortex and indicated by a double-headed arrow) is continuous with a reversal line in the anterior cortex, relating to the marked posterior cortical drift indicated for this individual. *Right:* CPL image, showing moderate brightness of LAM cortex, and the slightly brighter signal of cement lines compared to surrounding tissues.

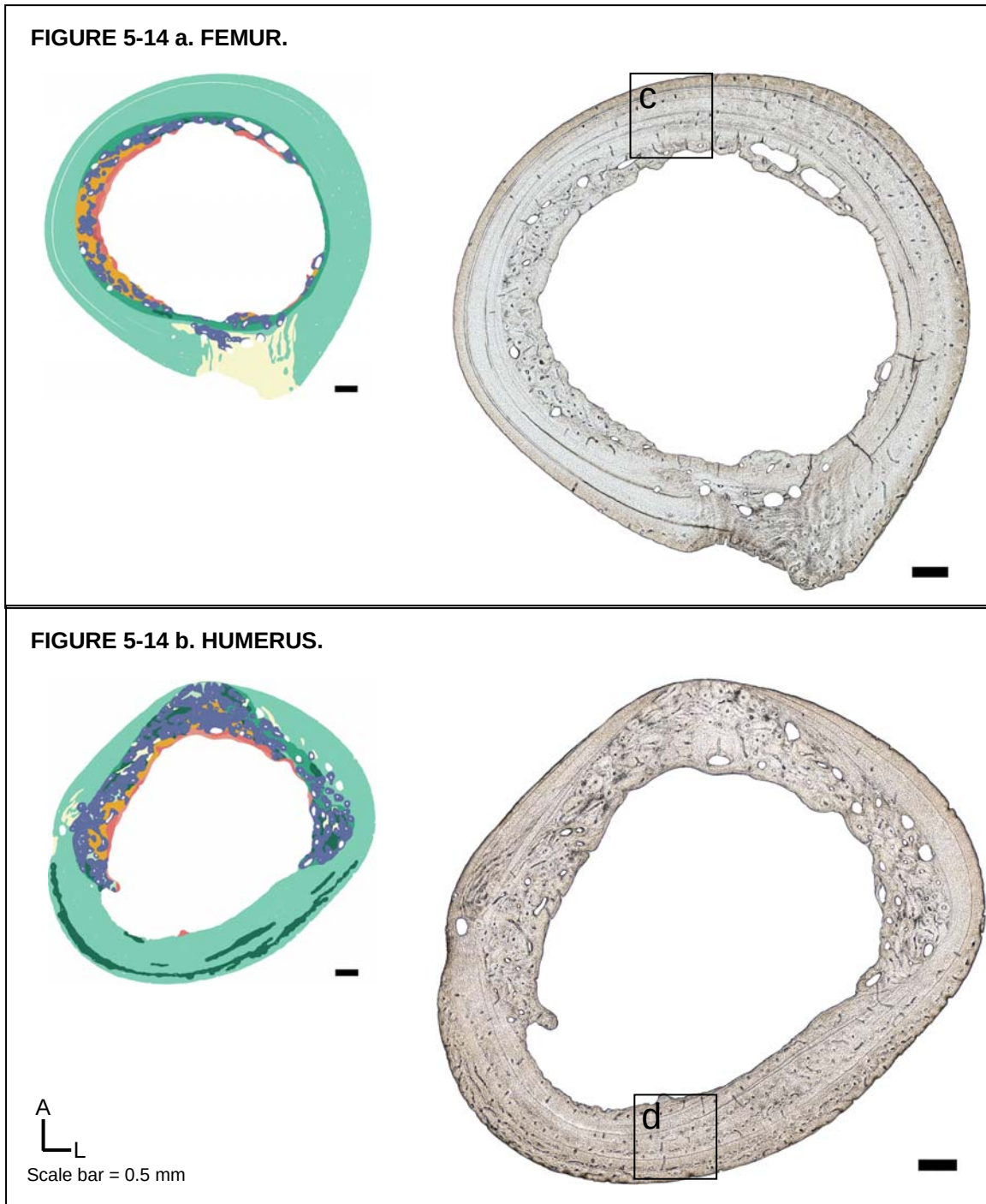


FIGURE 5-14 a and b. *C. aethiops* midshaft femur and humerus images: M2 female (SLW 34). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM images. Note the decrease in overall vascularity of periosteal cortex compared to younger individuals, particularly in the femur. Prominent resting lines are observed in both elements in this individual. Insets show location of FIGURES 5-14 c and d.

FIGURE 5-14 c.

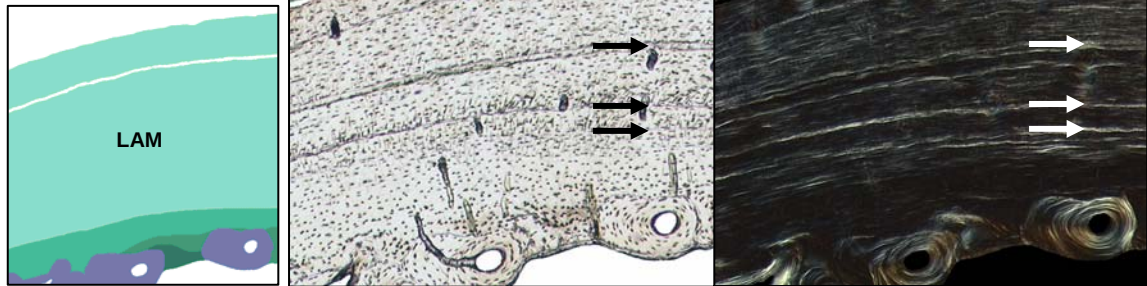


FIGURE 5-14 d.

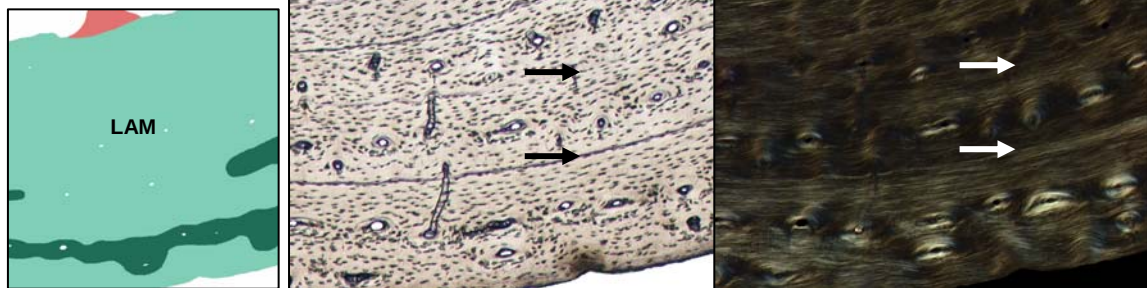


FIGURE 5-14 c. *C. aethiops* SLW 34 midshaft femur images: Growth marks in the anterior cortex. *Left:* Tissue type map. *Middle:* LM image, showing annuli and resting lines (arrows) in sparsely-vascularized LAM cortex. *Right:* CPL image. Bright appearance of resting lines is less evident in LAM tissue having a predominantly transverse collagen fiber orientation.

FIGURE 5-14 d. *C. aethiops* SLW 34 midshaft humerus images: Growth marks in the posterior cortex. *Left:* Tissue type map. *Middle:* LM image, showing annuli and resting lines (arrows). *Right:* CPL image. Growth marks exhibit a distribution similar to that observed at the midshaft femur of this individual. (Compare with FIGURE 5-14 c).

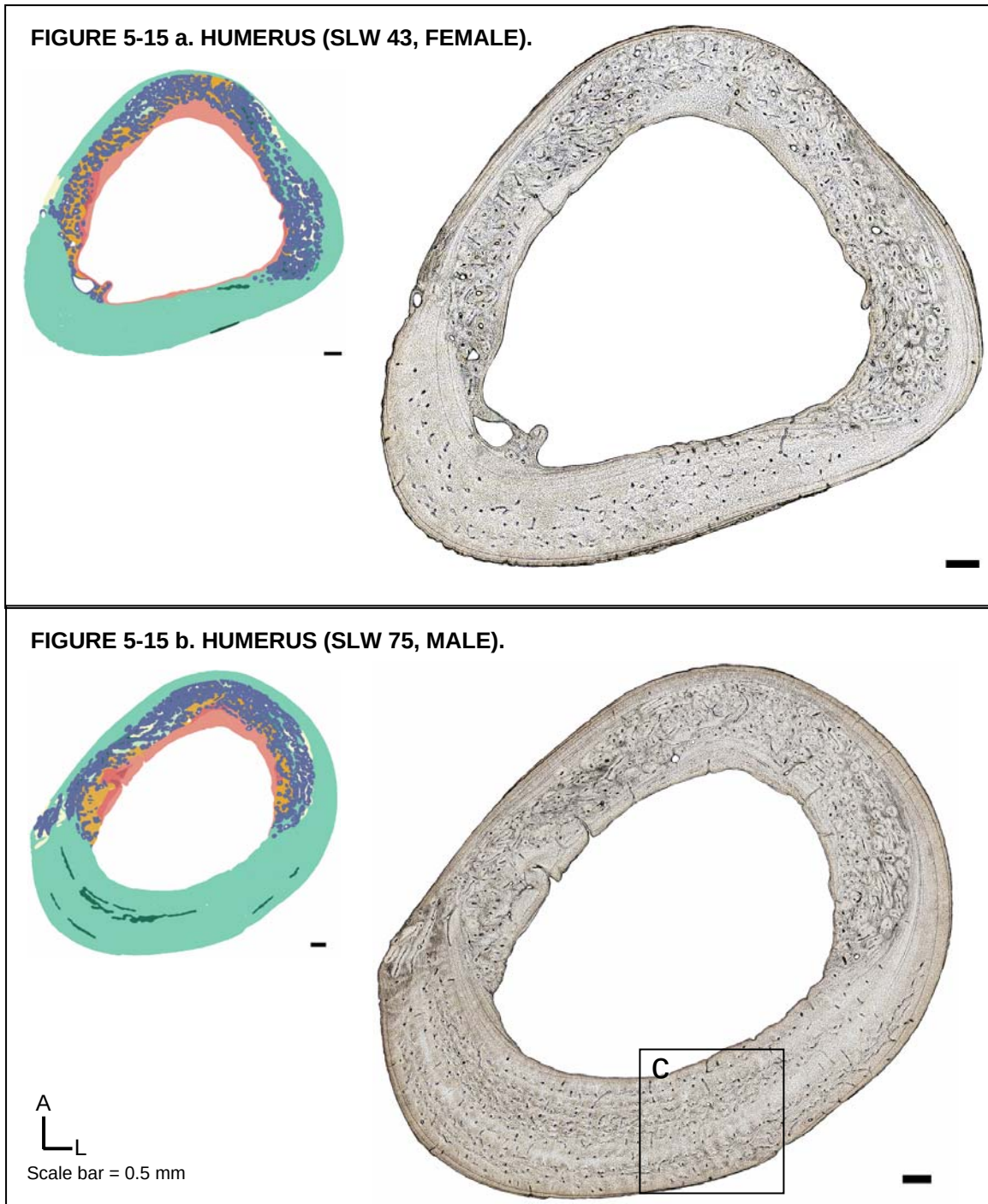


FIGURE 5-15 a and b. *C. aethiops* midshaft humerus images: Vascular patterns in EPIPH females (SLW 43) and males (SLW 75). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM images. SLW 43 and SLW 75 show a comparable degree of vascularization with other females and males, respectively, in their age class. The greater vascularity in the posterior cortex of males (SLW 75) compared to females (SLW 43) is apparent. Inset shows location of FIGURE 5-15 c.

FIGURE 5-15 c.

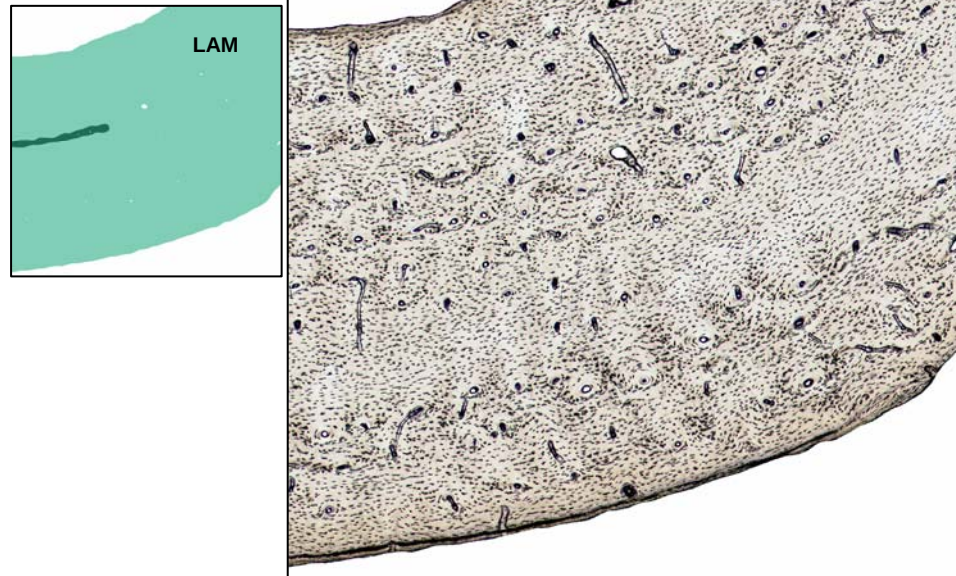


FIGURE 5-15 c. *C. aethiops* SLW 75 midshaft humerus images: Vascular patterns in the postero-medial cortex. *Left:* Tissue type map. *Top Right:* LM image, showing changes in vascularization across the thickness of the cortex. In this region, two zones of vascular LAM bone (with simple vascular canals and scattered primary osteons) alternate with zones of less vascular or avascular LAM. A similar pattern is observed in other individuals.

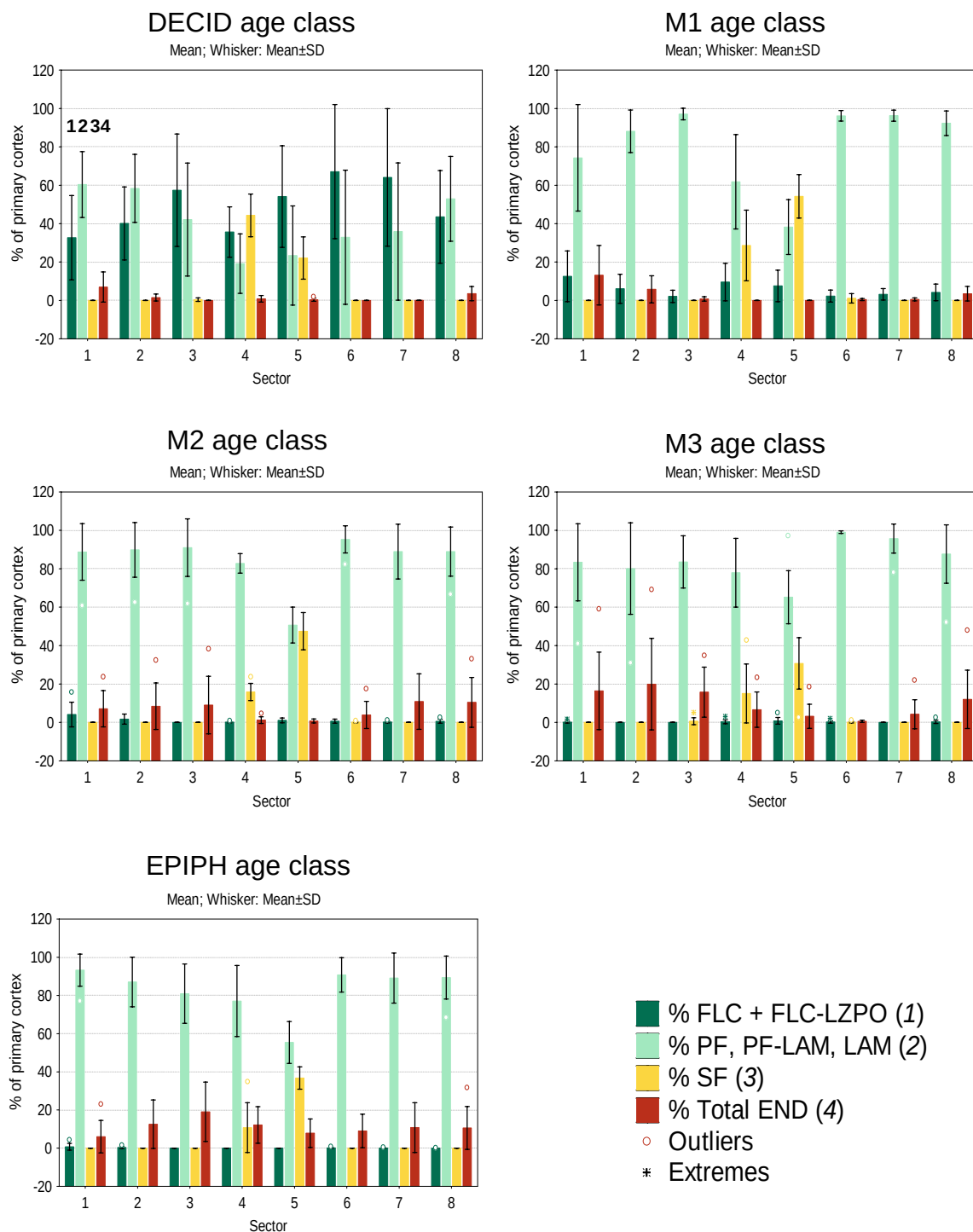


FIGURE 5-16 a. Plots of age class mean proportions of primary cortex area represented by different tissue types, across radial sectors: *C. aethiops* midshaft femur. (Ring data combined.) Fibro-lamellar (FLC, FLC-LZPO) and lamellar-zonal (PF, PF-LAM, LAM) tissue types are grouped to show general trends. Bars representing different tissue types are color coded to correspond with tissue type maps.

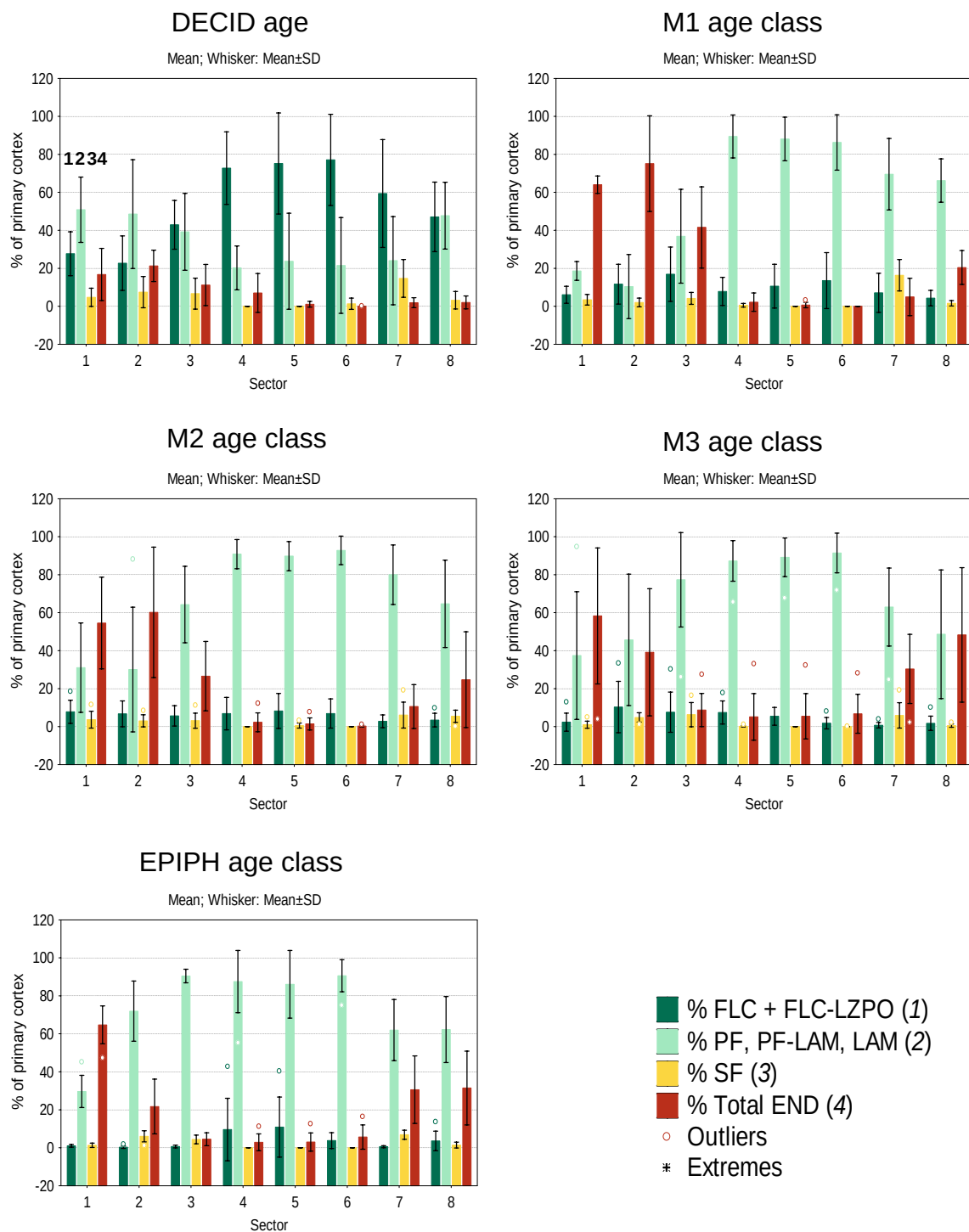


FIGURE 5-16 b. Plots of age class mean proportions of primary cortex area represented by different tissue types, across radial sectors: *C. aethiops* midshaft humerus. (Ring data combined.) Fibro-lamellar (FLC, FLC-LZPO) and lamellar-zonal (PF, PF-LAM, LAM) tissue types are grouped, to show general trends. Bars representing different tissue types are color coded to correspond with tissue type maps.

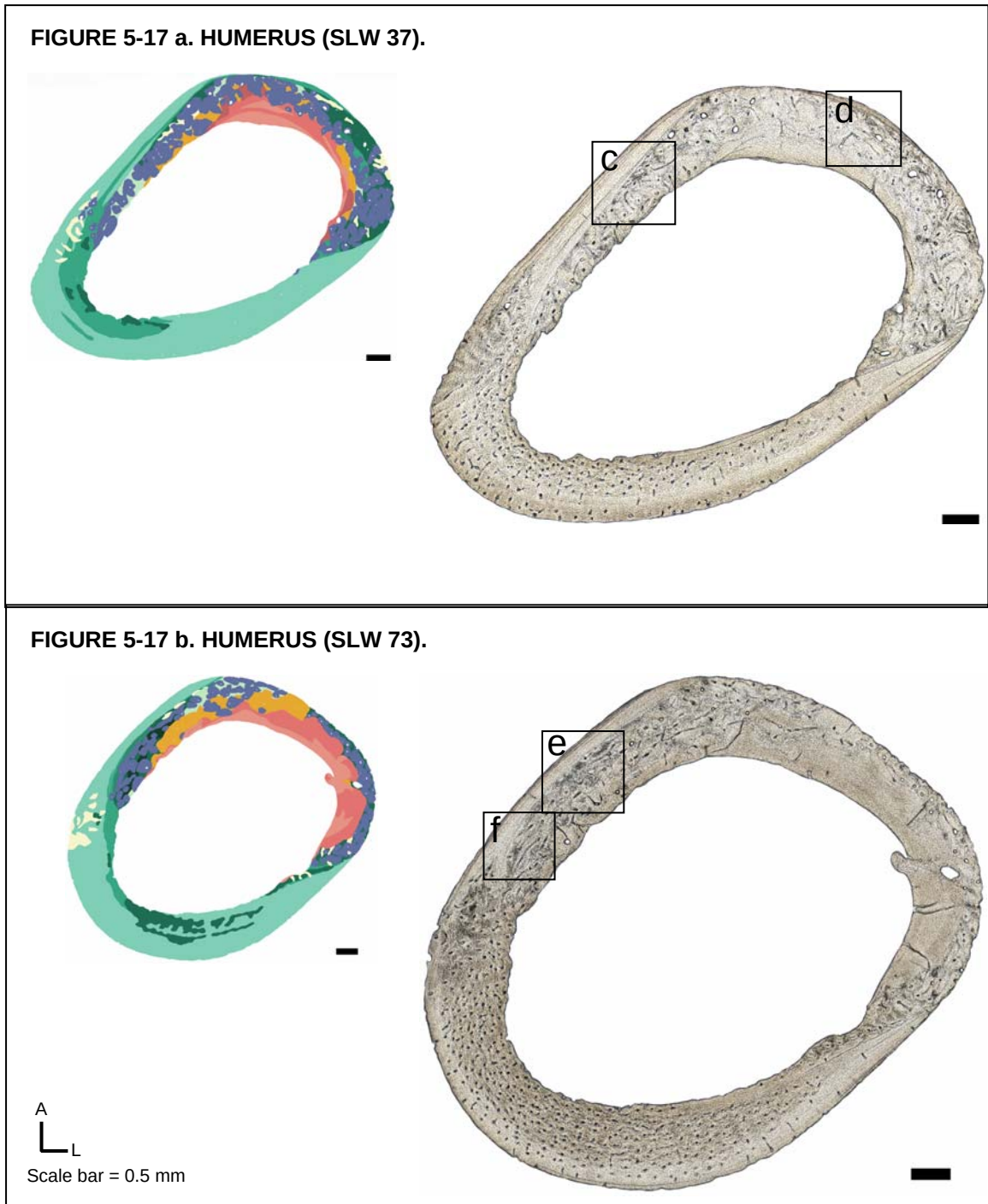


FIGURE 5-17 a and b. *C. aethiops* midshaft humerus images: Evidence of cortical drift in the M1 age class (SLW 37 and SLW 73). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM images. Insets show location of FIGURES 5-17 c – f.

FIGURE 5-17 c.

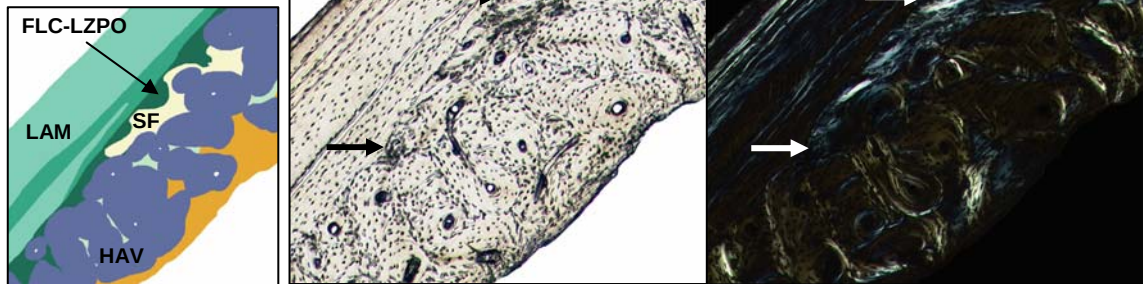


FIGURE 5-17 d.

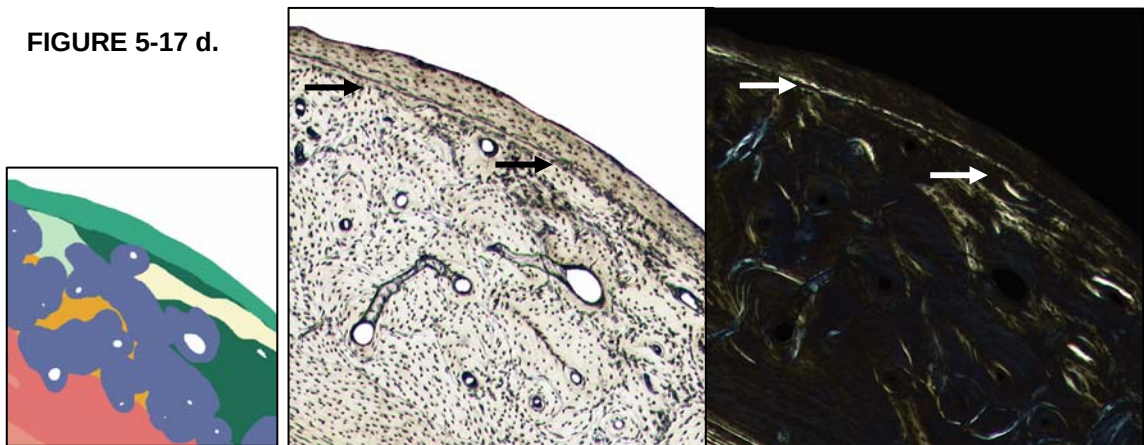


FIGURE 5-17 c. *C. aethiops* SLW 37 midshaft humerus images: Reversal in the antero-medial cortex. *Left:* Tissue type map. *Middle:* LM image. Arrows indicate the scalloped contour of a reversal line between an earlier period of FLC-LZPO deposition and a later period of LAM deposition. Sharpey Fibers in this region are indicative of bone of periosteal origin, though the boundary between endosteal and periosteal tissue is difficult to locate in areas of dense HAV. (Non-HAV is classified as INT of unknown origin in such regions.) *Right:* CPL image, showing discontinuity in collagen fiber matrix organization at the reversal line.

FIGURE 5-17 d. *C. aethiops* SLW 37 midshaft humerus images: Reversal in the antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image. Arrows indicate antero-lateral extent of reversal line shown in Figure 5-17 c. *Right:* CPL image.

FIGURE 5-17 e.

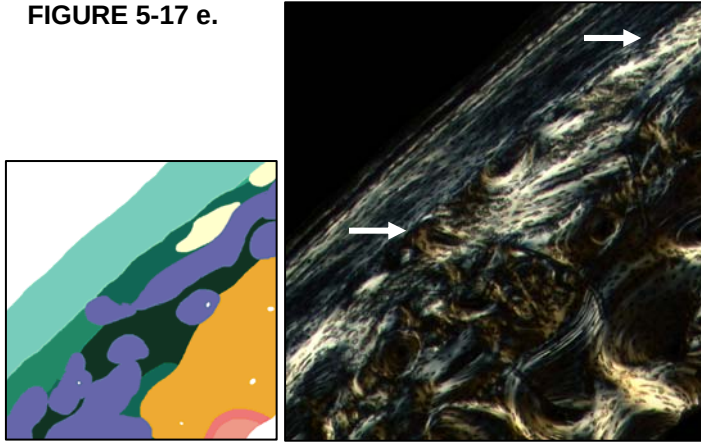


FIGURE 5-17 f.

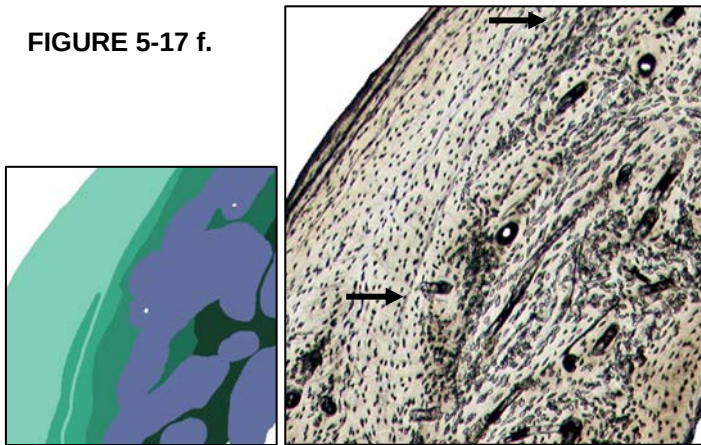


FIGURE 5-17 e. *C. aethiops* SLW 73 midshaft humerus images: Reversal in the antero-medial cortex. *Left:* Tissue type map. *Right:* CPL image, showing a discontinuity in collagen fiber matrix organization on either side of a reversal line (indicated by an arrow).

FIGURE 5-17 f. *C. aethiops* SLW 73 midshaft humerus images: Medial cortex. *Left:* Tissue type map. *Right:* LM image. The reversal region shown in FIGURE 5-17 e takes on the smooth appearance of a resting line as it continues medially (arrows), before disappearing entirely (towards the bottom of this image).

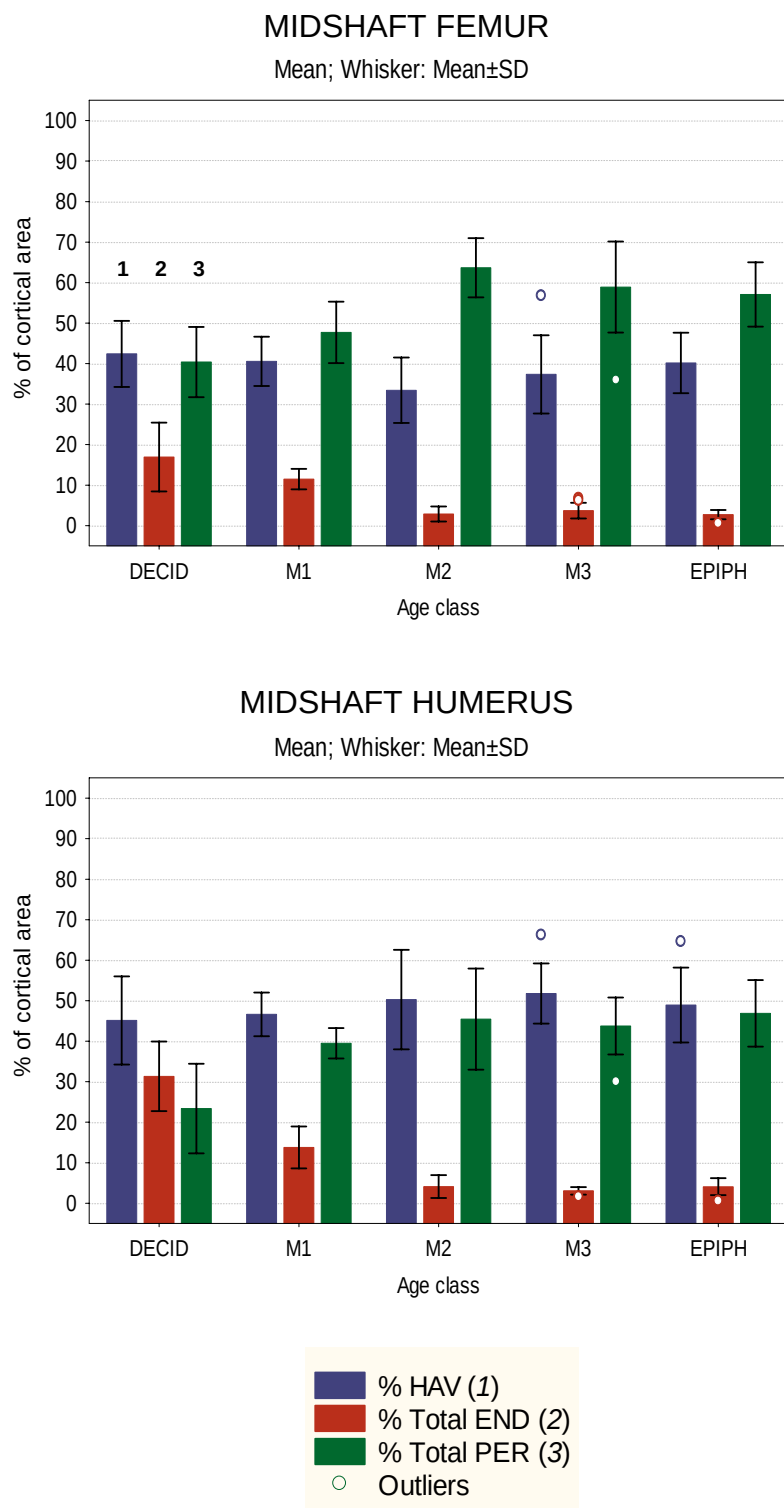


FIGURE 5-18. Plots of age class mean proportions of total cortical area represented by endosteal (END), periosteal (PER) and secondary osteonal (HAV) tissue: *H. lar* midshaft femur and humerus. Bars representing different tissue types are color coded to correspond with tissue type maps.

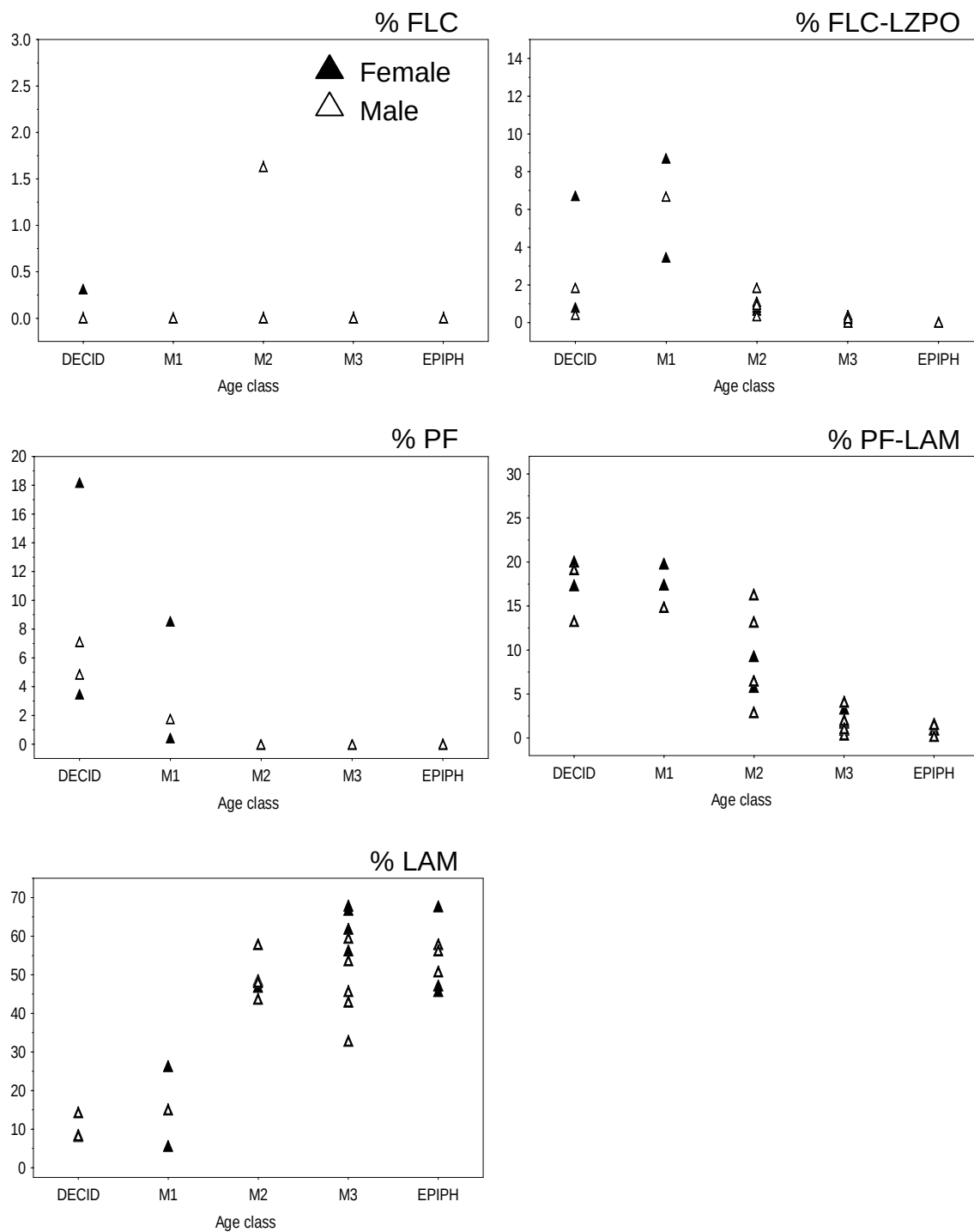


FIGURE 5-19 a. Scatterplots of periosteal tissue type proportions by age class, categorized by sex: *H. lar* midshaft femur.

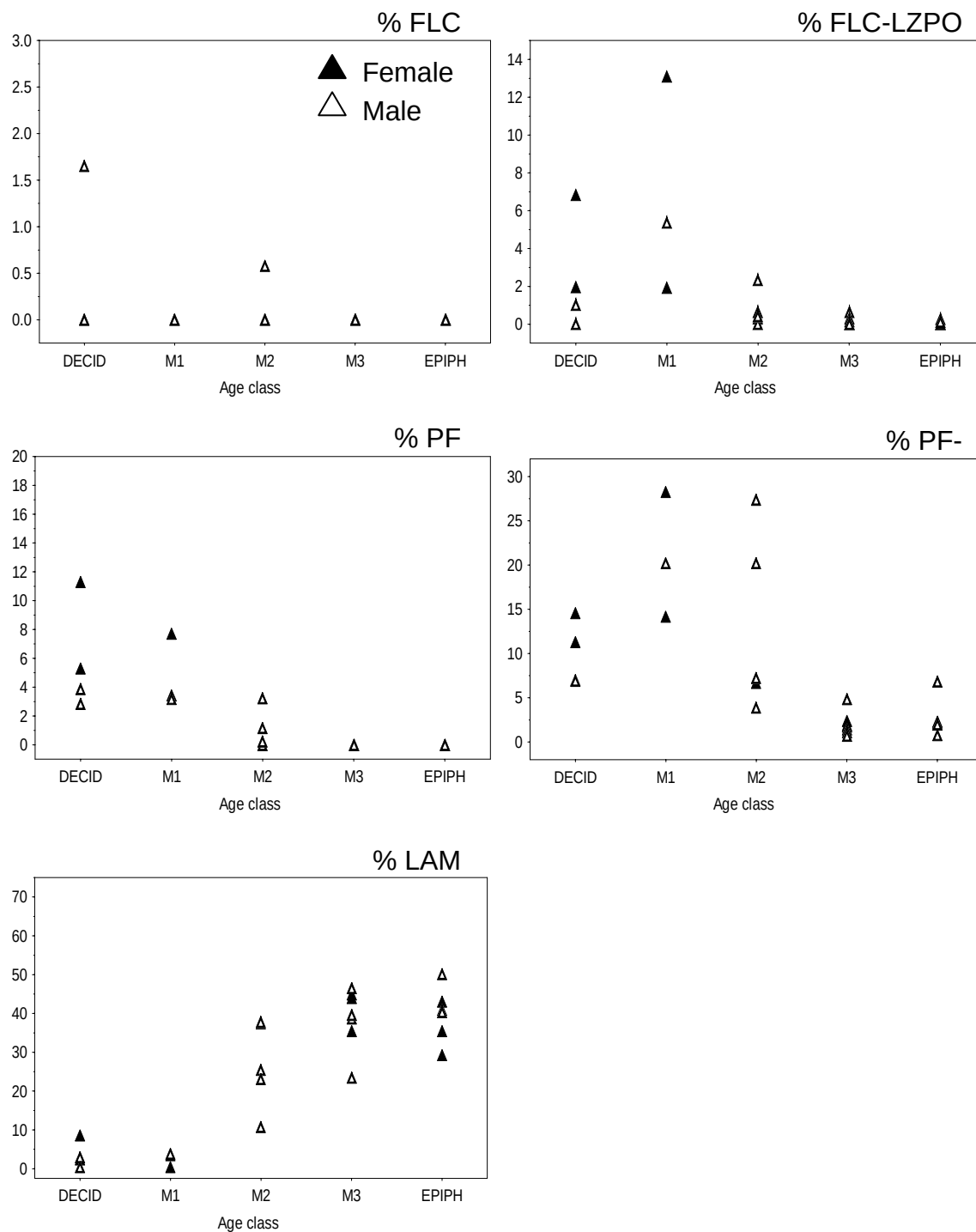


FIGURE 5-19 b. Scatterplots of periosteal tissue type proportions by age class, categorized by sex: *H. lar* midshaft humerus.

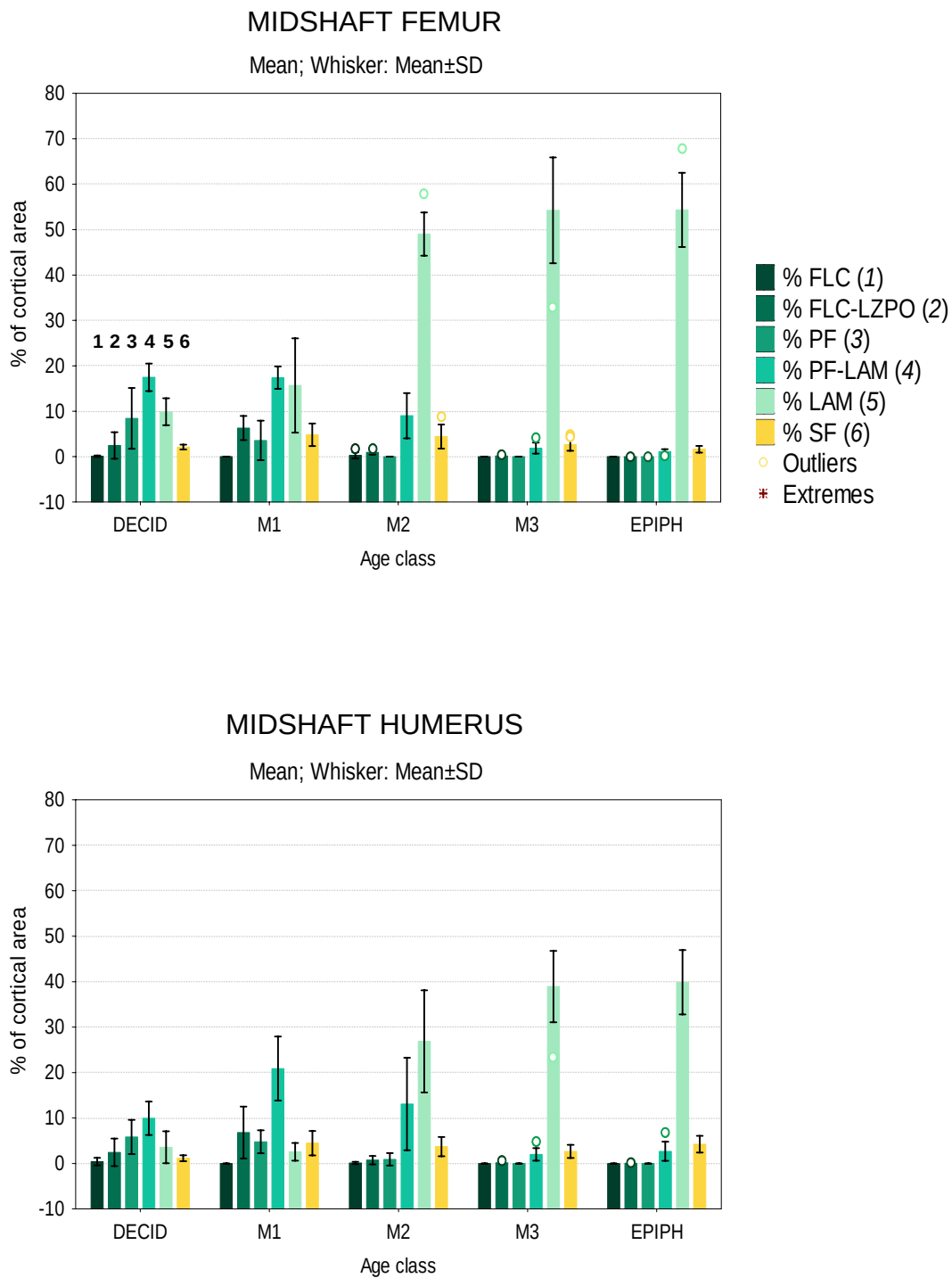


FIGURE 5-20. Plots of age class mean proportions of total cortical area represented by primary periosteal tissue types: *H. lar* midshaft femur and humerus. Bars representing different tissue types are color coded to correspond with tissue type maps.

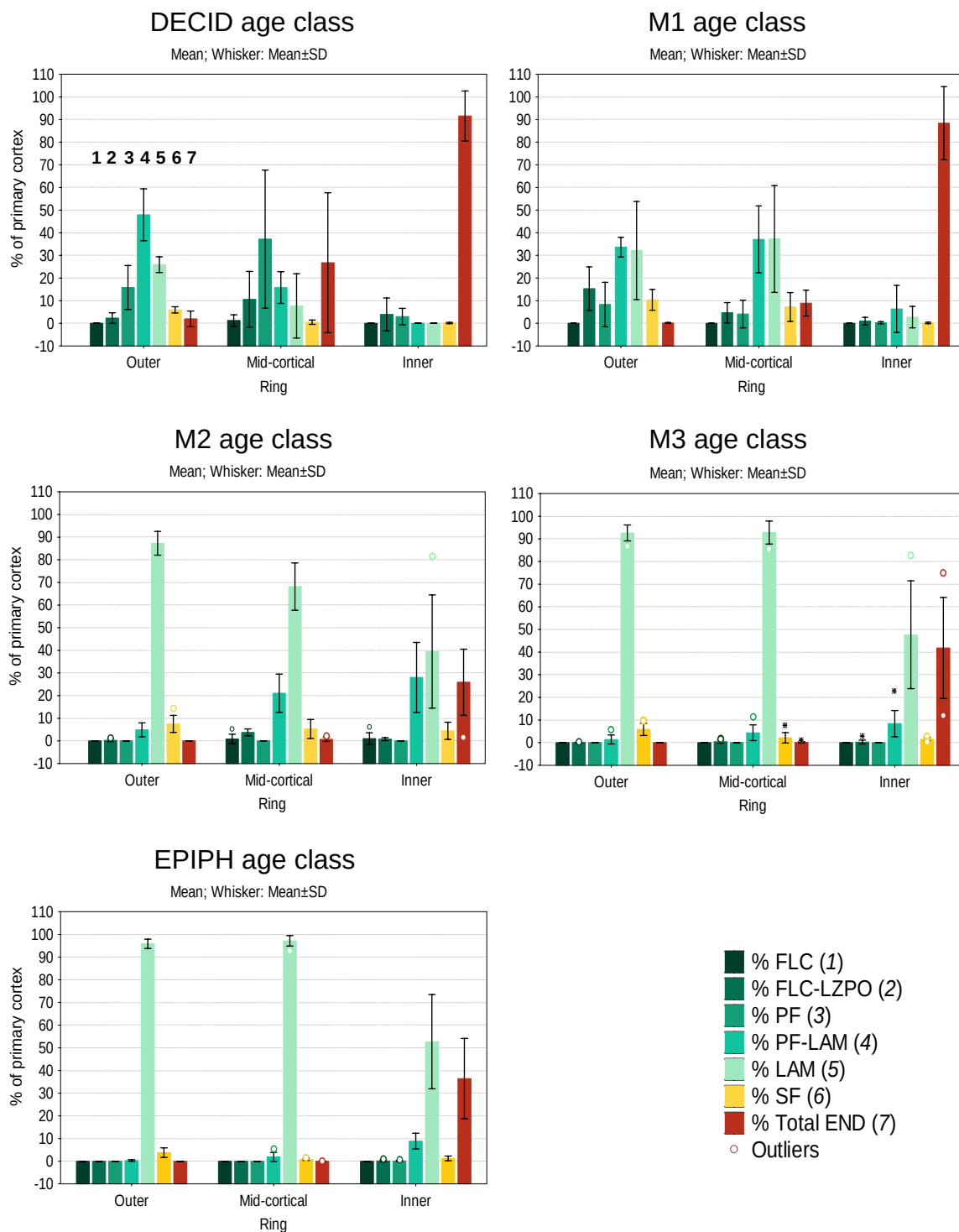


FIGURE 5-21 a. Plots of age class mean proportions of primary cortex area represented by different tissue types, across circumferential rings: *H. lar* midshaft femur. Earlier-deposited periosteal tissues (and, to a variable extent, endosteal tissues) are represented in the inner cortical ring, while recently-deposited periosteal tissues are represented in the outer cortical ring. Bars representing different tissue types are color coded to correspond with tissue type maps.

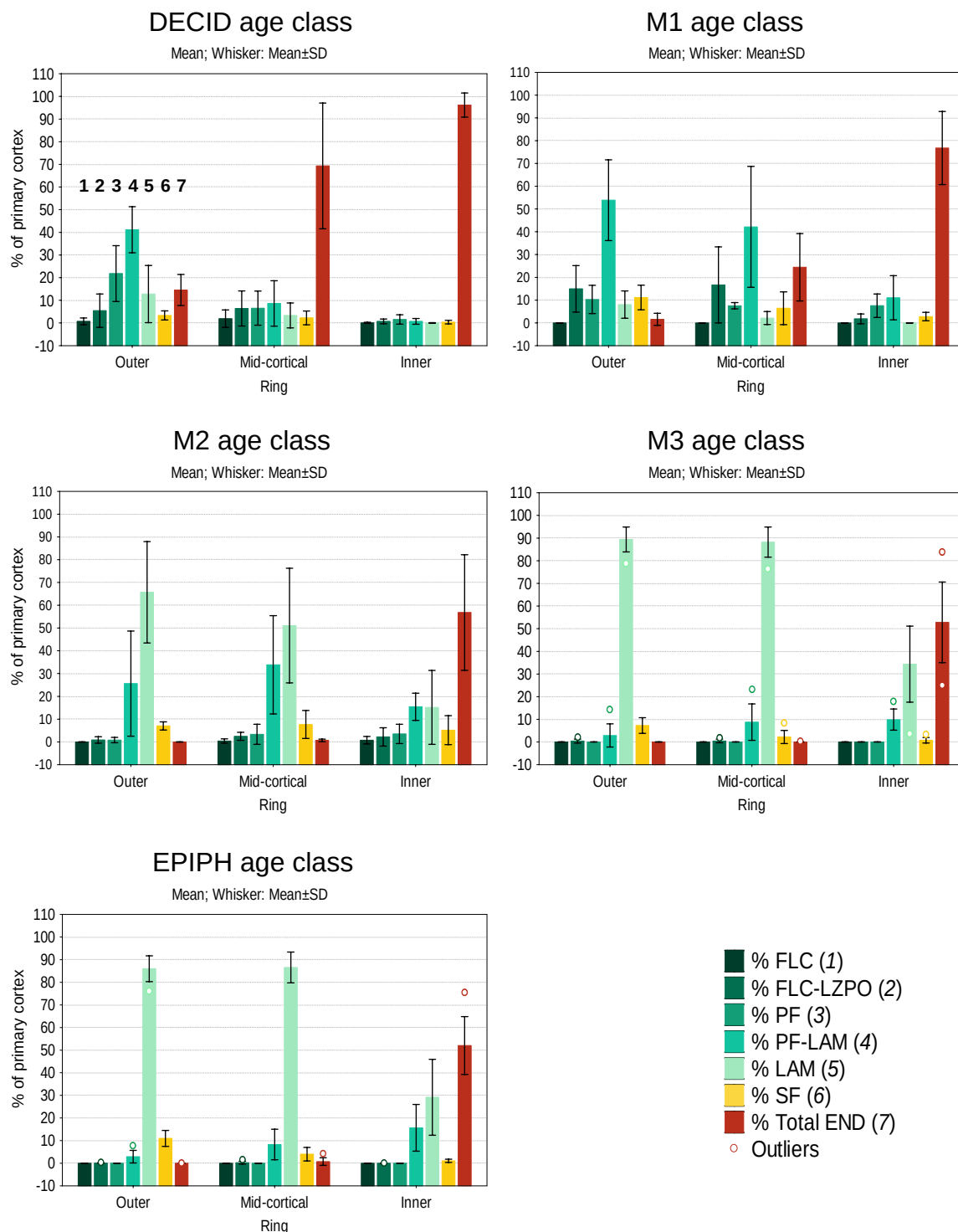


FIGURE 5-21 b. Plots of age class mean proportions of primary cortex area represented by different tissue types, across circumferential rings: *H. lar* midshaft humerus. Earlier-deposited periosteal tissues (and, to a variable extent, endosteal tissues) are represented in the inner cortical ring, while recently-deposited periosteal tissues are represented in the outer cortical ring. Bars representing different tissue types are color coded to correspond with tissue type maps.

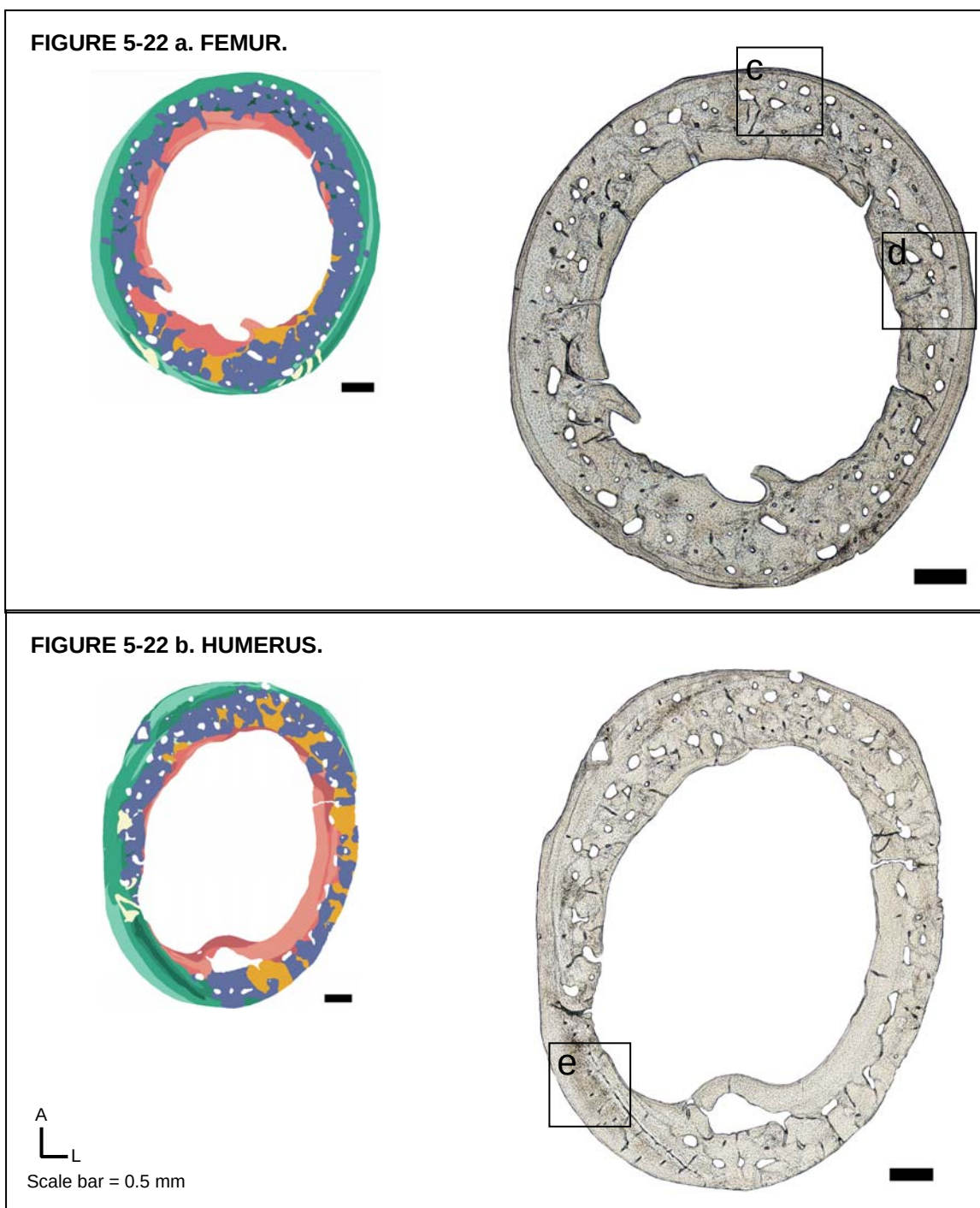


FIGURE 5-22 a and b. *H. lar* midshaft femur and humerus images: DECID female (MCZ 41462). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as depicted in Figure 5-2. *Right:* LM images. Note the relatively sparse vascularization of periosteal cortex. Insets show location of FIGURES 5-22 c, d, and e.

FIGURE 5-22 c.

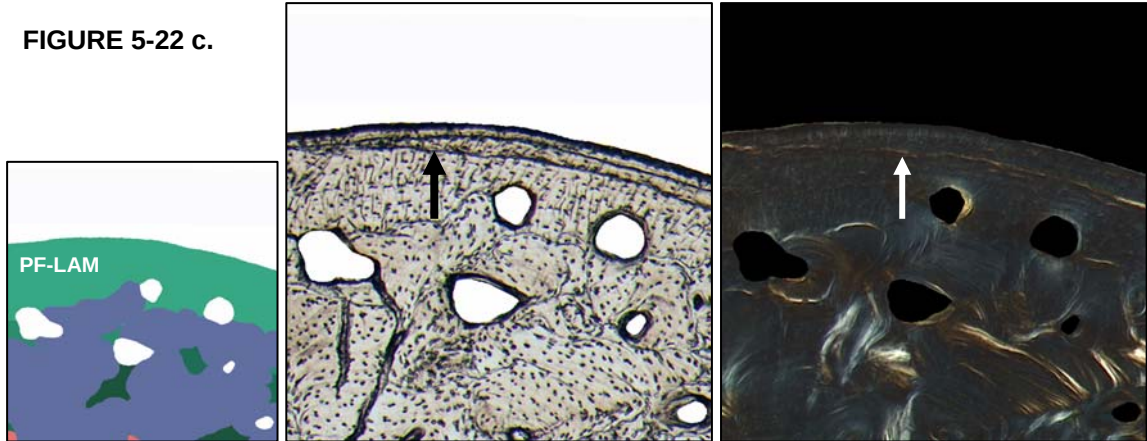


FIGURE 5-22 d.

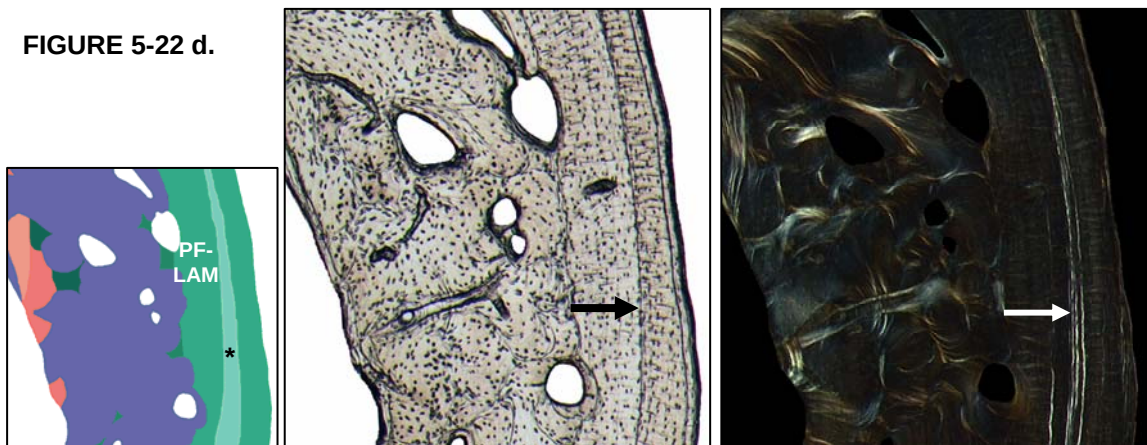


FIGURE 5-22 c. *H. lar* MCZ 41462 midshaft femur images: Reversal in the anterior cortex. *Left:* Tissue type map. *Middle:* LM image, showing the scalloped contour of a reversal line in PF-LAM cortex, indicated by arrows. *Right:* CPL image, showing predominantly dark appearance of PF-LAM. The reversal line has a brighter signal in CPL.

FIGURE 5-22 d. *H. lar* MCZ 41462 midshaft femur images: Lateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing lateral continuation of reversal line shown in Figure 5-22 c. The reversal line shown in FIGURE 5-22 c takes on a smooth contour as it passes laterally and posteriorly, suggesting that it represents a temporal discontinuity between cycles of deposition in this region. Lamellar-zonal cortex is sparsely vascularized, and is comprised of PF-LAM interrupted by a period of LAM deposition (*). *Right:* CPL image, showing predominantly dark appearance of lamellar-zonal cortex, with the exception of LAM having a brighter CPL signal. The cement line is associated with this brief period of LAM deposition.

FIGURE 5-22 e.



FIGURE 5-22 e. *H. lar* MCZ 41462 midshaft humerus images: FLC-LZPO in the postero-medial cortex. *Left:* Tissue type map. *Middle:* LM image, showing avascular lamellar-zonal cortex interrupted by a period of FLC-LZPO deposition (*). The latter is characterized by predominantly longitudinal vascularization with occasional circular canals and irregular anastomoses. *Right:* CPL image.

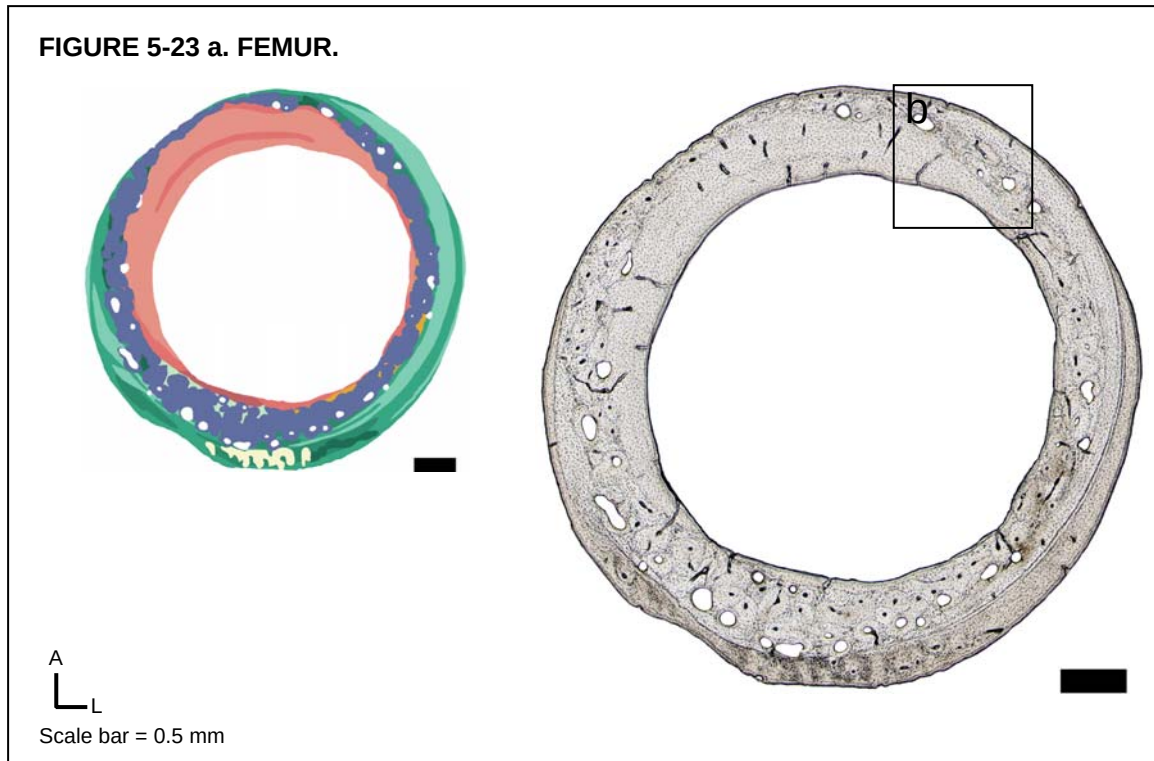


FIGURE 5-23 a. *H. lar* midshaft femur images: DECID male (MCZ 35456). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image. This specimen shows a pattern of postero-lateral cortical drift. Inset shows location of FIGURE 5-23 b.

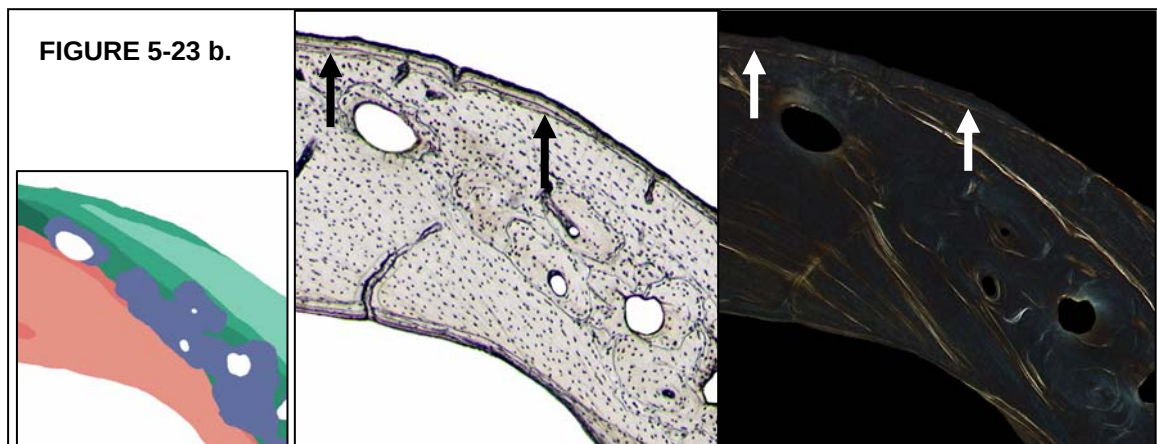


FIGURE 5-23 b. *H. lar* MCZ 35456 midshaft femur images: Reversal in the antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image. Arrows indicate scalloped contour of reversal line. *Right:* The reversal line appears bright in CPL. Discontinuous layers of PF-LAM and LAM can also be observed on either side.

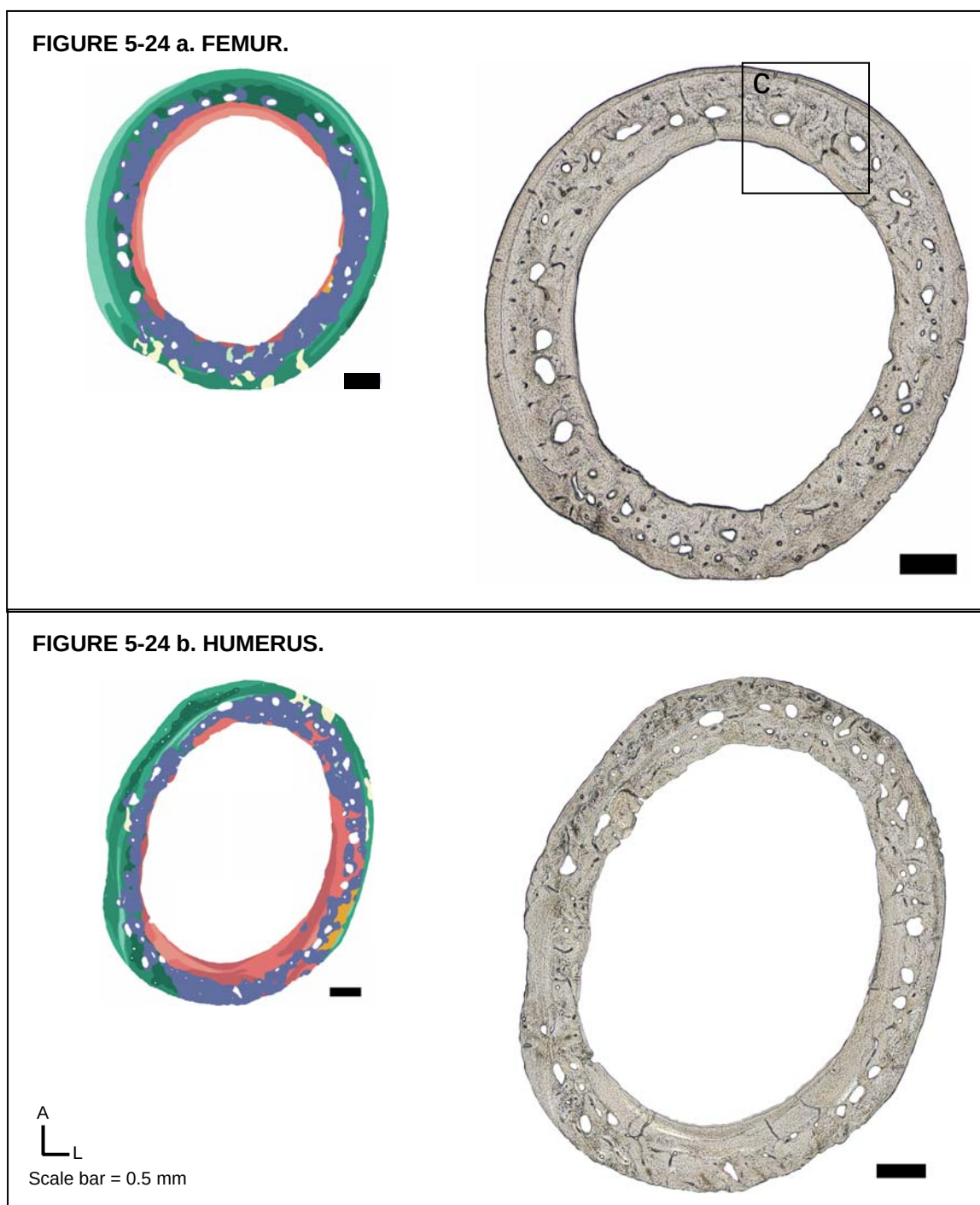


FIGURE 5-24 a and b. *H. lar* midshaft femur and humerus images: DECID female (MCZ 41466). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as depicted in Figure 5-2. *Right:* LM images. Inset shows location of FIGURE 5-24 c.

FIGURE 5-24 c.

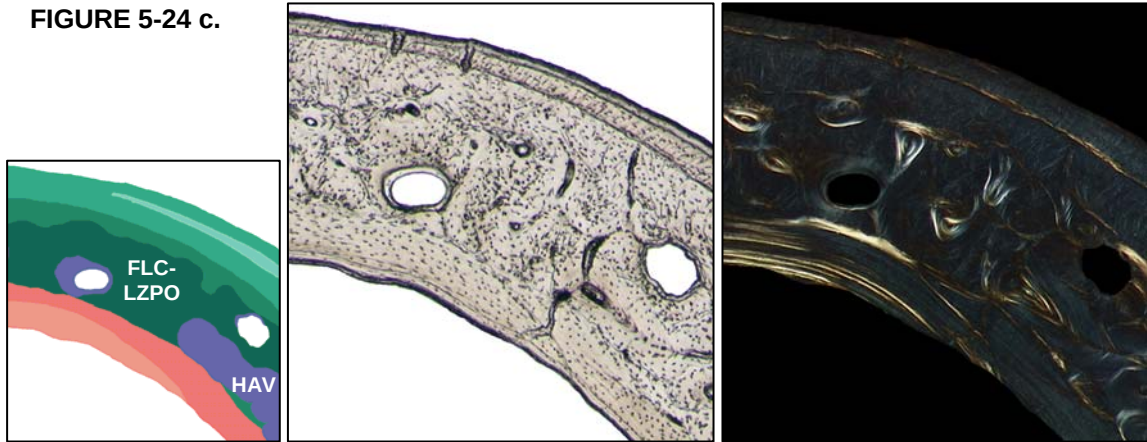


FIGURE 5-24 c. *H. lar* MCZ 41466 midshaft femur images: FLC-LZPO in the antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing FLC-LZPO with primary osteons having a predominantly longitudinal orientation. This tissue is located in a region also undergoing secondary intracortical remodeling (HAV). Large resorption spaces can also be observed. *Right:* CPL image.

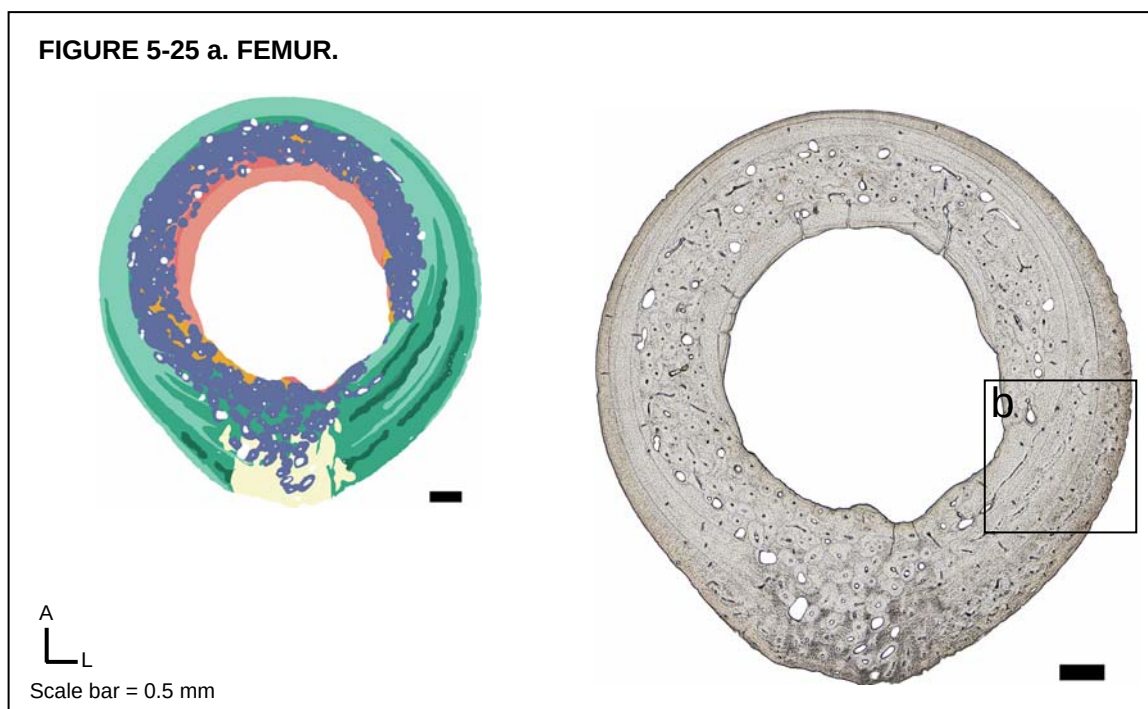


FIGURE 5-25 a. *H. lar* midshaft femur images: M1 female (MCZ 41502). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image. Note the distribution of END and PER tissues, indicating postero-lateral cortical drift. Inset shows location of FIGURE 5-25 b.

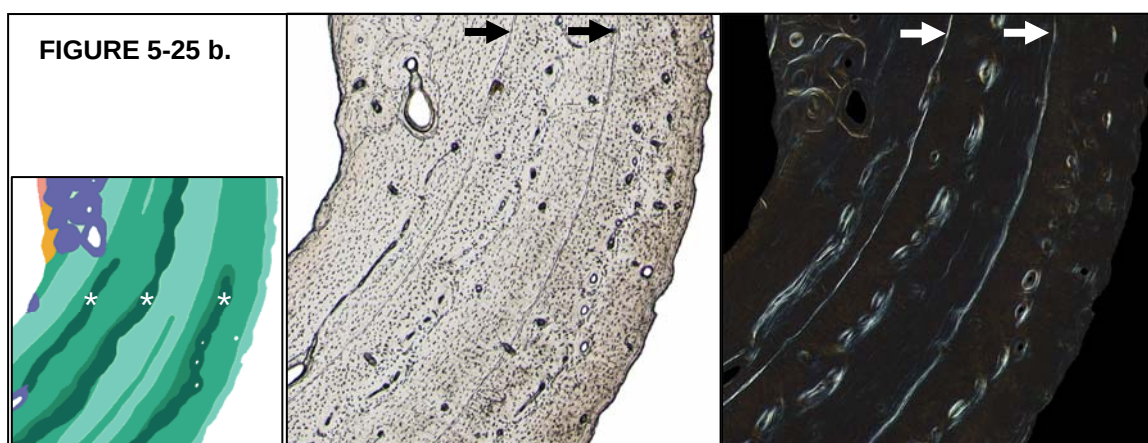


FIGURE 5-25 b. *H. lar* MCZ 41502 midshaft femur images: Postero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing lamellar-zonal cortex, interrupted by three periods of FLC-LZPO deposition (*). Arrows indicate the location of two resting lines. These structures are continuous with reversal lines anteriorly. *Right:* CPL image.

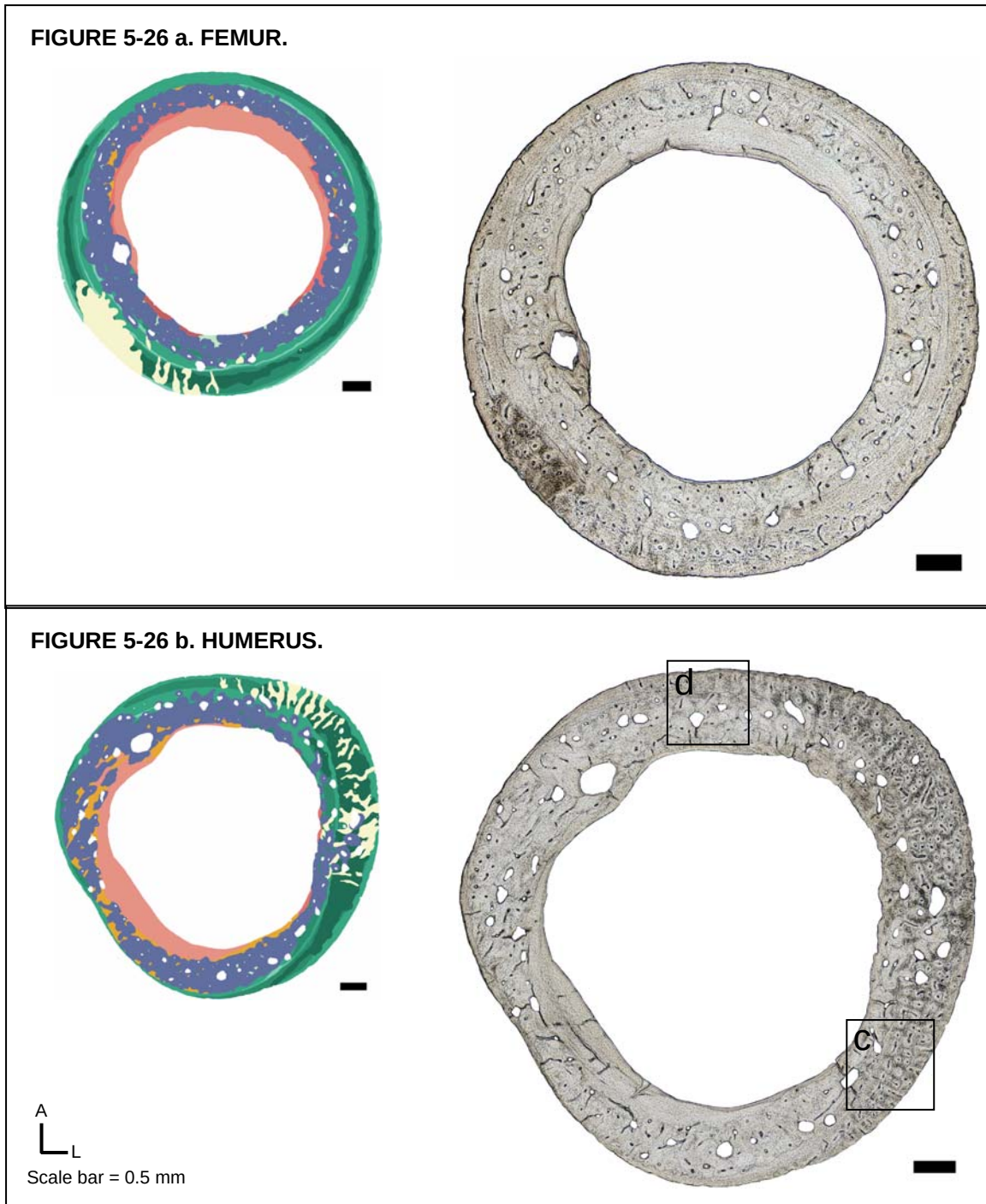


FIGURE 5-26 a and b. *H. lar* midshaft femur and humerus images: M1 female (MCZ 41482). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM images. The distribution of END and PER tissues in this individual indicates cortical drift in a predominantly antero-lateral direction. Insets show location of FIGURES 5-26 c and d.

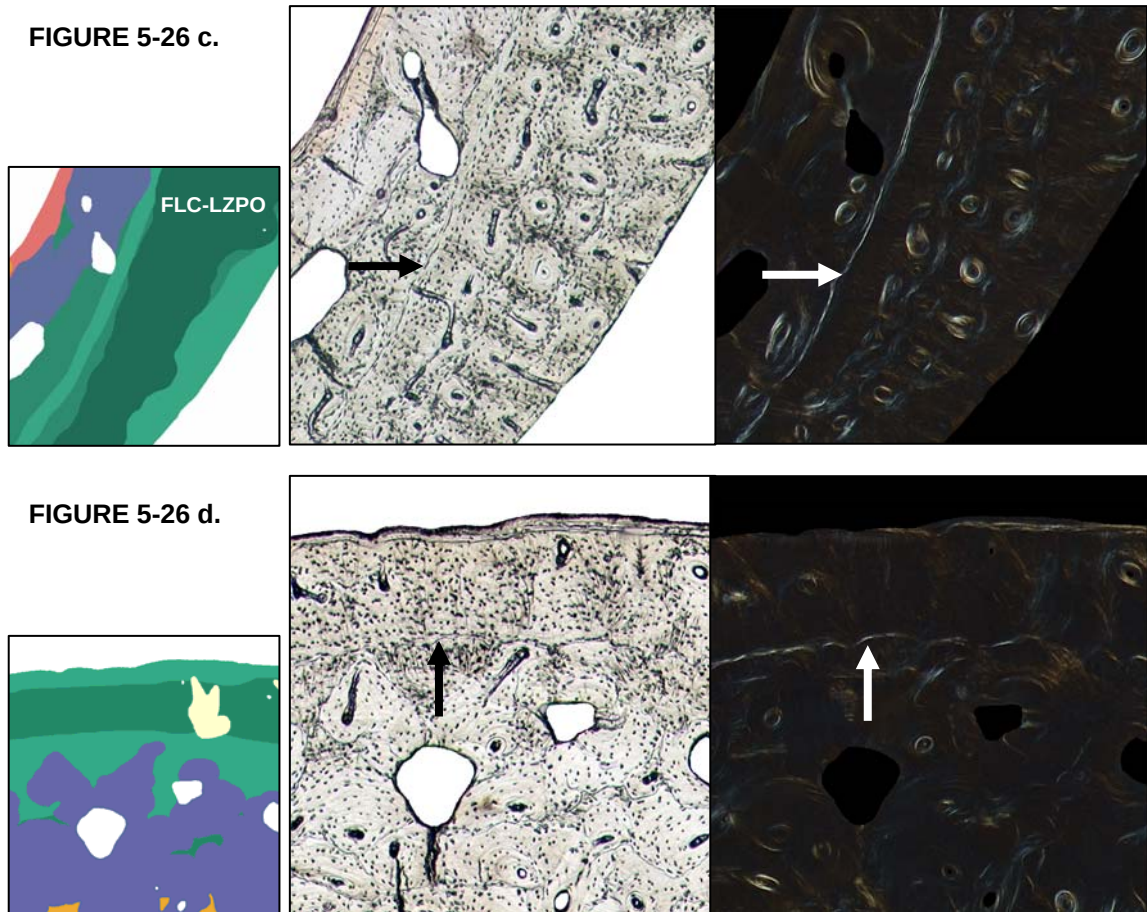


FIGURE 5-26 c. *H. lar* MCZ 41482 midshaft humerus images: FLC-LZPO in the postero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image showing the predominantly longitudinal vascular pattern of FLC-LZPO, and the presence of a smooth-contoured resting line (arrow). The latter is continuous with a reversal line anteriorly (Figure 6-26 d), and may reflect evidence of metaphyseal remodeling history. *Right:* CPL image, showing predominantly dark appearance of the extra-osteonal matrix. The resting line appears bright in CPL.

FIGURE 5-26 d. *H. lar* MCZ 41482 midshaft humerus: Reversal in the anterior cortex. *Left:* Tissue type map. *Middle:* LM showing the scalloped contour of a reversal line (arrow) in primary periosteal cortex. *Right:* CPL image, showing the brighter signal of the reversal line relative to adjacent tissue.

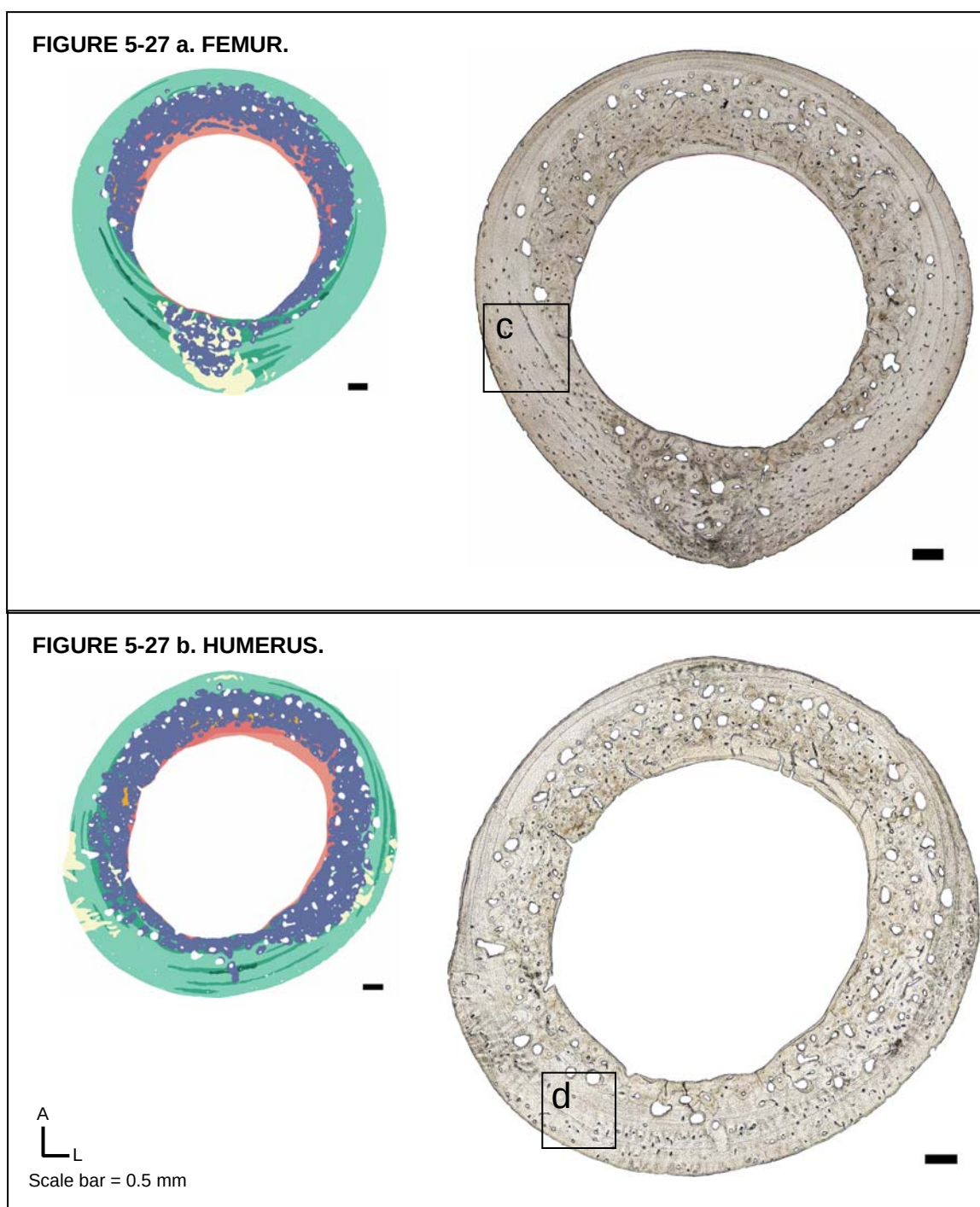


FIGURE 5-27 a and b. *H. lar* midshaft femur and humerus images: M2 female (MCZ 41452). *Left:* Whole cross-section tissue type maps. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM images. Insets show location of FIGURES 5-27 c and d.

FIGURE 5-27 c.

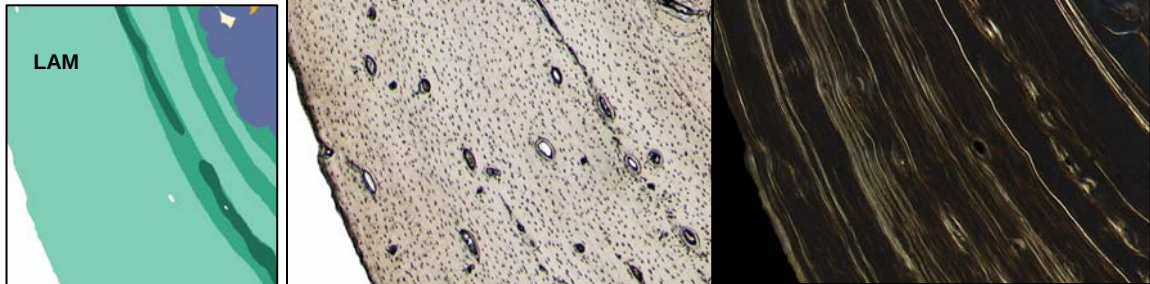


FIGURE 5-27 d.

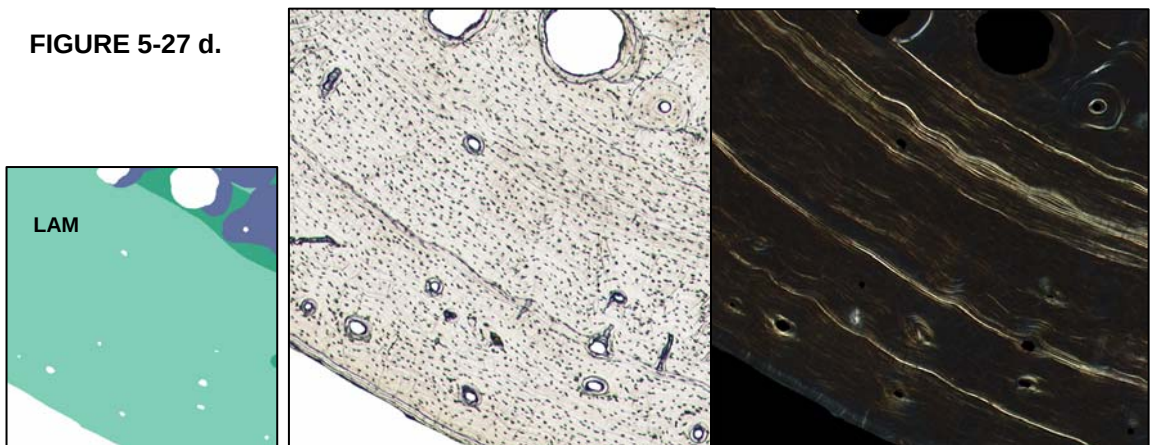


FIGURE 5-27 c. *H. lar* MCZ 41452 midshaft femur images: Polarization contrast of LAM tissue in the medial cortex. Primary cortex is comprised largely of LAM tissue. *Left:* Tissue type map. *Middle:* LM image, showing sparse vascularization by scattered small primary osteons and simple vascular canals. *Right:* CPL image, showing LAM matrix with variable brightness.

FIGURE 5-27 d. *H. lar* MCZ 41452 midshaft humerus images: Polarization contrast of LAM tissue in the postero-medial cortex. *Left:* Tissue type map. Primary cortex is comprised largely of LAM tissue. *Middle:* LM image. *Right:* CPL image, showing LAM matrix with alternating dark and bright appearance.

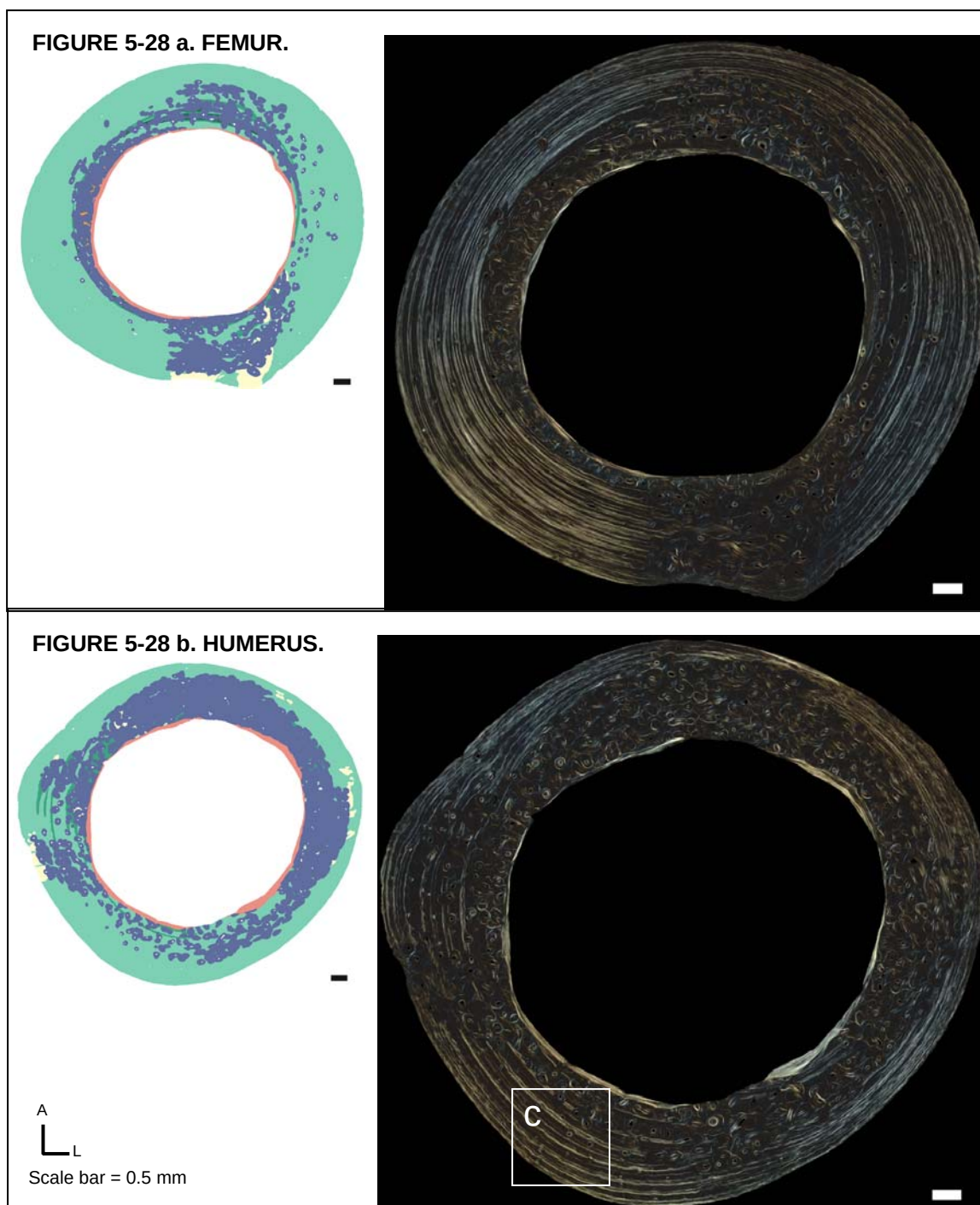


FIGURE 5-28 a and b. Midshaft femur and humerus of *H. lar*: M3 FEMALE (SLW 10).
Left: Whole cross-section tissue type maps. Colors denote different tissue types, as shown in Figure 5-2. *Right:* CPL images. Inset shows location of FIGURE 5-28 c.

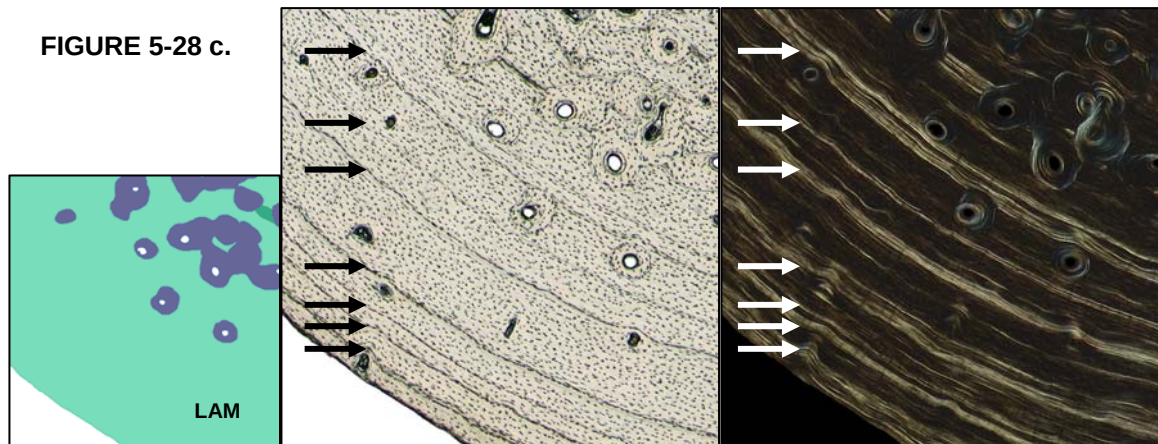
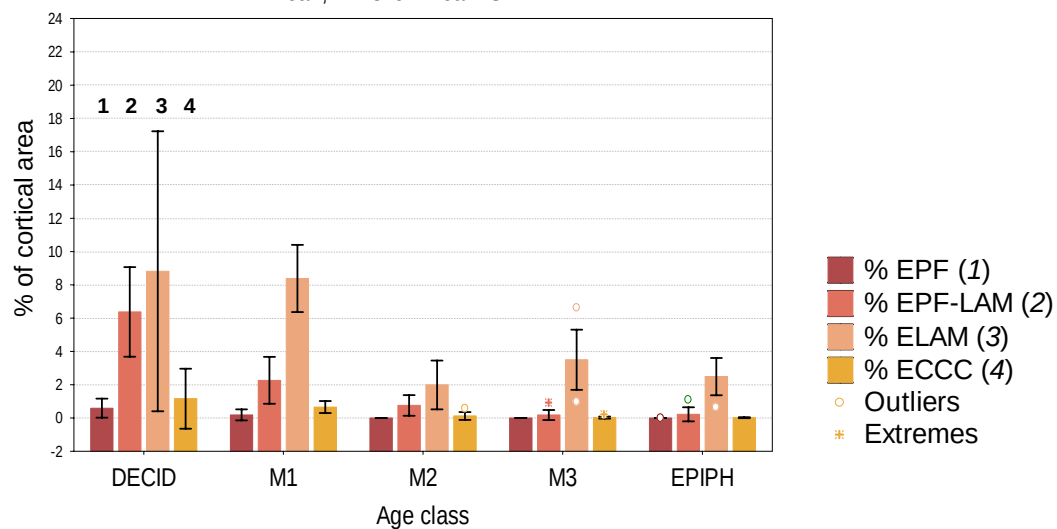


FIGURE 5-28 c. *H. lar* LSW 10 midshaft humerus images: Growth marks and LAM tissue in the postero-lateral cortex. Primary cortex is comprised largely of LAM tissue of varying brightness in CPL. *Left:* Tissue type map. *Middle:* LM image, showing sparse vascularization by scattered simple vascular canals. *Right:* CPL image, showing LAM matrix with alternating dark and bright appearance. Bright lamellar patches are frequently associated with resting lines, as indicated by arrows.

MIDSHAFT FEMUR

Mean; Whisker: Mean $\pm$ SD

MIDSHAFT HUMERUS

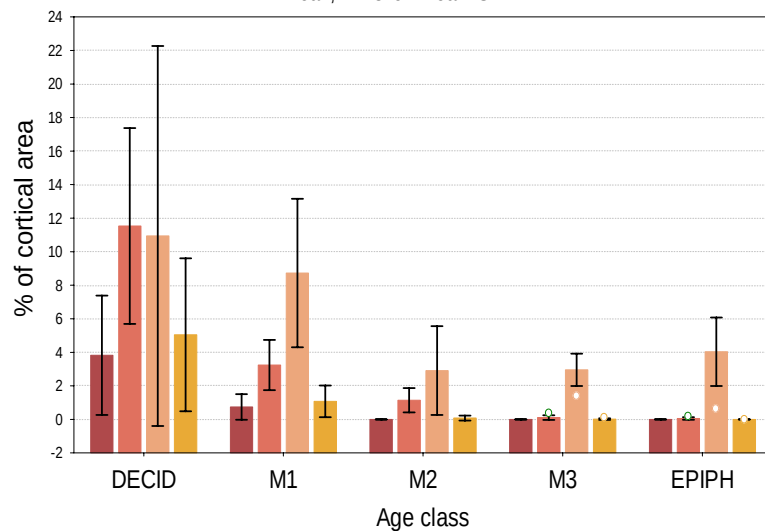
Mean; Whisker: Mean $\pm$ SD

FIGURE 5-29. Plots of age class mean proportions of total cortical area represented by endosteal tissue types *H. lar* midshaft femur and humerus. Bars representing different tissue types are color coded to correspond with tissue type maps.

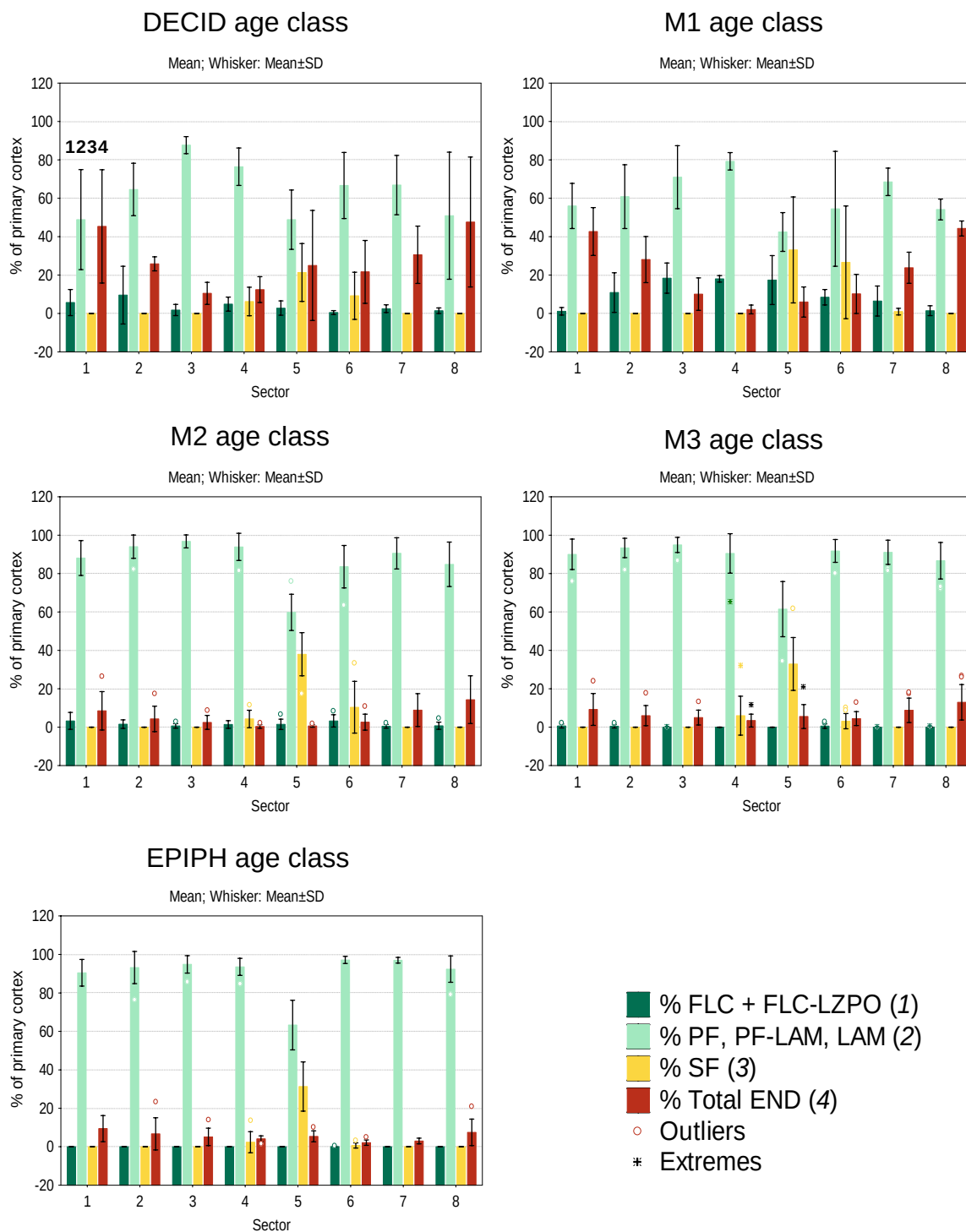


FIGURE 5-30 a. Plots of age class mean proportions of primary cortex area represented by different tissue types, across radial sectors: *H. lar* midshaft femur. (Ring data combined.) Fibro-lamellar (FLC, FLC-LZPO) and lamellar-zonal (PF, PF-LAM, LAM) tissue types are grouped, to show general trends. Bars representing different tissue types are color coded to correspond with tissue type maps.

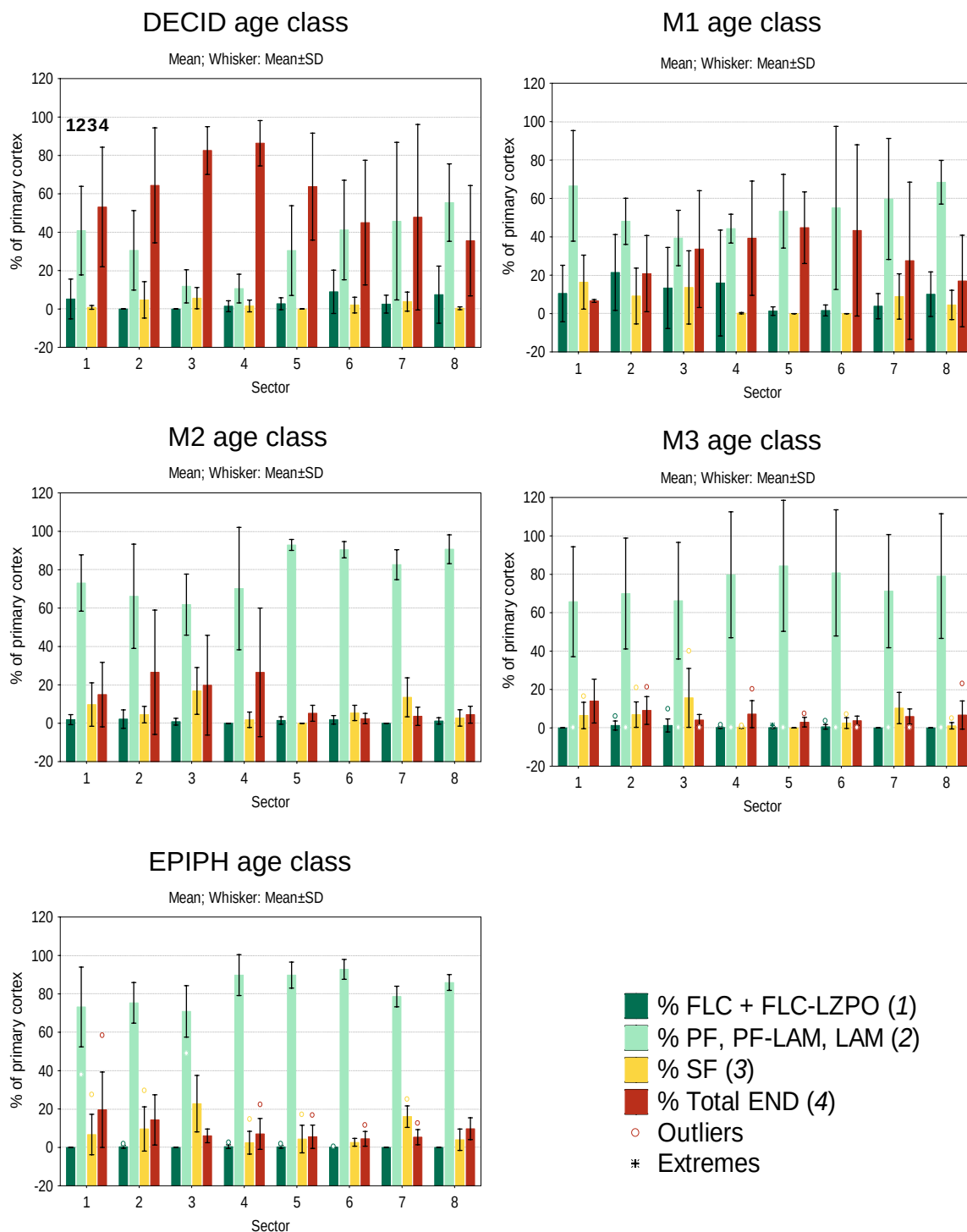


FIGURE 5-31 b. Plots of age class mean proportions of primary cortex area represented by different tissue types, across radial sectors: *H. lar* midshaft humerus. (Ring data combined.) Fibro-lamellar (FLC, FLC-LZPO) and lamellar-zonal (PF, PF-LAM, LAM) tissue types are grouped, to show general trends. Bars representing different tissue types are color coded to correspond with tissue type maps.

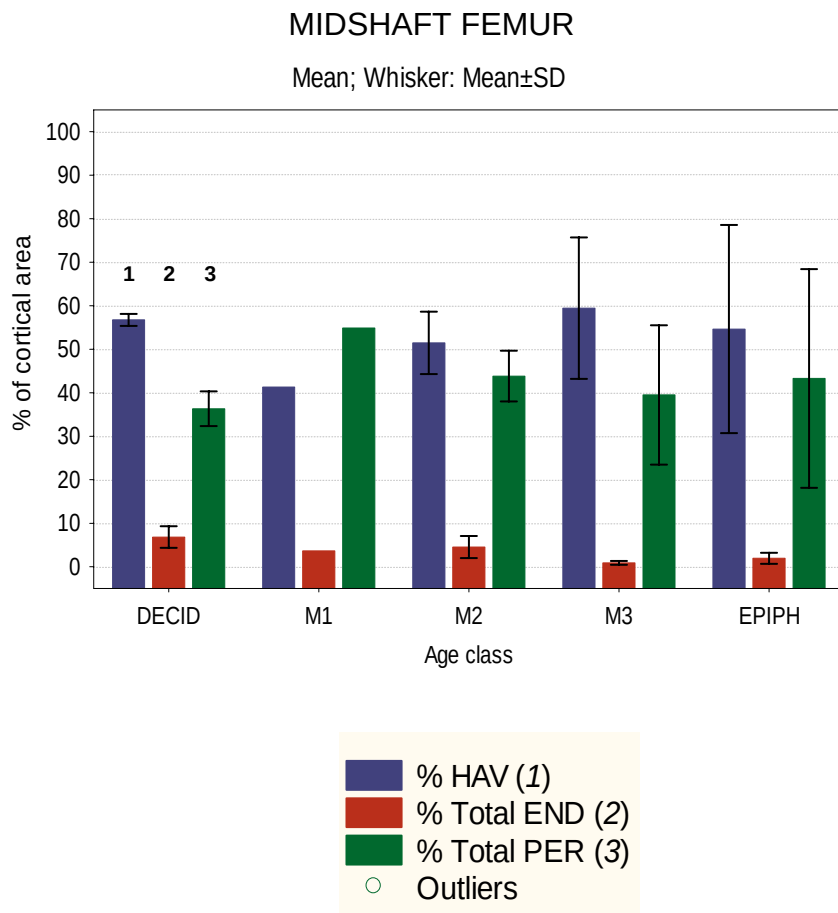


FIGURE 5-31. Plot of age class mean proportions of total cortical area represented by endosteal (END), periosteal (PER) and secondary osteonal (HAV) tissue: *P. troglodytes* midshaft femur. Bars representing different tissue types are color coded to correspond with tissue type maps.

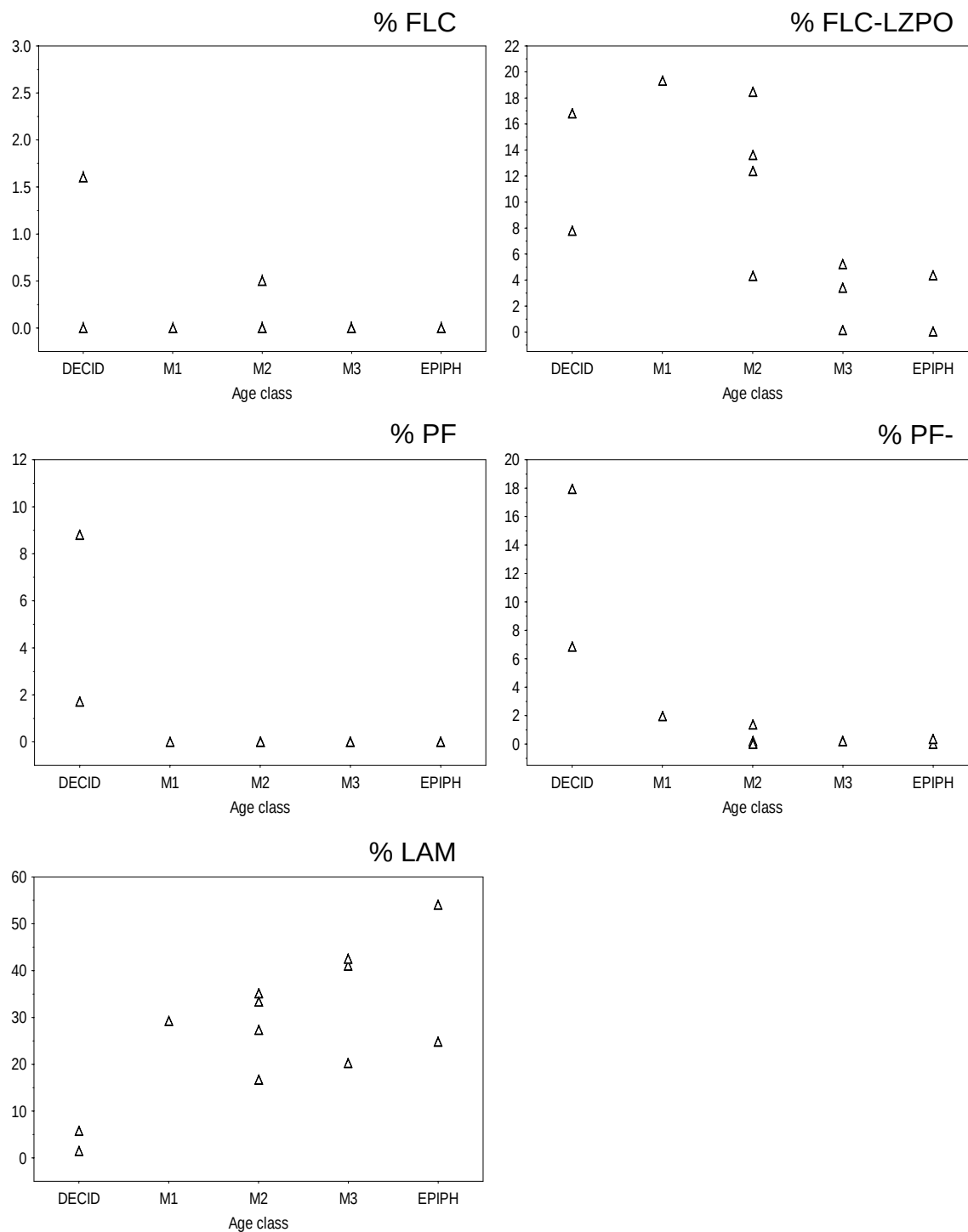


FIGURE 5-32. Scatterplots of periosteal tissue type proportions by age class: *P. troglodytes* midshaft femur.

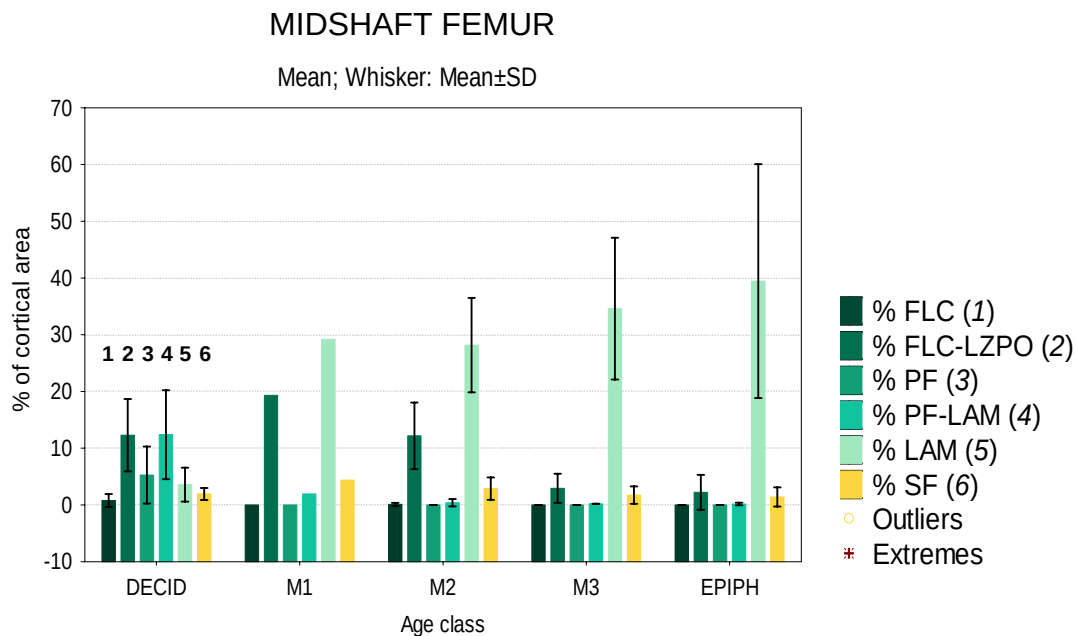


FIGURE 5-33. Plot of age class mean proportions of total cortical area represented by primary periosteal tissue types: *P. troglodytes* midshaft femur. Bars representing different tissue types are color coded to correspond with tissue type maps.



FIGURE 5-34. Plots of age class mean proportions of primary cortex area represented by different tissue types, across circumferential rings: *P. troglodytes* midshaft femur. Earlier-deposited periosteal tissues (and, to a variable extent, endosteal tissues) are represented in the inner cortical ring, while recently-deposited periosteal tissues are represented in the outer cortical ring. Bars representing different tissue types are color coded to correspond with tissue type maps.

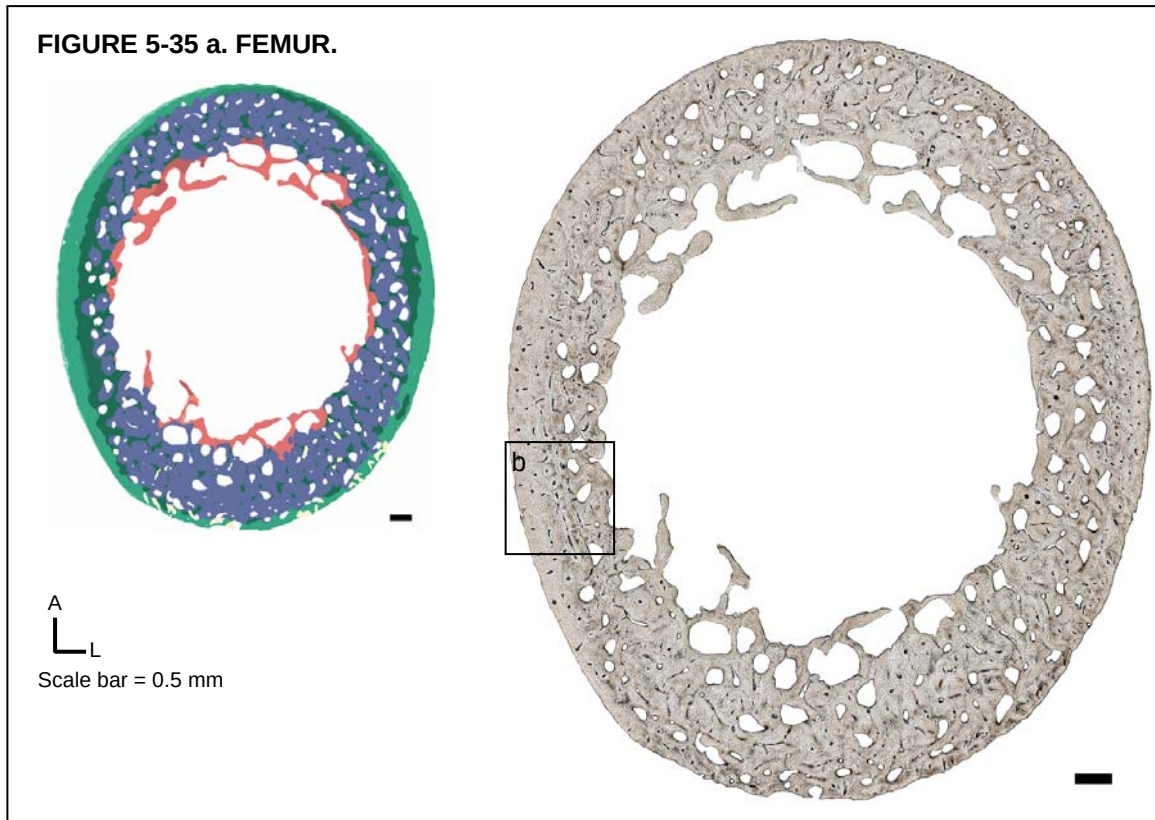


FIGURE 5-35 a. *P. troglodytes* midshaft femur images: DECID (MNH 83618). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image. Inset shows location of FIGURE 5-35 b.

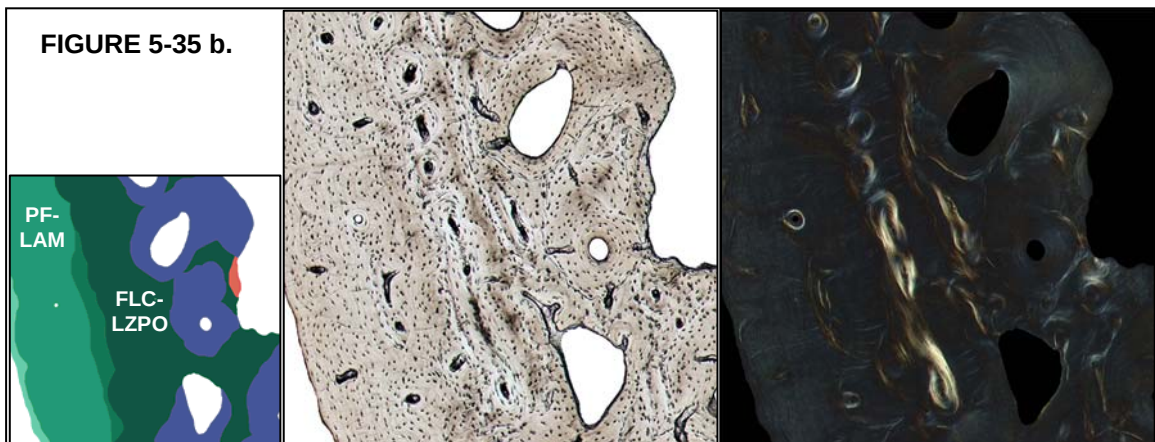


FIGURE 5-35 b. *P. troglodytes* MNH 83618 midshaft femur images: Lamellar-zonal to fibro-lamellar transition in the medial cortex. *Left:* Tissue type map. *Middle:* LM image, showing predominantly longitudinal vascularization of FLC-LZPO. Lamellar-zonal cortex (largely PF-LAM) is sparsely vascularized. The transition between these two tissue types is fairly abrupt. *Right:* CPL image, showing predominantly dark appearance of lamellar-zonal cortex.

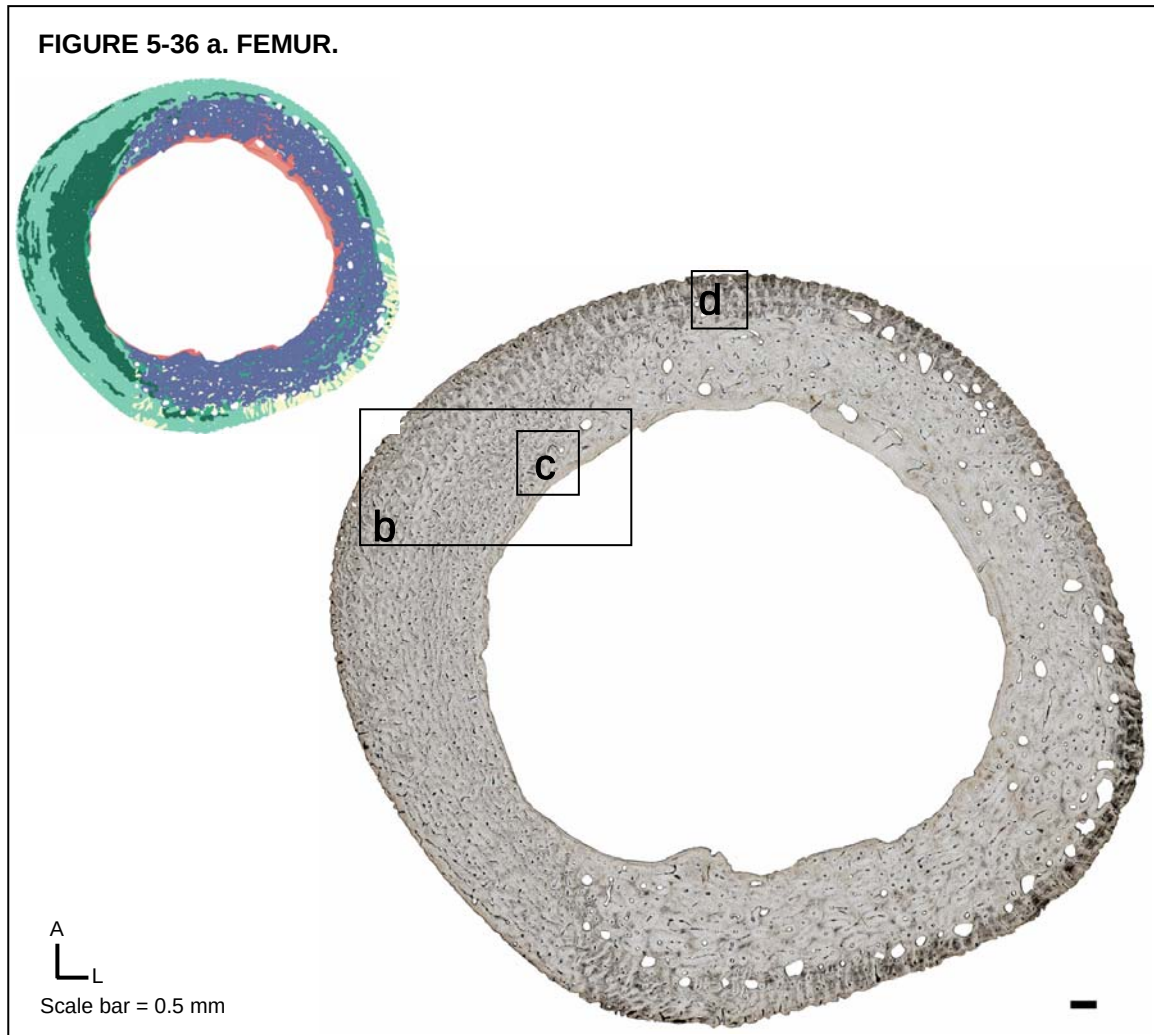


FIGURE 5-36 a. *P. troglodytes* midshaft femur images: M1 (MNH 83604). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image. Insets show location of FIGURES 5-36 b - d.

FIGURE 5-36 b. *P. troglodytes* MNH 83604 midshaft femur images: Antero-medial cortex. *Top:* Tissue type map. Inset shows location of FIGURE 5-36 c. *Middle:* LM image, showing vascularization by predominantly longitudinal primary osteons with scattered oblique canals and irregular anastomoses (forming a reticular vascular pattern in some cortical regions). FLC-LZPO tissue in the inner 1/2 of the cortex is bordered on both sides by lamellar-zonal tissue. This transition towards the outer cortex is commensurate with changes in primary osteon size (i.e., diameter and number of concentric lamellae) and density. *Bottom:* CPL image, showing features described above.

FIGURE 5-36 c. Close-up of FIGURE 5-36 b. Transition from earlier-deposited lamellar-zonal tissue to FLC-LZPO tissue. This transition is associated with a resting line (indicated by arrows), which is characterized by a smooth contour in LM (*Left*) and a bright signal in CPL (*Right*). This individual is also characterized by resting lines in the mid-periosteal anterior cortex, which are continuous with a reversal anteriorly and laterally. (See Figure 5-36 d).

FIGURE 5-36 b.



FIGURE 5-36 c.

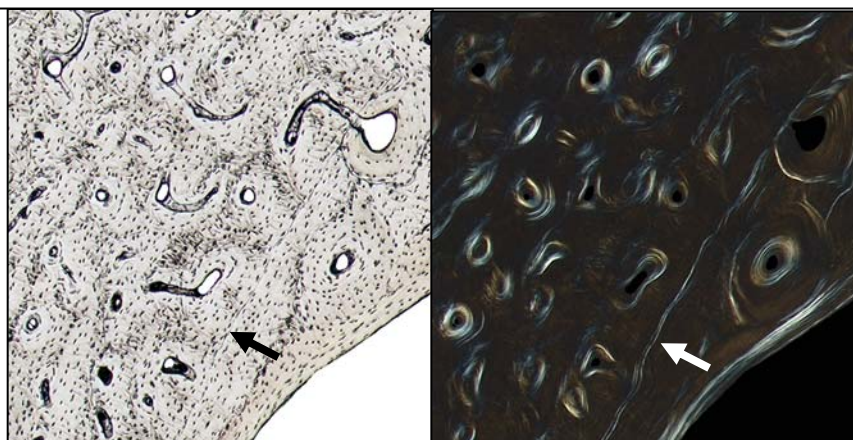


FIGURE 5-36 d.

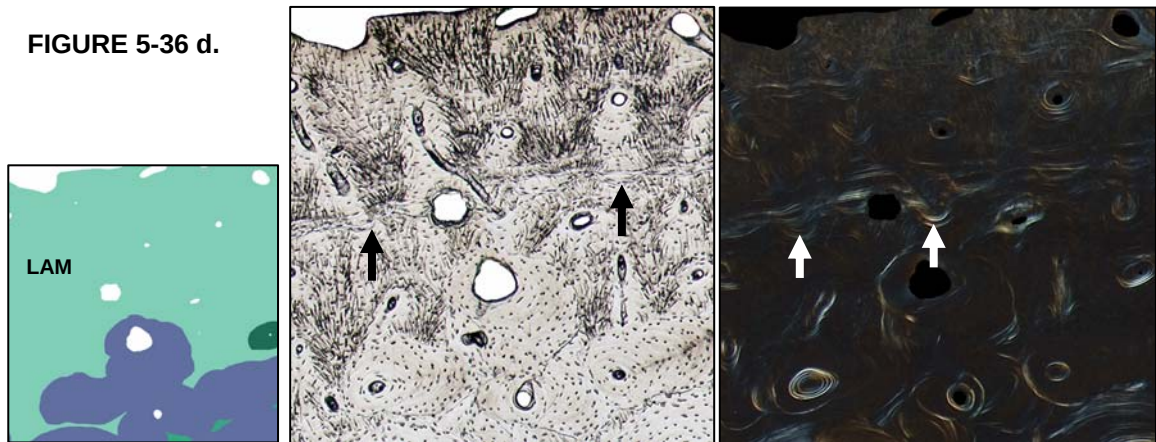


FIGURE 5-36 d. *P. troglodytes* MNH 83604 midshaft femur images: Antero-medial cortex. *Left:* Tissue type map. *Middle:* LM image showing scalloped contour of reversal line (indicated by arrows) in sparsely vascularized primary periosteal cortex. *Right:* CPL image showing bright signal of reversal line in a largely LAM cortex appearing dark. It can also be recognized by the appearance of microstructural discontinuity, particularly by the partial resorption of primary osteons embedded in older tissue (indicated by arrows).

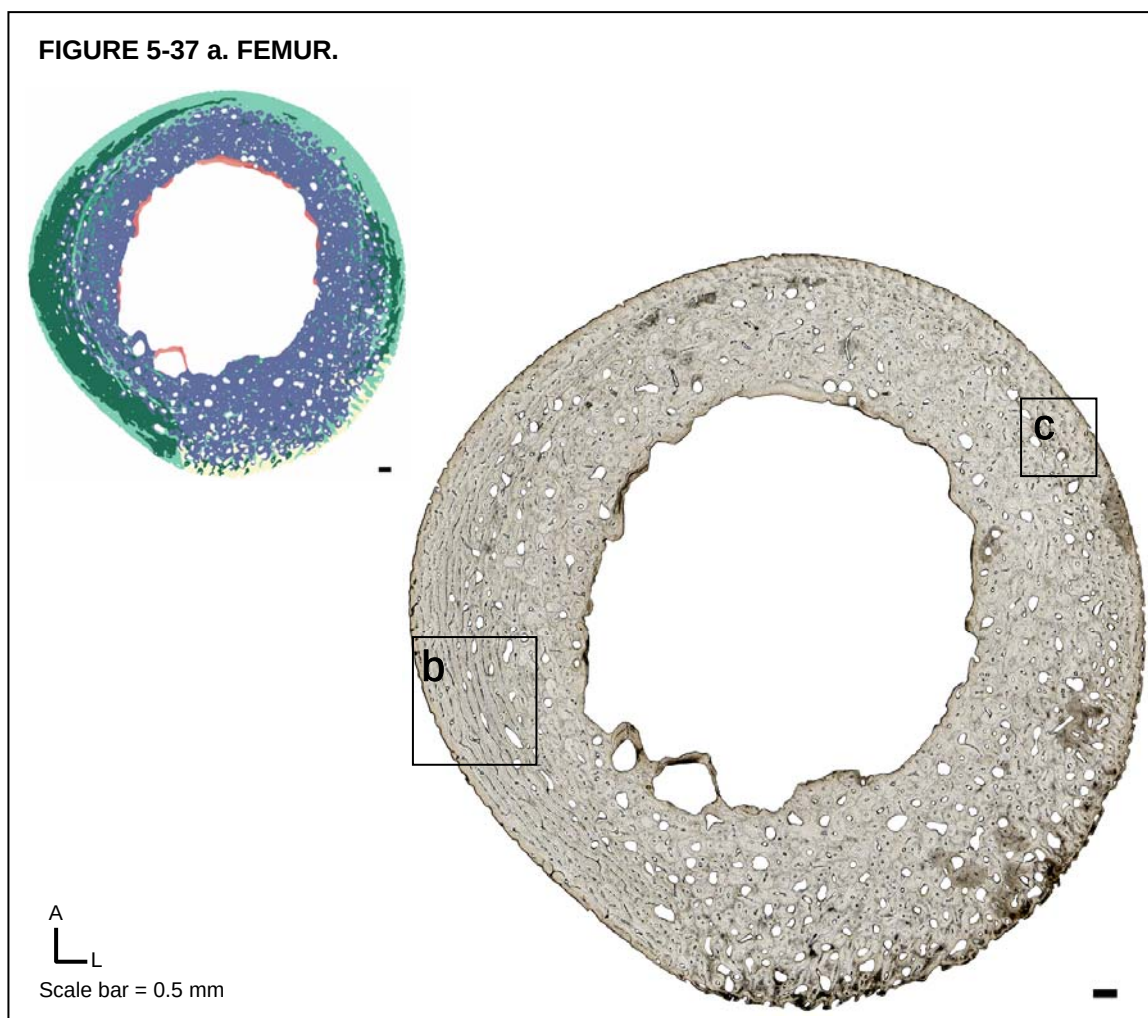


FIGURE 5-37 a. *P. troglodytes* midshaft femur images: M2 female (MNH 32052). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image, shows longitudinal, reticular and laminar vascular patterns in primary periosteal cortex. Insets show location of FIGURES 5-37 b and c.

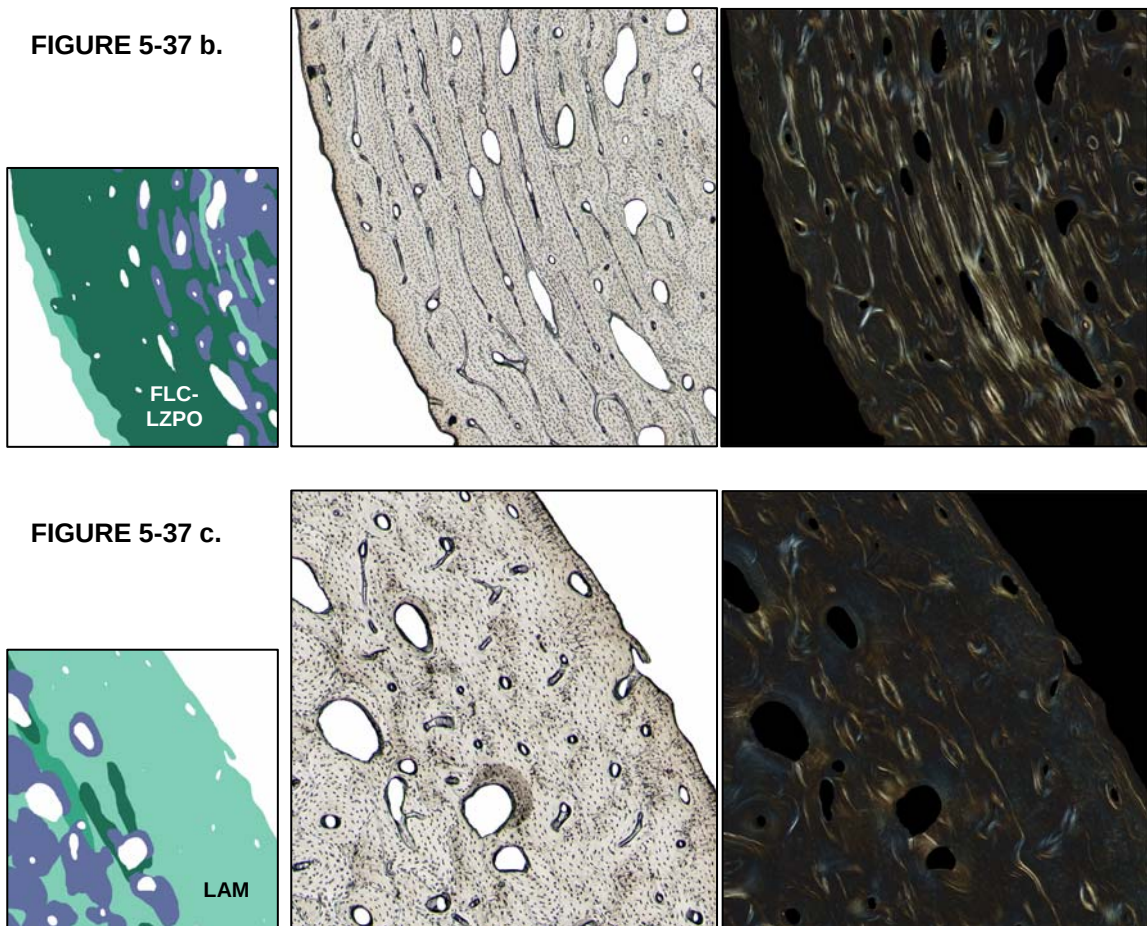


FIGURE 5-37 b. *P. troglodytes* MNH 32052 midshaft femur images: Lamellar FLC-LZPO tissue in the medial cortex. *Left:* Tissue type map. *Middle:* LM image, showing densely-distributed primary osteons having predominantly circular and longitudinal orientations in the medial and postero-medial cortex, forming a lamellar vascular pattern. *Right:* CPL image, showing collagen fiber organization in lamellar FLC-LZPO tissue. Extra-osteonal matrix is PF and LAM in its organization.

FIGURE 5-37 c. *P. troglodytes* MNH 32052 midshaft femur images: Antero-lateral cortex. *Left:* Tissue type map. *Middle:* LM image, showing vascularization of LAM cortex by scattered longitudinal primary osteons and simple vascular canals. *Right:* CPL image, showing primary osteons embedded in a predominantly LAM cortex, the latter appearing dark in CPL.

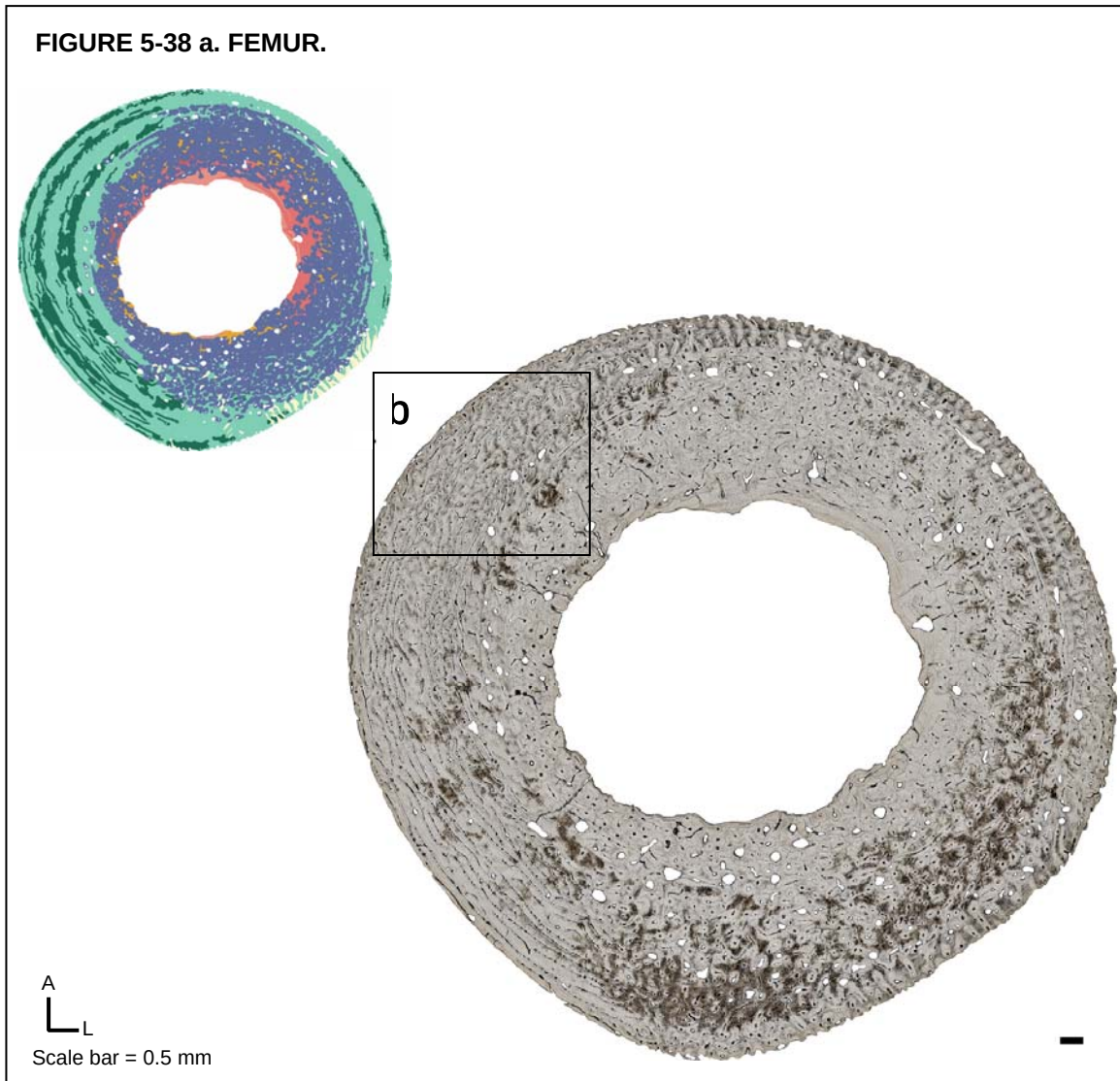
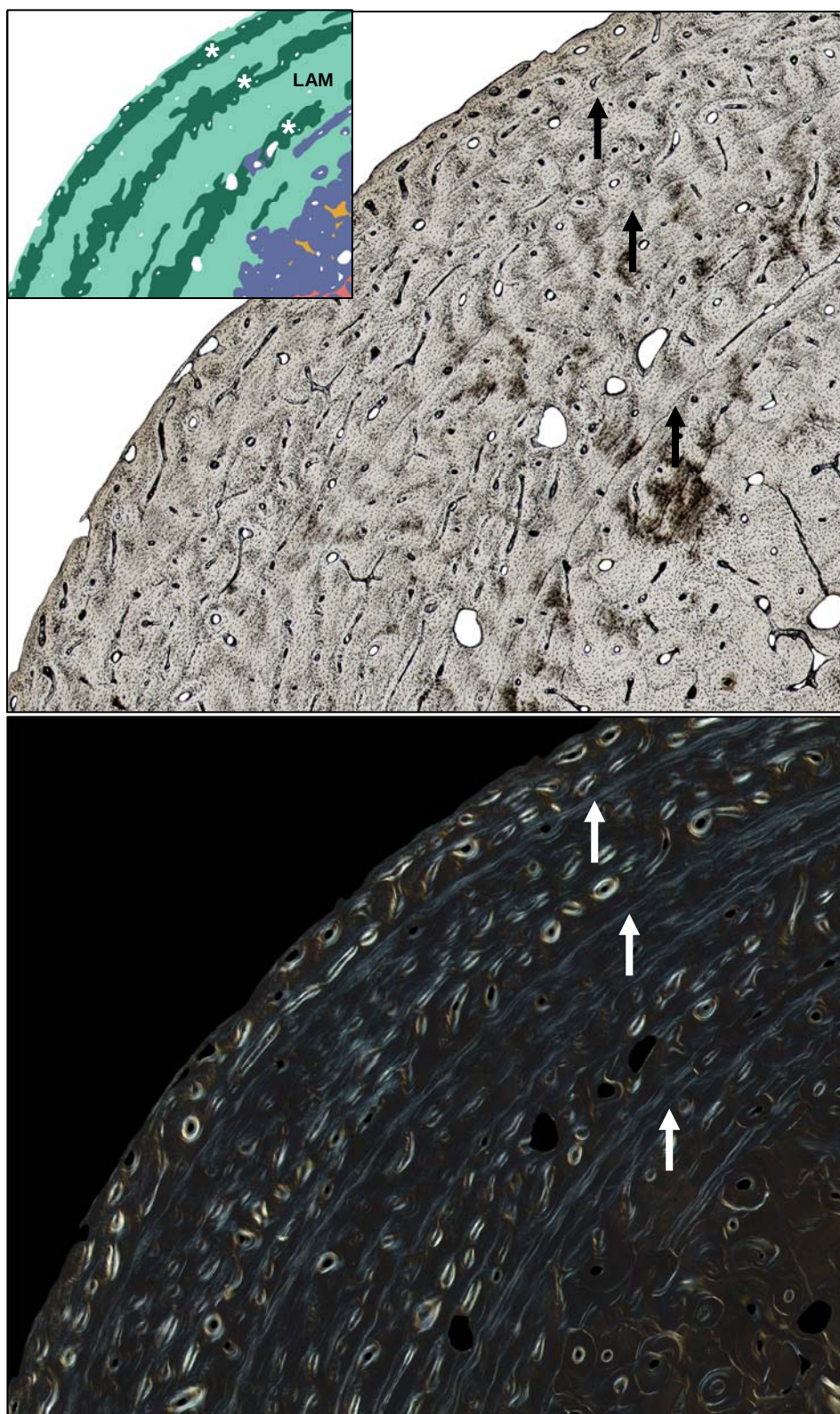


FIGURE 5-38 a. *P. troglodytes* midshaft femur images: M2 male (MNH 83673). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image, showing longitudinal, reticular and laminar vascular patterns in primary periosteal cortex. Inset shows location of FIGURE 5-38 b.

FIGURE 5-38 b. *P. troglodytes* MNH 83673 midshaft femur images: Antero-medial cortex. This image shows an apparently repetitive microstructural pattern; growth layers comprised of FLC-LZPO (*) and vascular LAM tissues are interspersed with layers of less vascular LAM and resting lines. *Top left:* Tissue type map. *Top:* LM image, showing vascularization by predominantly longitudinal and oblique primary osteons with irregular anastomoses. FLC-LZPO tissue (*) shows an apparently regular alternation with LAM tissue. These transitions are commensurate with changes in the density and size of primary osteons. Towards the anterior cortex (top of this image), periods of LAM deposition are associated with resting lines. Three resting lines can be observed here, indicated by arrows. *Bottom:* CPL image, showing features described above.

FIGURE 5-38 b.



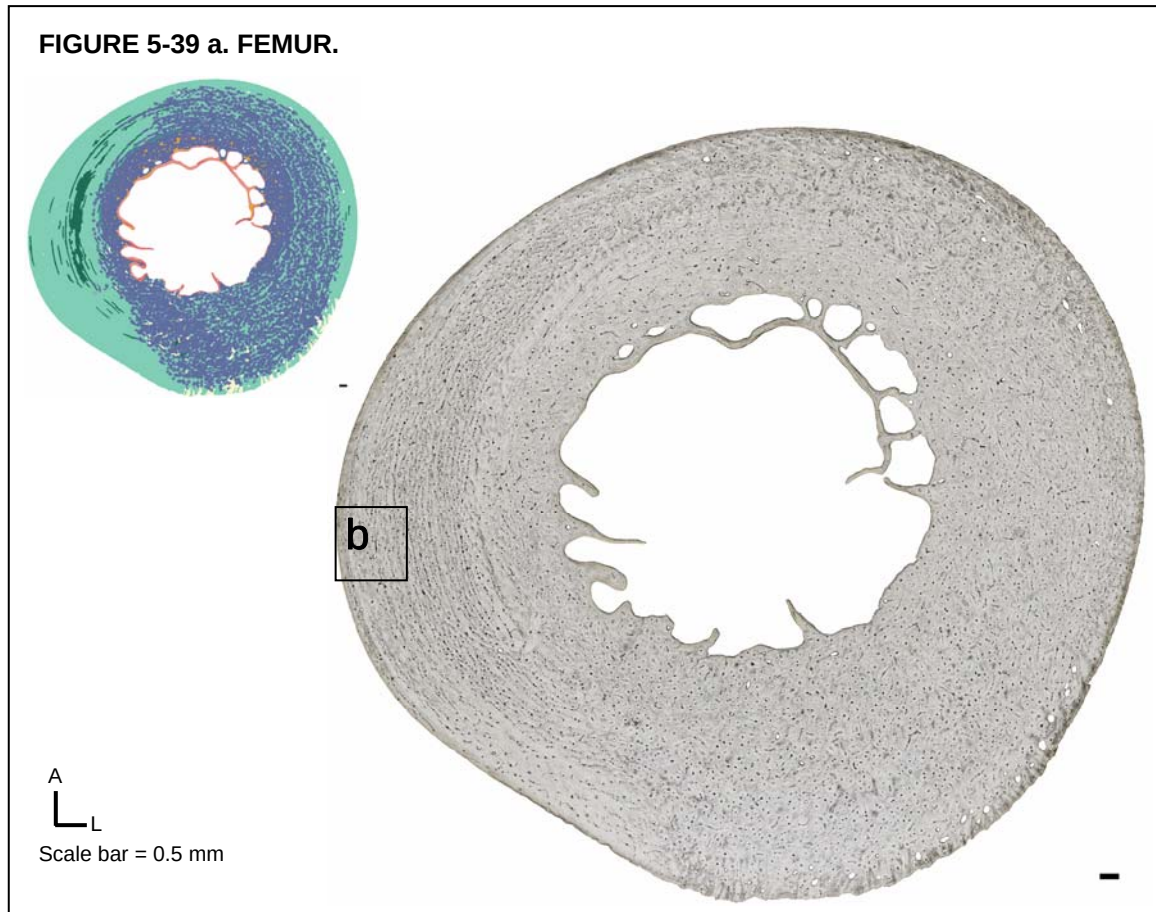


FIGURE 5-39 a. *P. troglodytes* midshaft femur images: M3 female (MNH 83651). *Left:* Whole cross-section tissue type map. Colors denote different tissue types, as shown in Figure 5-2. *Right:* LM image. Inset shows location of FIGURE 5-39 b.

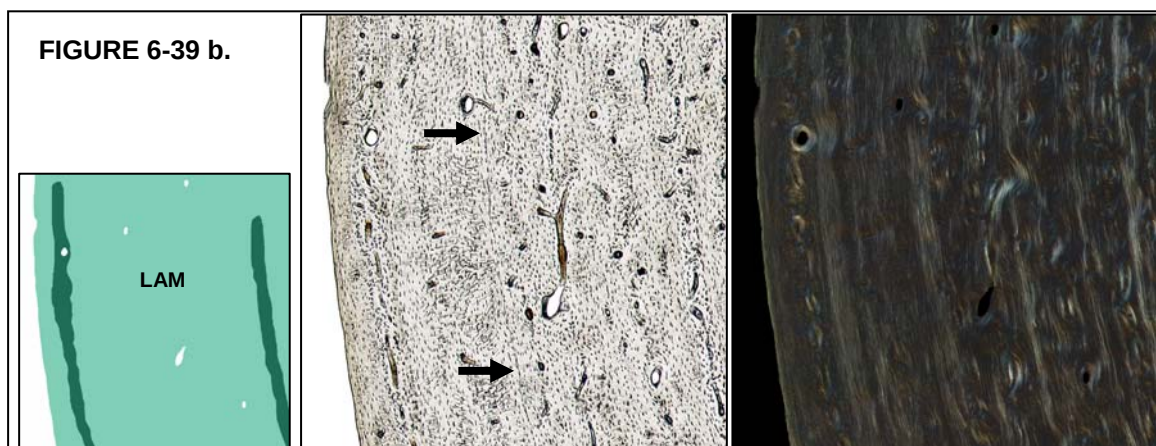


FIGURE 5-39 b. *P. troglodytes* MNH 83651 midshaft femur images: Medial cortex. *Left:* Tissue type map. *Middle:* LM image, showing vascularization of LAM by simple primary vascular canals and scattered primary osteons having a longitudinal orientation. An annulus is also observed (indicated by arrows). *Right:* CPL image showing predominantly LAM matrix. A similar pattern also characterizes the opposing cortex.

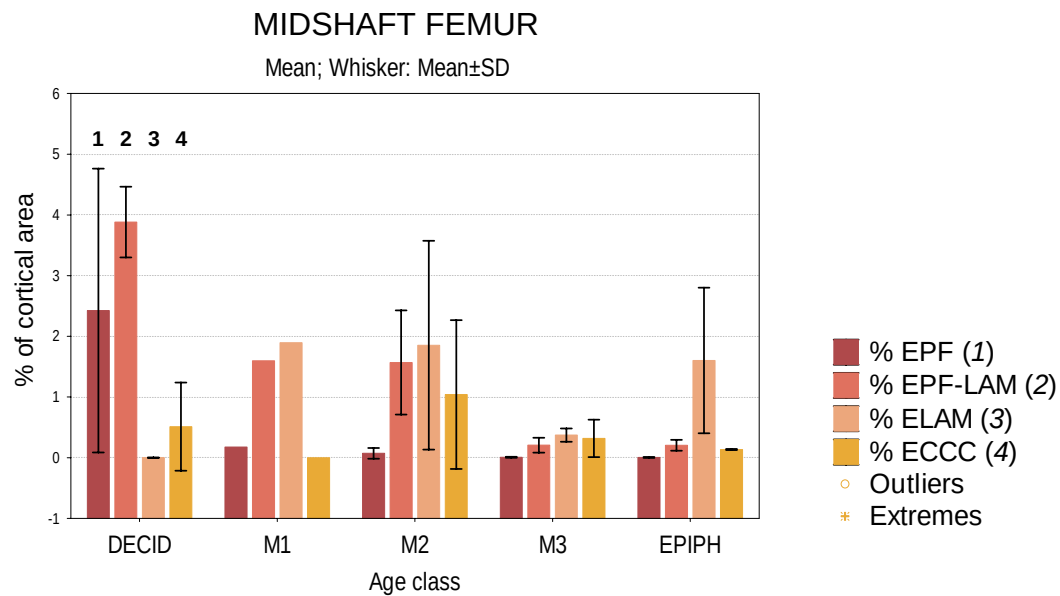


FIGURE 5-40. Plot of age class mean proportions of total cortical area represented by primary endosteal tissue types: *P. troglodytes* midshaft femur. Bars representing different tissue types are color coded to correspond with tissue type maps.

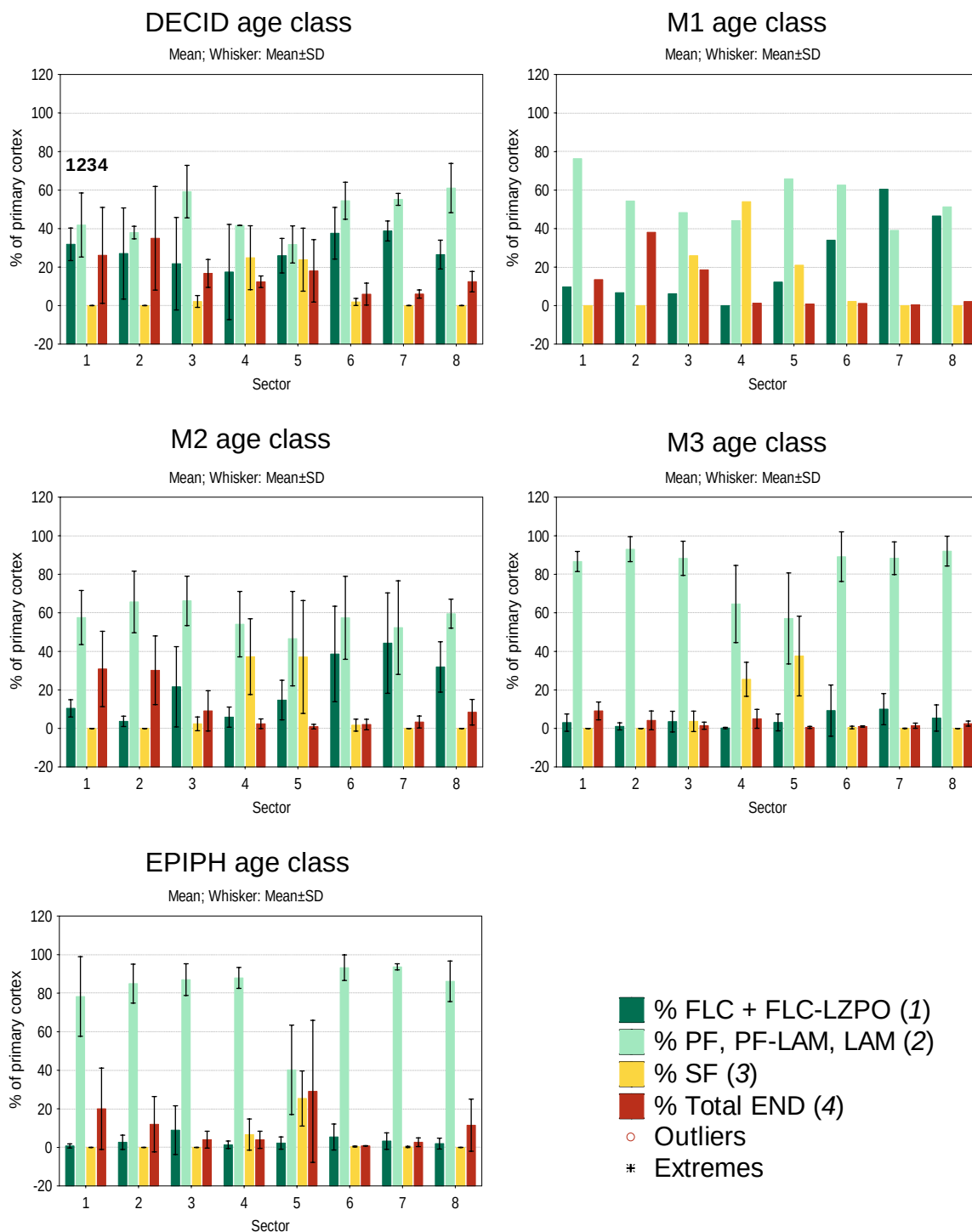


FIGURE 5-41. Plots of age class mean proportions of primary cortex area represented by different tissue types, across radial sectors: *P. troglodytes* midshaft femur. (Ring data combined.) Fibro-lamellar (FLC, FLC-LZPO) and lamellar-zonal (PF, PF-LAM, LAM) tissue types are grouped, to show general trends. Bars representing different tissue types are color coded to correspond with tissue type maps.

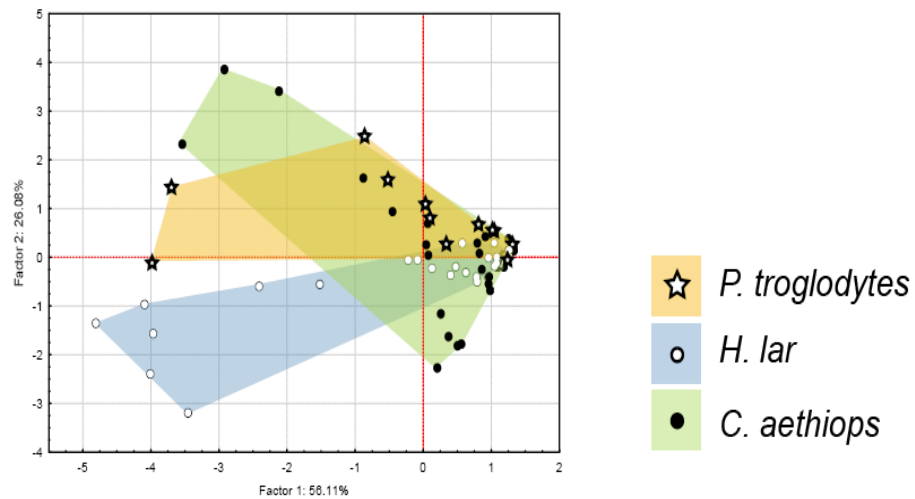
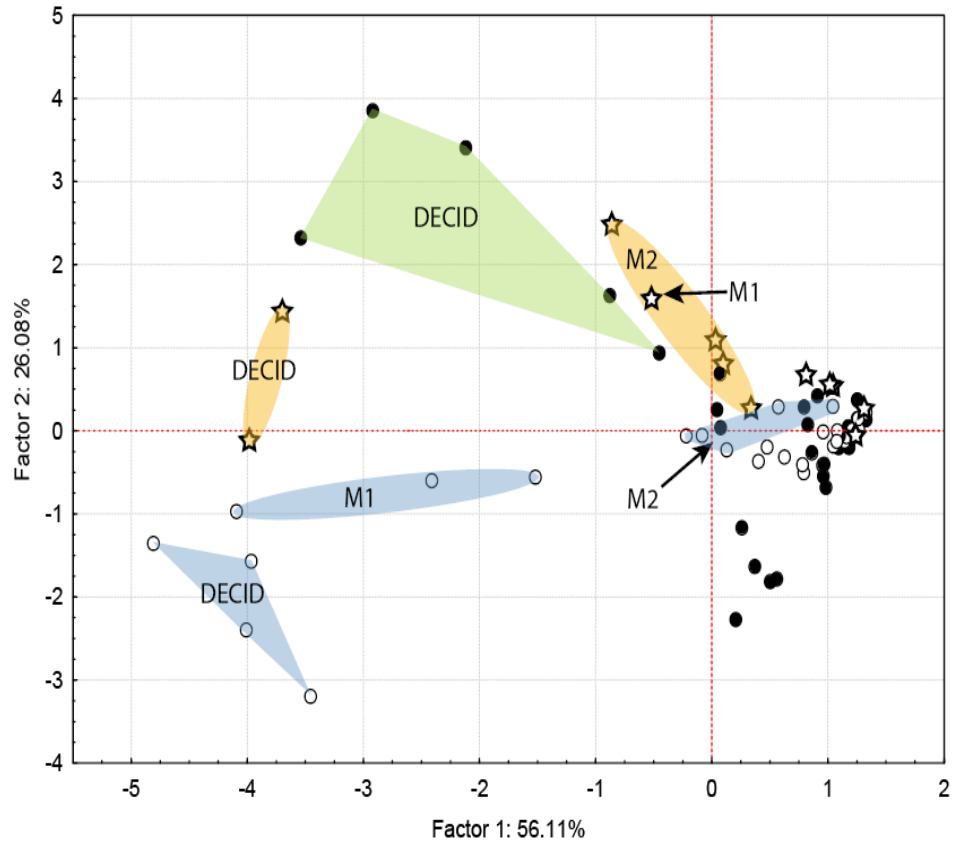


FIGURE 5-42. Principal components 1 and 2: midshaft femur of *C. aethiops*, *H. lar* and *P. troglodytes*. *Top:* Minimum polygons or ovals were drawn to delineate age classes within taxa, which show non-overlapping distributions on PC 1. *Bottom left:* Minimum polygons were drawn to delineate whole-sample taxon distributions on PC 2.

CHAPTER 6.

SECONDARY INTRACORTICAL REMODELING

INTRODUCTION

Secondary intracortical remodeling, as a partially destructive process, may complicate interpretations of growth-related aspects of life history from features in primary bone. This is particularly true given that it varies in its extent and distribution among taxa, within single skeletons, and among individuals of different ages (e.g., Amprino and Godina, 1947; Burr, 1992; Currey, 2002; Enlow and Brown, 1956, 1957, 1958; Havill, 2004; Paine, 1994; Schaffler and Burr, 1984; Smith, 1961). However, analyses of secondary intracortical remodeling within and among vertebrate skeletons can also provide insight into functional aspects of life history, particularly mechanical loading patterns associated with species-specific positional behaviors. This is based on the premise that the distribution of intracortical remodeling within cortices reflects a history of loading-induced *in vivo* strains and / or microdamage accumulation (see reviews by Currey, 2002; Martin et al., 1998).

Studies of intracortical remodeling in nonhuman primates have tended to focus either on its age-related dynamics (Burr, 1992; Burr et al., 1989; Glassman, 1985; Havill, 2004; Mulhern and Ubelaker, 2003) or on the extent to which patterns of secondary intracortical remodeling in adult animals varies in accordance with species-specific locomotor patterns (Paine, 1994; Paine and Godfrey, 1997; Schaffler and Burr, 1984). With a few exceptions (Bouvier and Hylander, 1981; Enlow, 1962b, 1963; Newell-Morris and Sirianni, 1982), ontogenetic variation in the distribution of secondary

intracortical remodeling within nonhuman primate long bones is poorly understood, as is the manner in which regional variation in intracortical remodeling may be influenced by other processes of bone remodeling (i.e., at periosteal and endosteal surfaces) during growth. The current study begins to address this issue by examining ontogenetic and regional variation in secondary intracortical remodeling at the midshaft femur and humerus of *Chlorocebus aethiops* and *Hylobates lar*, and at the midshaft femur of *Pan troglodytes*. This variability is interpreted within the context of results from analyses of cross-sectional geometry presented in Chapter 4 of this thesis, and microstructural evidence of local bone growth dynamics discussed in Chapter 5.

BACKGROUND

Secondary remodeling refers to the coupled and sequential action of osteoblasts and osteoclasts at single bone sites, resulting in the resorption of an existing bone volume and its immediate replacement by bone of more recent origin (see recent reviews by Currey, 2002; Dempster, 1999; Martin et al., 1998; Parfitt, 1994). Together, groups of osteoclasts and osteoblasts involved in a local secondary remodeling event comprise a *basic multicellular unit (BMU)*. Three sequential stages in the activity of a BMU are recognized (Frost, 1986, 1990): 1) *activation* in response to a hormonal or mechanical stimulus, involving changes in bone lining cells and local differentiation and recruitment of osteoclasts to the bone surface; 2) *resorption* of a bone volume by osteoclasts, creating a resorption space; and 3) *formation* of a new volume of bone at the same bone site by recently-differentiated osteoblasts. The sequential activities of a given BMU are similar whether secondary remodeling occurs at endosteal, cancellous, intracortical, or periosteal

surfaces of bone. Factors influencing BMU activation and cell activity are not entirely understood, but they include hormones, circulating levels of calcium and other minerals, and mechanical stimuli (e.g., Martin et al., 1998; Riggs et al., 2002; works in Seibel et al., 1999).

The current study examines secondary remodeling at intracortical surfaces in bone (hereafter referred to as *intracortical remodeling*), which results in the formation of a cylindrical structure, termed a *secondary osteon* or *Haversian system* (reviewed by Currey, 2002; Dempster, 1999; Martin et al., 1998; Parfitt, 1994). (See Chapter 2, Figure 2-3). Newly-differentiated osteoclasts form a cutting cone (rather than a trench-like depression, as on other bone surfaces) approximately 200 μm in diameter, which tunnels through existing bone in a predominantly longitudinal direction, leaving behind a resorption space. This resorptive phase occurs over a duration of approximately 3 weeks in humans (Martin et al., 1998). The BMU brings with it a capillary loop, which supplies nourishment and progenitor cells for both osteoclasts and osteoblasts. Reversal from osteoclastic resorption to osteoblastic formation occurs over a period of several days, and results in the formation of a *cement line*. The formative phase begins with the appearance of differentiated osteoblasts around the periphery of the resorption space, and it takes place over a period of approximately 3 months in humans. Inward concentric deposition of lamellar bone follows at an average rate of 1-2 $\mu\text{m}/\text{day}$ (Martin et al., 1998), refilling the space up to the blood vessel wall. The entire bone volume is not replaced, but a *Haversian canal* is left in the center of the secondary osteon for passage of blood vessels and nerves. This vascular supply provides nourishment for adjacent osteocytes and bone lining cells, and a passageway for exchange of minerals between the skeleton and the

bloodstream (Martin et al., 1998; Parfitt, 1994).

In areas of recurrent intracortical remodeling, old secondary osteons may be subsequently resorbed (in whole or in part) and replaced by more recent secondary osteons. In such areas of densely-distributed secondary osteons, bone regions located between secondary osteons are often referred to as *interstitial bone* (Francillon-Vieillot et al., 1990; Martin et al., 1998).

Consequences of intracortical remodeling for bone material properties

The consequences of secondary intracortical remodeling are not entirely understood. Secondary osteonal bone is characterized by reduced material strength under monotonic loading in tension, compression, and shear compared to primary bone (Currey, 1959, 1975; Currey, 2002; Evans and Bang, 1967; Margel-Robertson and Smith, 1978; Martin et al., 1998; Martin and Ishida, 1989; Reilly et al., 1974; Saha and Hayes, 1977; Vincentelli and Grigorov, 1985; Walmsley and Smith, 1957). However, while examinations of the material properties of secondary osteonal bone under cyclic loading have reached varying conclusions (Carter and Hayes, 1976; Carter et al., 1976; Evans and Riolo, 1970; Gibson et al., 2006; Schaffler et al., 1990), recent evidence indicates that it actually improves the resistance of bone to fatigue failure (Gibson et al., 2006; Martin et al., 1998).

These effects of intracortical remodeling on bone material properties may be attributed in part to its consequences for porosity, decreased mineralization relative to adjacent older bone tissues, and the presence of a cement line interface (Burr et al., 1988; Currey, 1959, 1975; Martin and Boardman, 1993; Martin and Ishida, 1989; Schaffler and

Burr, 1988; Schaffler et al., 1987). Intracortical remodeling may also influence material properties of bone by altering the preferred collagen fiber orientation (Bromage and Boyde, n.d.; Martin et al., 1996; Mason et al., 1995; McMahon et al., 1995; Riggs et al., 1993a; Riggs et al., 1993b).¹

Functions of intracortical remodeling

Based on extensive developmental and comparative investigations of vertebrate bone histology (Enlow, 1962a, 1963; Enlow and Brown, 1956, 1957, 1958), Enlow observed that intracortical remodeling occurs in “definite and predictable locations within the bone” (Enlow, 1963: 70). He proposed that intracortical remodeling of bone occurs as part of a suite of remodeling activities (involving coordinated patterns of resorption and formation on bone surfaces) which are fundamental to the bone growth process, and which serve either to maintain or to make local adjustments in bone shape with increases in overall size. For instance, he suggested that secondary osteons may be produced as a consequence of tissue-level mechanisms of endosteal (i.e., inward) growth of the cortex. Further, he noted that secondary osteons are commonly observed at sites of muscle attachment, where he proposed that the sequential resorptive and depositional activity associated with their formation provides a mechanism by which these attachments can be maintained or relocated despite ongoing circumstances of growth remodeling at the bone surface (also see Hoyte and Enlow, 1966). Finally, in the older skeleton, Enlow suggested that intracortical remodeling functions in mineral homeostasis, and in the

¹ For reviews of the influence of these variables of bone material properties, see Currey (2002) and Martin et al. (1998).

replacement of older cortical regions characterized by osteocyte necrosis or accumulated mechanical fatigue (Enlow, 1962a, 1962b, 1963, 1976, 1982; Enlow and Hans, 1996).

While some intracortical remodeling may contribute to short-term adjustments in mineral concentration, this function is thought to be fulfilled primarily by remodeling at cancellous surfaces in bone, particularly in regions of the axial skeleton and pelvis (Parfitt, 1994, 2001). Recent efforts in the study of intracortical remodeling have been largely based on the premise its occurrence in the peripheral skeleton primarily serves a mechanical function. Intracortical remodeling is suggested to maintain mechanical integrity of the skeleton by altering the preferred orientation of collagen fibers and other mechanical properties of bone in response to *in vivo* strains (Bouvier and Hylander, 1981; Lanyon, 1987; Riggs et al., 1993a), and by preventing and/or repairing fatigue-induced microdamage (e.g., Frost, 1986, 1990; Martin et al., 1998). Evidence concerning the mechanical significance of intracortical remodeling is discussed further below.

The mechanical significance of intracortical remodeling. Theories of intracortical remodeling are largely based on the premise that it is regulated by *in vivo* strains. Initiation of intracortical remodeling is either increased or decreased when strain magnitudes fall above or below strain thresholds defined by normal physiologic loading conditions (the latter as characterized, for example, by Biewener, 1991; Biewener and Taylor, 1986; Biewener et al., 1988; Rubin and Lanyon, 1982; Rubin and Lanyon, 1984). Lanyon and colleagues (Lanyon, 1987; Lanyon and Baggott, 1976; Lanyon et al., 1982; Lanyon et al., 1979) proposed that intracortical remodeling occurs as part of an adaptive process, whereby adjustments in bone maintain *in vivo* strains within appropriate limits

with respect to the current loading environment. While such adaptive changes are thought to be primarily accomplished by alterations in structural properties of bone (i.e., via growth remodeling at periosteal and endosteal surfaces), intracortical remodeling in regions experiencing *high* physiologic strain magnitudes may function to maintain an acceptable fatigue life (Lanyon, 1987; Lanyon and Baggott, 1976; Lanyon et al., 1979).

Frost's (Frost, 1986, 1990, 2001, 2003) "mechanostat" paradigm makes explicit predictions concerning the manner in which *both* growth remodeling (at periosteal, endosteal and cancellous surfaces; termed 'modeling' by Frost) and BMU-based secondary remodeling (at periosteal, endosteal, cancellous and intracortical surfaces) act in concert to maintain bone strain magnitudes within threshold limits consistent with normal physiologic loading. According to this model, activation of secondary remodeling is predicted to increase under two conditions: 1) in low-strain environments, when *in vivo* strains fall below a minimum effective strain threshold of $\sim 50\text{-}100\ \mu\epsilon$, possibly as high as $400\ \mu\epsilon$ (i.e., the "MESr", which defines disuse-activated remodeling); and 2) in high-strain environments exceeding normal physiologic strain magnitudes, defined by a minimum effective strain threshold of $\sim 3000\ \mu\epsilon$ (i.e., the "MESp", which defines repair-activated secondary remodeling). Secondary remodeling in the first condition serves to reduce bone mass, until optimum strain conditions are restored. Secondary remodeling in the latter condition is a damage-initiated response. Activation of secondary remodeling is predicted to decrease under *mild overload* conditions ($1,000\text{-}1,500\ \mu\epsilon < \textit{in vivo} strains $< \sim 3,000\ \mu\epsilon$). In this latter case, increased growth remodeling and decreased secondary remodeling serves to increase bone mass and reduce peak strains to acceptable levels. The implication of the mechanostat model is that, during$

normal physiologic loading, increases in mechanical strain will be associated with a *decrease* in the initiation of BMU-based secondary remodeling.

While the mechanostat paradigm has received much support, inconsistencies with the model include observations that regional increases in intracortical remodeling activity are not limited to the strain conditions summarized above, defined by the MESr and MESp, respectively (see reviews by Martin et al., 1998; and Skedros et al., 2001). In fact, many observations indicate that intracortical remodeling is initiated in response to high cyclic strain magnitudes during normal functional activities (Bouvier and Hylander, 1996; Lanyon and Baggott, 1976; Lanyon et al., 1979; Lieberman et al., 2003; Martin et al., 1998).

Remodeling to prevent or repair accumulation of microdamage. Considerable attention has focused on the role of intracortical remodeling in the repair and prevention of microdamage due to bone fatigue. Fatigue occurs as a normal consequence of cyclic loading during normal activities (reviewed by Martin et al., 1998). It is associated with degradation of material properties, including a decline in elastic modulus (i.e., material stiffness) and yield strength, and the accumulation of microdamage (Carter et al., 1981; Carter and Hayes, 1977a, 1977b; Schaffler et al., 1989, 1990).

Several factors influence fatigue behavior and accumulation of microdamage in bone. Tissue age is a major determinant of bone fatigue. Elastic modulus degradation and generation of microdamage become more pronounced with increasing numbers of loading cycles experienced, as bone approaches the end of its fatigue life (i.e., number of cycles to failure) (Martin et al., 1998). Further, loading at higher cyclic strain magnitudes and strain rates is associated with increased damage and reduced fatigue life (Burr et al.,

1985; Carter and Caler, 1985; Carter et al., 1981; Schaffler et al., 1989). Fatigue behavior and microdamage accumulation in bone also varies considerably according to strain mode (e.g., tension, compression) (Carter et al., 1981; Currey, 2002; Griffin et al., 1997; Martin et al., 1998; Pattin et al., 1996).

Fatigue damage is apparent at ultrastructural levels (Bentolila et al., 1998; Schaffler et al., 1996), and at the light microscope level in the form of linear microcracks (Frost, 1960). Microcracks are most frequently located in interstitial areas between secondary osteons (Frost, 1960; Norman and Wang, 1997; Schaffler et al., 1995), and their growth may result in separations along lamellar boundaries or osteonal cement lines (Carter and Hayes, 1977b; Schaffler et al., 1989). Significant microdamage can be produced as a result of cyclic loading under normal physiologic strain conditions (Burr et al., 1985; Norman and Wang, 1997; Schaffler et al., 1995; Schaffler et al., 1989, 1990). If this damage is allowed to accumulate without repair, bones may eventually progress to failure at stresses lower than the ultimate stress under monotonic loading (Martin et al., 1998).

Although secondary osteonal bone is weaker than primary bone in its material strength, evidence indicates that it improves resistance to fatigue failure and extends fatigue life in two ways (reviewed by Martin et al., 1998). First, the microscopic structure of secondary osteonal bone is particularly well suited for slowing or preventing the growth of microcracks. Cement lines and interfaces between concentric lamellae serve as discontinuities in bone, which introduce local variations in material stiffness. Together with eventual osteonal separation at cement line boundaries, this slows and/or deflects crack propagation, and effectively increases the amount of energy required to

propagate microcracks to failure (Burr et al., 1988; Gibson et al., 2006; Martin and Burr, 1982; Martin et al., 1998; Saha and Hayes, 1977; Schaffler et al., 1987). The reduced elastic modulus of recently-formed secondary osteons (due to their lower mineralization density) may also contribute to controlling crack growth (Gibson et al., 2006).

Secondly, intracortical remodeling is suggested to function in the repair of bone regions characterized by accumulated microdamage, thereby extending fatigue life (Burr, 1993; Carter and Hayes, 1977b; Chamay, 1970; Frost, 1960, 1990; Martin and Burr, 1982). Experimental studies have shown that intracortical remodeling events occur in spatial association with microcracks statistically more frequently than expected by chance, and that new secondary remodeling events are initiated following the production of loading-induced microdamage (Bentolila et al., 1998; Burr and Martin, 1993; Burr et al., 1985; Mori and Burr, 1993). These and other observations indicate that microdamage provides a direct stimulus for the preferential intracortical remodeling of specific skeletal sites (Burr, 1993, 2002; Lee et al., 2002; Martin, 2002), although the extent to which intracortical remodeling may also occur stochastically (i.e., in a manner that is not site-specific) is not entirely understood (Burr, 2002; Parfitt, 2002).

The influence of strain mode on intracortical remodeling. The manner in which initiation of secondary intracortical remodeling may be influenced by strain mode is not well understood. While intracortical remodeling is commonly reported to be more extensive in compressive cortical regions (Lanyon and Baggott, 1976; Lanyon et al., 1982; Lanyon et al., 1979; Mason et al., 1995; Skedros et al., 1996), it may also be preferentially distributed in tensile cortical regions (Skedros et al., 1994; Skedros et al., 2001), depending on the skeletal element and taxon examined. As most attention has

focused on the role of strain magnitude in the initiation of intracortical remodeling (as discussed above), differential remodeling of compression and tension cortices may be determined in part by differences in strain magnitudes associated with these loading modes (Martin and Burr, 1989; Skedros et al., 2001). In terrestrial quadrupeds, for example, limb elements are subjected to both axial compression and bending loads. As a result, the neutral axis of bending is shifted towards the tensile side of the cortex, and maximum compressive strains exceed maximum tensile strains (Biewener et al., 1988; Demes et al., 1998; Lanyon and Baggott, 1976; Lieberman et al., 2004; Rubin and Lanyon, 1982).

However, material properties and fatigue behavior of bone vary considerably in tension and compression, and it is likely that strain mode exerts an influence on intracortical remodeling independently of strain magnitude. Bone is weaker in tension, and fatigue life and strain threshold (i.e., strain magnitude above which material property degradation increases) are lower when loaded cyclically in tension versus compression (Carter et al., 1981; Currey, 2002; Griffin et al., 1997; Martin et al., 1998; Pattin et al., 1996). Different patterns of microdamage accumulation are also observed in tensile and compressive loading (Carter and Hayes, 1977b). Frost (1986) suggested there may be strain mode differences in thresholds governing BMU activation, with increased secondary remodeling being initiated at lower strain magnitudes under tension compared to compression. Additionally, secondary intracortical remodeling has also been proposed to function in altering bone material properties, namely by altering the preferred collagen fiber orientation, in response to changes in strain mode conditions (Bromage and Boyde, n.d.; Martin et al., 1996; McMahon et al., 1995; Riggs et al., 1993a; Riggs et al., 1993b).

Intracortical remodeling in long bone diaphyses of nonhuman primates

Several researchers have examined intracortical remodeling in long bone diaphyses of nonhuman primates. These studies have focused on two main lines of inquiry. First, studies have sought to characterize age and sex – related dynamics within species [rhesus and pig-tailed macaques (Burr, 1992; Burr et al., 1989; Havill, 2004; Newell-Morris and Sirianni, 1982); saddle-back tamarins (Glassman, 1985); and common chimpanzees (Kerley, 1966; Mulhern and Ubelaker, 2003, 2006)]. Nonhuman primates examined thus far exhibited significant age-related variability in such parameters as osteon number and population density, osteon size, BMU activation frequency, and bone formation rate (Burr, 1992; Burr et al., 1989; Glassman, 1985; Havill, 2004; Kerley, 1966; Mulhern and Ubelaker, 2003). However, age accounted for little (if any) of the variation observed in percent area of secondary osteonal bone during postnatal ontogeny. As several researchers noted that intracortical remodeling was distributed in inner cortical regions (Burr, 1992; Mulhern and Ubelaker, 2006), this observation may reflect a consequence of growth remodeling at endosteal and periosteal surfaces (also see Havill, 2004).

Secondly, studies have aimed to determine the influence of skeletal function and/or body size on variation in intracortical remodeling (Paine, 1994; Paine and Godfrey, 1997; Schaffler and Burr, 1984). These latter studies have focused almost exclusively on adults. Schaffler and Burr (1984) published the first systematic comparative examination of intracortical remodeling in nonhuman primates, focusing on individuals representing 20 different species ($n=1$ captive individual per species). They

observed significant variability in the percent area of *in tact* secondary osteons at the midshaft femur among groups defined on the basis of general categories of locomotor behavior. The percent area of *in tact* secondary osteons was lowest in arboreal quadrupeds, followed (with some overlap) by terrestrial quadrupeds. Suspensory primates were characterized by a significantly greater percent area of *in tact* secondary osteons than quadrupeds, but significantly less than humans.

In a more restricted comparative sample of primates, Paine and colleague (Paine, 1994; Paine and Godfrey, 1997) found that indices of relative intracortical remodeling in the femur and humerus (osteon % area in the humerus / osteon % area in the femur) also varied in accordance with general categories of locomotor behavior. Relative femoral remodeling was lowest in quadrupeds, compared to leapers and slow climbers. Among the former group, vervet monkeys (*C. aethiops*) and patas monkeys (*Erythrocebus patas*), characterized as more terrestrial than other quadrupeds examined, exhibited the lowest index of relative intracortical remodeling (indicating more humeral remodeling). These results indicate that, not only may the distribution of intracortical remodeling reflect functional limb differentiation; it may also reflect substrate preferences within broad locomotor categories.

These two studies revealed a less than straightforward relationship between body size and intracortical remodeling in nonhuman primates, however. While there was a tendency for larger-bodied taxa to intracortically-remodel their femora and humeri to a greater extent than smaller-bodied taxa, differences in positional behavior were also important in accounting for interspecific variation (Paine, 1994; Paine and Godfrey, 1997; Schaffler and Burr, 1984). Paine and colleague (Paine, 1994; Paine and Godfrey,

1997) demonstrated a positively allometric relationship between secondary osteonal area and cortical area in the femur and humerus. However, different scaling patterns were observed when subsets of the sample were considered separately, in accordance with locomotor differences.

Since Enlow's (Enlow, 1962b, 1963) investigations of rhesus macaques (and other vertebrates), only a few systematic studies have examined regional variability in intracortical remodeling in long bone diaphyses of nonhuman primates. As a component of the study described above, Paine and colleague (Paine, 1994; Paine and Godfrey, 1997) demonstrated that primate taxa practicing different categories of locomotor behavior were characterized by distinct and highly patterned regional distributions of secondary osteons across four anatomically-defined quadrants at the midshaft femur and humerus. However, they were not able to suggest a mechanical explanation that accounted for observations at the midshaft femur and humerus equally well. Newell-Morris and Sirianni (1982) observed a similar distribution of secondary osteons in the midshaft humerus of pig-tailed macaques (*M. nemestrina*) as reported by Paine (1994) for other Old World monkeys. However, in contrast to the latter study, this pattern was attributed by Newell-Morris and Sirianni (1982) to local growth remodeling history; intracortical remodeling was concentrated among endosteal tissues in the anterior and lateral cortex, opposing the direction of most rapid periosteal growth and cortical drift. A similar pattern of intracortical remodeling in older cortical regions was reported for the midshaft fibula (and mandible) of rhesus macaques (Bouvier and Hylander, 1981). Findings of these latter studies demonstrate that the relationship between patterns of intracortical remodeling and skeletal function may not be as straightforward as is

sometimes assumed, and that local conditions of bone growth remodeling may also be important in explaining intra-skeletal and taxonomic variability in intracortical remodeling in primates.

RESEARCH OBJECTIVES

The current study examines ontogenetic and regional variability in secondary intracortical remodeling at the midshaft femur and humerus of *C. aethiops* and *H. lar* and at the midshaft femur of *P. troglodytes*. As intracortical remodeling is a partially destructive process, characterization of variability in whole cross-section proportions of secondary osteonal bone provides an important context for investigation of primary bone tissue types presented in Chapter 5 of this thesis. Further, as few studies have examined both ontogenetic and regional variation in intracortical remodeling in nonhuman primates, the current study also provides important insight into the manner in which its distribution may reflect local dynamics of bone growth as well as skeletal function. Two specific questions are addressed in the current study:

1) *Does the percent area of secondary osteonal bone at the midshaft femur and humerus vary with respect to age during postnatal ontogeny?* The proportion of secondary osteonal bone (i.e., as a percentage of cortical area) is quantified in whole cross-sections, and analyzed within taxa with respect to age.

2) *Does the percent area of secondary osteonal bone show a regional distribution within cross-sections?* Regional variability in the proportion of secondary

osteonal bone within single cross-sections is quantified across circumferential rings and radial sectors. Partitioning of cross-sections by circumferential rings provides a means of examining variation in intracortical remodeling across cortical regions deposited during different growth stages. Two specific aims are addressed:

A) To determine whether the percent area of secondary osteonal bone varies among circumferential rings within cross-sections. Tissue age is suggested have a particularly important influence on the distribution of intracortical remodeling within cross-sections, in part due to its consequences for increased loading cycles and accumulation of fatigue damage. Tissues deposited at the periosteal surface are sequentially relocated to inner cortical regions during growth. Thus, older tissues comprising the inner and mid-cortical circumferential rings are expected to exhibit higher proportions of secondary osteonal bone than recently-deposited tissues comprising the outer circumferential ring. Results are considered within the context of other circumstances of bone growth remodeling (e.g., patterns of cortical drift, described in Chapter 5), which may also be important in explaining this variation.

B) To determine whether the percent area of secondary osteonal bone varies among radial sectors within cross-sections. As material properties and fatigue behavior of bone vary considerably in tension and compression, long bones subjected to bending at midshaft are expected to exhibit significant variability across cortical regions (i.e., radial sectors) in the extent of intracortical remodeling. In the absence of available *in vivo* strain data, the mechanical significance of regional variability in intracortical remodeling is considered within the context of results from analysis of cross-sectional geometry

presented in Chapter 4 of this thesis². Specifically, the current study will consider whether the proportion of secondary osteonal bone is increased in the plane of greatest structural rigidity to bending (i.e., the *maximum second moment of area*, or $I_{\max}$).

MATERIALS AND METHODS

This study was conducted as part of a larger examination of cortical bone macro- and micro-structural development at the midshaft femur and humerus of *Chlorocebus aethiops* (vervet monkeys, $N=32$) and *Hylobates lar* (white-handed gibbons, $N=28$) and at the midshaft femur of *Pan troglodytes* (common chimpanzees, $N=12$).

Materials

Sample selection. As described in detail in Chapter 3 of this thesis, several criteria were prioritized in the selection of collections from which specimens would be taken for the current study: 1) collections comprising wild-caught individuals from restricted geographic areas; 2) collections comprising an equal distribution of individuals among different age classes throughout the postnatal growth period (discussed below), an equal distribution of males and females, and associated body mass data; 3) collections

² Engineering beam theory predicts that several cross-sectional geometric properties, which characterize the amount and distribution of bone material around a given cross section, are proportional to the resistance of long bones to the strains engendered by different kinds of loading (Biewener, 1992; Ruff and Hayes, 1983; Wainwright et al., 1976). According to principles of cross-sectional geometric analysis, greater structural reinforcement is expected in the plane where maximal strains occur in a long bone diaphysis subjected to bending. The most critical cross-sectional property for estimating maximum bending rigidity in the section is the *maximum second moment of area* ($I_{\max}$), the orientation of which is specified by the angle theta (θ). See Chapter 4 of this thesis for further description of cross-sectional geometric analysis.

comprising immature individuals and non-geriatric adults. Geriatric adults were excluded on the basis of tooth wear and evidence of osteoarthritis. Individuals showing obvious evidence of skeletal fractures and pathologies (as described by White, 1991) were also avoided.

Both the invasive nature of the sampling protocol required for histological processing and the importance of the above selection criteria meant that the taxonomic range and appropriate samples available for this study were limited. Though it was generally possible to meet criteria 1-3 for the *C. aethiops* and *H. lar* samples, many *H. lar* individuals selected are missing associated body mass data. Further, many criteria were difficult to meet when selecting appropriate specimens for the *P. troglodytes* sample. However, given what little is known of *P. troglodytes*, the sample available for this study still provides invaluable preliminary data on ontogenetic and regional variability in secondary intracortical remodeling.

Biological age classes. In the absence of chronological age information for individuals examined in the current study, it was necessary to rely on other criteria to estimate biological age. Dental alveolar emergence was chosen as the most appropriate criterion for estimating biological age in the current study for several reasons (e.g., Smith, 1989; Smith, 1992). For statistical analysis in the current study, individuals were classified into five biological age classes, presented in Table 6-1 and described in detail in Chapter 3 of this thesis.

Tooth eruption and long bone epiphyseal union status were assessed according to the methods of Wintheiser et al. (1977) and Bolter and Zihlman (2003). Individuals

continue skeletal growth after full eruption of the dentition (e.g. personal observations, and Bolter and Zihlman, 2003; Watts, 1990). Thus, individuals were only considered adult when they had reached both dental and skeletal maturity, defined by complete permanent dentitions and complete fusion of all long bone epiphyses, respectively.

Among adults, geriatric individuals were excluded on the basis of extreme tooth wear or evidence of osteoarthritis as described by White (1991).

Description of the sample. Table 6-2 provides details about sample representation across biological age classes in the current study. The femur of *C. aethiops* SLW 47 was excluded from this analysis due to preparation artifacts, and resulting difficulty in the interpretation of microstructural details necessary for accurate identification of secondary osteons. Further, anteroposterior orientation of the *C. aethiops* SLW 21 humerus was lost during processing, and this particular bone sample was therefore excluded from analysis of orientation-dependent variables (i.e., radial sectors). Further detailed information about individuals comprising each species sample can be found in Chapter 3 and in Appendix B.

Sample location

Bone samples extracted from the midshaft femur and humerus were utilized in this study. The midshaft location is commonly chosen for investigations of bone structural properties and histocompositional variables, making results more comparable to other published studies (e.g., Burr, 1992; Goldman et al., 2003; Schaffler and Burr, 1984; Schaffler et al., 1985)

Identification of the midshaft of long bones under investigation is usually based on a measurement of bone length, which includes the epiphyses of a long bone (e.g., Ruff and Hayes, 1983). However, this traditional measure of bone length prevents the inclusion of specimens for which epiphyses are either unfused, broken or otherwise missing. To maintain consistency in our definition of midshaft between immature (unfused) and adult specimens, a measure of bone length was developed for the current study which excluded the epiphyses. Bone length measurements and orientations were completed as described in Chapter 3 of this thesis.

Image acquisition and image processing

The sample removal, preparation and imaging methods are described in detail in Chapter 3 of this thesis, and are briefly summarized below. Bone samples were imaged in both conventional transmitted light microscopy (LM) and circularly-polarized light microscopy (CPL) modalities. This facilitated examination of microscopic details necessary for the discrimination of secondary intracortical remodeling; cement lines and Haversian canals could be readily viewed in LM, while the organization of the collagen fiber matrix could be readily examined using CPL. Acquired LM and CPL images were then imported into several commercially available software packages for image processing, image visualization and data acquisition.

Image acquisition. All images were acquired at low magnification using a Leica DMRX/E Universal microscope (Leica Inc., Wetzlar, Germany.) configured with an automated Marzhauser stage (0.1 μm accuracy in 'X' and 'Y') and a circularly polarized

light filter arrangement. Images were captured using a JVC KY55BU (5X objective; 1 pixel = 1.4970 μm) or JVC KY55BE (5X objective; 1 pixel = 1.2563 μm) color CCD camera, and were automatically transferred to a Syncroscopy Montage Explorer system (Synoptics Ltd., Cambridge, UK.). (The JVC KY55BU camera was upgraded to a JVC KY55BE model in the middle of this study.) The Syncroscopy Montage Explorer system relies on pattern-recognition algorithms to automate the montaging of large field-of-view, high-definition images in real-time. This capability overcomes the limitation of a restricted field-of-view in conventional imaging techniques, and produces a single image of an entire cross section of bone (up to 30,000 x 30,000 pixels in size) that retains original microscopic detail afforded by the optical conditions of the microscope.

Polarized light imaging of bone samples in this study was completed using a Leica DMRX/E Universal microscope configured with a circularly-polarized light (CPL) filter arrangement in place, for the strict purpose of visualizing the organization of the collagen fiber matrix in bone to facilitate identification of secondary intracortical remodeling. While linear polarized light microscopy would have been adequate for the purpose of the current study, visualization was further improved with CPL as this technique eliminates the “Maltese Cross” artifact, in which reduced light intensity occurs at each 45-225° and 135-315° of arc relative to the transmission axes of the polarizer (Bromage et al., 2003). Polarized light microscopy and the CPL filter arrangement are described in further detail in Chapter 3 of this thesis.

LM imaging was undertaken with the bottom linear polarizing filter of the CPL filter arrangement removed, creating conventional (unpolarized) transmitted light illumination. To improve LM image quality, individual image fields were corrected in

Montage Explorer for illumination and color unevenness using shade correction functions as the montage was assembled.

Following acquisition, all images of left-side elements were horizontally flipped in Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.) to maintain a common orientation for analysis.

Image processing and creation of bone tissue maps. LM and CPL images were imported into Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA.), where they were superimposed for visualization purposes and for the creation of tissue type maps. Image processing functions are outlined in detail in Appendix D of this thesis, and are summarized below.

Sequential image processing steps were performed using the ‘Layers’ function. First, a binary overlay was created from the original LM image, on which most non-bone areas (e.g., medullary canals, large resorption spaces, large cracks, but not small vascular canals and Haversian spaces) were assigned to a background color (black) so they could be deleted in later quantitative analysis. Secondly, bone areas comprised of secondary intracortical remodeling (described below) and primary bone tissues (as described in Chapter 5) were manually traced and color-coded on separate layers. (Figure 6-1).

This composite tissue type map was collapsed into one layer, using the ‘Flatten’ function. Flattened images were reduced to 25 % or 12.5 % (for *P. troglodytes* samples) of their original pixel dimensions. Finally, these images were converted to gray-scale, in which bone area representing secondary intracortical remodeling was assigned a gray-level value of 114. Non-bone areas were assigned a gray-level value of 0 (= black), and

thus could be eliminated from quantitative analysis.

Identification of secondary intracortical remodeling

The current study examined variability in a measure of cumulative intracortical remodeling, as represented by the total area of secondary osteonal bone, including both *in tact* and fragmentary secondary osteons (e.g., Paine and Godfrey, 1997). While this variable does not provide information about the rate at which new secondary remodeling events are initiated, it does provide a measure of the overall extent to which bone at the midshaft femur and humerus has been intracortically-remodeled.

In tact and fragmentary secondary osteons were identified on the basis of the following criteria: 1) presence of a complete or partial Haversian canal, surrounded by centripetally-arranged lamellar bone, and 2) presence of a complete or partial cement (i.e. reversal) line, delimiting a secondary osteon from adjacent tissue. In areas of dense intracortical remodeling, fragmentary osteons were also identified by the presence of lamellae following a distinct arc-like trajectory and demonstrating features consistent with observations of adjacent *in tact* secondary osteons. (Figure 6-2).

Image data acquisition

Gray-scale tissue maps were imported into Optimas 6.5 Image Analysis software (Media Cybernetics Inc., Silver Spring, MD.), where data relating to the pixel dimensions of bone areas comprised of secondary osteons were collected using a customized image segmentation macro described by Feik et al. (2000) and Goldman (2001). This macro relied on Fourier shape descriptor techniques to automate the determination of periosteal

and endosteal surface outlines of each cross-section.³ Following determination of bone outlines, the section centroid was then calculated and, based on a user-defined orientation point ('anterior', as described in Chapter 3), the antero-posterior axis of the section was determined. Seven additional lines were then placed, resulting in 8 transects located at equal angles from one another, which divided the cross-section into 16 radial sectors. Following this, periosteal and endosteal outlines were used to define two additional circumferential outlines, equally spaced from one another and from surface outlines, to divide the cross-section into three circumferential rings: an inner ring bordering the endosteal margin, a mid-cortical ring, and an outer ring bordering the periosteal margin. (See Figure 6-3). As a result, this macro divided each bone cross-section into a total of 48 segments (16 radial sectors x 3 circumferential rings), from which quantitative gray-level data were extracted. Within each bone segment, the number of pixels representing secondary osteonal bone was calculated, and data were transferred to a Microsoft Excel spreadsheet. This method permitted the quantitative analysis of regional variation in primary bone tissue within bone cross sections. Additionally, segment data could be collapsed for analysis of whole bone cross-section proportions.⁴

The decision was made to reduce the number of radial sectors analyzed, in order

³ On occasion, it was necessary to edit original images in Adobe Photoshop prior to importing into Optimas. Using a small black line of 2 pixel diameter, small cracks open to the bone surface were closed off and / or large trabeculae which protruded into the medullary space were detached from the endosteal surface.

⁴ This method provided the most useful means available of reliably identifying bone segments for quantitative analysis of regional variability in cortical microstructure. However, it is also noted that bone segments defined on this basis may not be developmentally-homologous. For instance, bone tissues of endosteal and periosteal origin were variably-represented in the inner circumferential ring, dependent upon local growth circumstances associated with specimen age, element and taxon representation.

to improve statistical power. Qualitative observations indicated that some regional patterns may be lost by reducing the number of sectors to 4 (corresponding to anterior, posterior, medial and lateral cortices). Thus, the number of sectors was reduced to 8, permitting examination of variation in tissue type proportions across anterior, antero-medial, medial, postero-medial, posterior, postero-lateral, lateral, and antero-lateral cortices.

Statistical analysis

The proportion of secondary osteonal bone (%HAV) was calculated as a percentage of total cortical area from raw pixel dimensions generated in Optimas according to the following equation:

$$\% \text{ HAV} = (\text{pixel dimensions of HAV} / \text{total pixel dimensions of cortical area}) * 100$$

% HAV was examined in analyses of tissue type distributions in whole bone cross-sections and in circumferential rings and radial sectors within cross-sections. Spearman correlations were also calculated based on absolute area (mm²) dimensions of secondary osteonal bone.

In many cases, assumptions of parametric statistical analysis were not met or could not be verified due to small sample sizes ($n < 5$) within age classes and species groups. Thus, all statistical analyses reported here were performed using nonparametric techniques, in the Statistica 6.0 (Statsoft, Inc.) software package. Except where indicated, results associated with a probability value of $p \leq 0.05$ were considered

statistically significant.

Association of secondary intracortical remodeling with age class and cortical area.

Associations of % HAV with age class and cortical area were examined using nonparametric Spearman rank order correlation. This method assigns ranks to original variates based on their relative magnitudes, and provides a measure of the degree to which the rank order of paired variables is the same. Therefore, this method is appropriate for variables measured on an ordinal scale, and for cases in which bivariate distributions do not meet parametric assumptions of linearity and normality (Sokal and Rohlf, 1995).

Statistical comparisons among groups. Contrasts among group medians were made using Kruskal-Wallis (for two independent samples) and Mann-Whitney (for three or more independent samples) tests. Age class and sex were factors in these analyses. Where Kruskal-Wallis tests revealed significant contrasts among groups, post-hoc pairwise Mann-Whitney tests were performed to identify significant group contrasts. These methods assume that the distributions among groups are similar in shape. Contrasts among groups were also made using Kolmogorov-Smirnov (for two independent samples) and Median tests (for three or more independent samples), which test for overall differences in the shape and location of distributions among groups. Results of Mann-Whitney and Kruskal-Wallis tests are presented here.

Within single cross-sections, growth remodeling processes (e.g., cortical drift) may be expected to affect multiple rings and/or sectors in a similar way. Thus, contrasts

in % HAV among elements, circumferential rings and radial sectors were conducted using nonparametric tests for dependent samples: Wilcoxon matched pairs (for two groups) and Friedman ANOVA (for three or more groups). Where Friedman ANOVA tests revealed significant contrasts among groups, post-hoc pair-wise Wilcoxon matched pairs tests were performed to identify significant group contrasts.

For post-hoc comparisons using Mann-Whitney and Wilcoxon matched pairs tests, significance values were corrected for repeated tests using the Bonferroni method, in which p is divided by the number of comparisons (Sokal and Rohlf, 1995). Many post-hoc contrasts were no longer significant at $p \leq 0.05$ after Bonferroni correction. Because small sample sizes may partly account for this result, significant contrasts at $p \leq 0.10$ are also reported.

RESULTS

Raw data (reported in pixel dimensions) for whole cross-section HAV in *C. aethiops*, *H. lar* and *P. troglodytes* are presented in Appendix F (Table F-1), and descriptive statistics for each taxon sample are provided (Table F-2). Ontogenetic and regional variability in % HAV is examined by taxon below.

C. aethiops

Complete descriptive statistics for whole cross-section % HAV are summarized by age class in Appendix F (Table F-3 a). Tissue maps derived from all individuals in the sample are provided in Appendix G-1.

Whole cross-section % HAV. In the total sample with all age classes combined, HAV comprised a mean 7.7 % (range = 0.0 – 22.4, SD = 5.4527) of cortical area at the midshaft femur and 21.2 % (range = 2.6 – 36.5, SD = 8.4245) of cortical area at the midshaft humerus. Figure 6-4 a depicts bivariate plots of % HAV at the midshaft femur and humerus, respectively, plotted against cortical area and categorized by age class. An overall increase in % HAV was observed in both elements across age classes. Results of Spearman correlation analysis demonstrated a statistically-significant positive correlation of % HAV with age class and cortical area (CA) at the midshaft femur ($r = 0.6175$, $p = 0.0003$) and humerus ($r = 0.47757$, $p = 0.0076$). (Table 6-3).

These results were also consistent with Kruskal-Wallis tests, although most post-hoc contrasts between age classes were not significant ($p \leq 0.10$) after Bonferroni correction. (Table 6-4). The midshaft femur showed an increase from a mean of 1.8 % (range = 0.0 – 4.0) in the DECID age class, to a mean of 11.6 % (range = 5.6 – 22.4) in the EPIPH age class. At the midshaft humerus, % HAV increased from a mean of 11.3 % (range = 2.6 – 24.6) in the DECID age class to 26.5 % in the EPIPH age class (range = 22.8 – 30.2). The humerus exhibited a significantly greater % HAV compared to the femur, and this pattern was observed across all age classes. (Table 6-5).

Inspection of bivariate plots shown in Figure 6-4 b revealed substantial overlap among sexes in % HAV, and results of Kruskal-Wallis tests showed that sex was not a significant factor contributing to variation in % HAV. (Table 6-6). Therefore, analyses to follow were performed on combined-sex samples.

Regional variability in % HAV at the midshaft femur. Variability in % HAV among

circumferential rings. In the total sample with all age classes combined, % HAV was greatest in the inner ring (mean = 17.1 %), intermediate in the mid-cortical ring (mean = 7.11 %) and lowest in the outer ring (mean = 1.4 %). This trend was also apparent when age classes were considered separately. (Figure 6-5 a and b). When circumferential rings were considered separately, all showed evidence of an increase in % HAV across age classes, although there was substantial overlap among age classes. However, even in the EIPPH age class, HAV only comprised a mean 2.9 % of cortical area in the outer ring compared to 23.2 % of cortical area in the inner ring.

Friedman's ANOVA revealed statistically-significant variation in % HAV among circumferential rings in the total sample, and when age classes were considered separately. (Table 6-7). When rings were considered separately, Kruskal-Wallis tests detected statistically-significant variability among age classes for the outer and mid-cortical rings. (Table 6-8). Post-hoc contrasts were statistically significant at $p \leq 0.10$ after Bonferroni correction for those comparisons between the youngest (DECID or M1) and oldest (M3 or EIPPH) age classes.

Variability in % HAV among radial sectors. Age class and circumferential ring were significant factors in explaining variation in % HAV. Thus, analysis of variation among radial sectors was undertaken for each ring separately, in the total sample and with age classes considered separately.

Inspection of mean plots (Figure 6-6 a) of % HAV in the DECID and M1 age classes revealed increased proportions of HAV in sectors 8, 1, and 2 (anterior and adjacent sectors) and in sector 5 (posterior) of the inner and, to a lesser extent, mid-cortical rings. In the M2, M3 and EIPPH age classes, a pattern of increased % HAV in

sector 5 (posterior cortex) compared to all other sectors was observed across all circumferential rings. This trend was also observed in the total sample (all age classes combined). Increased % HAV in sector 5 compared to other cortical regions, particularly during later ontogeny, was often most pronounced in the mid-cortical and outer rings, where there was less overlap in % HAV among radial sectors. (Figure 6-6 b).

Friedman's ANOVA detected statistically-significant variability in % HAV among radial sectors in the total sample, and in the M2 (outer ring only), M3 (mid-cortical and outer rings) and EPIPH (all rings) age classes. (Table 6-9). Post-hoc pairwise comparisons among radial sectors in the total sample (all age classes combined) were statistically-significant at $p \leq 0.05$ for those contrasts including sector 5, with a few additional significant contrasts identified at $p \leq 0.10$. Significant contrasts among sectors were most commonly observed in the outer ring. Post-hoc contrasts among radial sectors were not statistically significant at $p \leq 0.10$ after Bonferroni correction when age classes were considered separately.

Qualitative observations. Qualitative observations indicated that secondary osteons were most commonly distributed in older periosteal tissues and at the boundary between endosteal and periosteal cortex, where they invaded cortical tissues of endosteal origin. (See Figure 6-7 a and Appendix G-1). Within periosteal cortex, intracortical remodeling often occurred in association with primary vascular interstitial bone in younger age classes. (Figure 6-7 b). Secondary osteons were generally sparsely distributed among recently-deposited periosteal tissues, with the exception of the posterior cortex. In the latter cortical region, secondary osteons often occurred in association with Sharpey fibers. (Figures 6-7 c and d). In the M2 and older age classes,

intracortical remodeling was largely restricted to regions of endosteal compacted coarse cancellous bone, with the exception of the posterior cortex. (Figure 6-7 e).

Regional variability in % HAV at the midshaft humerus. Variability in % HAV among circumferential rings. Figure 6-8 depicts age class means of % HAV categorized by circumferential ring. A tendency for % HAV to be highest in the mid-cortical ring was visually apparent in the total sample. (Also see Table 6-10). However, inner (mean = 22.07 % in the total sample), mid-cortical (mean = 27.6 % in the total sample), and outer (mean = 15.1 % in the total sample) rings exhibited considerable variation and overlap in % HAV. When rings were considered separately, both the inner and mid-cortical rings showed an overall increase in the proportion of secondary osteonal bone with age, reaching mean proportions of 29.6 % and 36.2 %, respectively, in the EPIPH age class. The proportion of secondary osteonal bone in the outer ring increased substantially between the DECID and M1 age classes, and then was stable thereafter.

Friedman's ANOVA revealed statistically-significant variation in % HAV among circumferential rings in the total sample, and in the DECID and EPIPH age classes when considered separately. (Table 6-10). When rings were considered separately, Kruskal-Wallis tests detected statistically-significant variability among age classes for the outer ring only. (Table 6-11). Post-hoc statistical contrasts were largely non-significant after Bonferroni correction.

Variability in % HAV among radial sectors. As described above, age class and circumferential ring were significant factors in explaining variation in % HAV. Thus, analysis of variation among radial sectors was undertaken for each ring separately, in the

total sample and with age classes considered separately.

Inspection of mean plots reveals that % HAV was lowest in sectors 4 - 6 (posterior and adjacent sectors) compared to all other sectors. (Figure 6-9). This pattern characterized all circumferential rings, and was observed across all age classes. Age class means of % HAV were below 10 % in sectors 4 - 6 in all rings, with the exception of the inner ring which frequently exhibited a greater proportion and more overlap in % HAV among sectors. Age class mean % HAV in the mid-cortical and outer rings was often highest in sectors 1, 2 and/or 3 (anterior to anterolateral). However, there were exceptions to this trend, with high proportions of HAV also observed in sectors 7 and/or 8.

Friedman's ANOVA detected statistically-significant variability in % HAV among radial sectors in the total sample, and when age classes were considered separately. (Table 6-12). Statistically-significant post-hoc contrasts at $p \leq 0.05$ in the total sample (all age classes combined) were most commonly identified for those comparisons involving sectors 4-6. Post-hoc contrasts were not significant after Bonferroni correction when age classes were considered separately.

Qualitative observations. Qualitative observations indicated that secondary osteons were commonly distributed at the boundary between endosteal and periosteal cortex in the medial, anterior and lateral cortices, where they were observed in cortical tissues of endosteal origin and older tissues of periosteal origin. In lateral and antero-lateral cortices, they were more commonly observed in older periosteal tissues. In some areas of the anterior and adjacent cortices, secondary osteons were also found in association with Sharpey fibers. Secondary osteons were sparsely distributed in the

posterior cortex. (Figure 6-10 a and Appendix G-1). Within periosteal cortex, intracortical remodeling was found in association with primary vascular tissue in young age classes. (Figure 6-10 b). However, in the M2 and older age classes, secondary osteons were also found in association with less vascularized interstitial bone, particularly in the anterolateral and lateral cortices. (Figure 6-10 e). Intracortical remodeling of primary endosteal cortex was often extensive, and found in areas of circumferential and compacted coarse cancellous tissue. (Figure 6-10 c and d).

H. lar

Complete descriptive statistics for whole cross-section % HAV are summarized by age class in Appendix F (Table F-3 b). Tissue maps derived from all individuals in the sample are provided in Appendix G-2.

Whole cross-section % HAV. In the total sample with all age classes combined, HAV comprised a mean 38.2 % (range = 25.5 – 57.0, SD = 8.3367) of cortical area at the midshaft femur and 49.2 % (range = 35.4 – 66.8, SD = 8.8862) of cortical area at the midshaft humerus. Figure 6-11 a depicts bivariate plots of % HAV at the midshaft femur and humerus, respectively, plotted against cortical area and color-coded for age class. Results of Spearman correlation analysis demonstrated no statistically-significant association of % HAV with age class or cortical area (CA) at the midshaft femur and humerus. However, absolute area of HAV did show a significant association. (Table 6-13). Results for % HAV were also consistent with Kruskal-Wallis tests. (Table 6-14). However, the humerus exhibited a significantly greater proportion of HAV compared to

the femur. This pattern was observed in total sample, and in older age classes when considered separately. (Table 6-15 and Appendix G-2).

Inspection of bivariate plots shown in Figure 6-11 b revealed substantial overlap among sexes in % HAV, and results of Kruskal-Wallis tests showed that sex was not a significant factor contributing to variation in % HAV. (Table 6-16). Therefore, analyses to follow were performed on combined-sex samples.

Regional variability in % HAV at the midshaft femur. Variability in % HAV among circumferential rings. Figure 6-12 depicts age class mean % HAV categorized by circumferential ring. % HAV was lowest in the outer ring (mean = 9.04 % in the total sample) compared to the mid-cortical (mean = 52.4 % in the total sample) and inner (mean = 63.6 % in the total sample) rings across all age classes. (Also see Table 6-17). When circumferential rings were considered separately, % HAV in the inner ring increased across age classes, from an age class mean of 49.6 % in the DECID age class and 71.4 % in the EPIPH age class. In the mid-cortical ring, mean proportions of secondary osteonal bone were highest in the DECID (71.2 %) and M1 (68.7 %) age classes, and declined to below 50% in older age classes.

Friedman's ANOVA revealed statistically-significant variation in % HAV among circumferential rings in the total sample, and when age classes were considered separately. (Table 6-17). When rings were considered separately, Kruskal-Wallis tests detected statistically-significant variability among age classes for the inner ring only. (Table 6-18). Post-hoc statistical contrasts among rings in the total sample and in the M2 – EPIPH age classes when considered separately were statistically significant at $p \leq 0.10$

after Bonferroni correction.

Variability in % HAV among radial sectors. As described above, age class and circumferential ring were significant factors in explaining variation in % HAV. Thus, analysis of variation among radial sectors was undertaken for each ring separately, in the total sample and with age classes considered separately.

Inspection of mean plots of % HAV depicted in Figure 6-13 a revealed several trends. In the inner ring, mean % HAV was substantially lower in sectors 7, 8, 1, and 2 (medial to anterolateral) compared to sectors 3 - 6 (lateral to posteromedial) in the DECID and M1 age classes. However, there was considerable overlap in mean % HAV among sectors in older age classes. (Figure 6-13 b). In the mid-cortical ring, the DECID age class showed substantial overlap in % HAV among sectors. However, older age classes (particularly M3 and EIPPH) showed increased mean % HAV in sector 5 (posterior) compared to adjacent sectors 4 and 6. Mean % HAV was greater than 70 % in sector 5 in the M3 and EIPPH age classes. Increased mean % HAV was also commonly observed in sectors 1 and 8 (and to a lesser extent, sectors 2 and 7), which surpassed proportions of HAV observed in sector 5 in some age classes. In the outer ring, mean % HAV was commonly highest in sector 5, reaching mean proportions greater than 30 % in the M3 and EIPPH age classes. This pattern was evident in the total sample and when age classes were considered separately, except in the DECID and M1 age classes where mean % HAV was high in sectors 1 and 8.

Friedman's ANOVA detected statistically-significant variability in % HAV among radial sectors in the total sample (outer and mid-cortical ring), and in the DECID (outer and inner ring), M1 (outer ring only), M2 (mid-cortical ring only), M3 (all rings)

and EPIPH (outer and mid-cortical ring) age classes. (Table 6-19). Statistically-significant post-hoc pair-wise comparisons among radial sectors were identified only in the total sample (all age classes combined), and supported observations of increased mean % HAV in sector 5 of the outer ring, and sectors 1, 2, 5 and 8 of the mid-cortical ring.

Qualitative observations. Qualitative observations indicated that secondary osteons were commonly distributed at the boundary between endosteal and periosteal cortex, where they were observed in cortical tissues of endosteal origin and older tissues of periosteal origin. (Figure 6-14 a and Appendix G-2). In fact, secondary osteons were often so densely distributed in this region, the precise location between endosteal and periosteal bone formation was difficult to reconstruct locally. Secondary osteons were often observed to occupy a mid-cortical zone, especially in younger individuals, where more recently deposited periosteal and endosteal tissues were not secondarily remodeled. (Figure 6-14 a - d). Large resorption spaces were common in DECID individuals, and secondary osteons in younger age classes were often eccentric or irregular in shape, showing evidence of drift. (Figures 6-14 b). In older age classes, intracortical remodeling also appeared to be extensive in some locations of the periosteal cortex, although it was preferentially distributed in older regions. An exception to this pattern was the posterior cortex, where secondary osteons were observed in more recently-deposited periosteal regions, often in association with Sharpey fibers. (Figure 6-14 e). Intracortical remodeling of periosteal cortex in older age classes was associated with less vascular primary interstitial bone. (Figure 6-14 f).

Regional variability in % HAV at the midshaft humerus. Variability in % HAV among circumferential rings. Figure 6-15 depicts age class means of % HAV categorized by circumferential ring. At the midshaft humerus, the mean % HAV was lowest in the outer ring (mean = 24.1 % in the total sample) compared to the mid-cortical (mean = 66.9 % in the total sample) and inner (mean = 63.6 % in the total sample) rings, except in the DECID age class. When circumferential rings were considered separately, considerable variability among age classes was found for the inner ring, in which the proportion of secondary osteonal bone increased substantially from the DECID (mean = 28.3 %) and M1 (mean = 49.3 %) age classes to comprise over 70 % of cortical area in all older age classes. The opposite trend was observed in the outer ring, where the percent area of secondary osteonal bone was greatest in the DECID age class (mean = 44.7 %) compared to all older age classes. No age-related variation was visually apparent in the mid-cortical ring.

Friedman's ANOVA revealed statistically-significant variation in % HAV among circumferential rings in the total sample, and when age classes were considered separately. (Table 6-20). When rings were considered separately, Kruskal-Wallis tests detected statistically-significant variability among age classes for the inner ring only. (Table 6-21). Post-hoc statistical contrasts among rings were statistically significant at $p \leq 0.10$ after Bonferroni correction for those contrasts involving the outer ring in the total sample and in the M3 and EIPH age classes when considered separately.

Variability in % HAV among radial sectors. As described above, age class and circumferential ring were significant factors in explaining variation in % HAV. Thus, analysis of variation among radial sectors was undertaken for each ring separately, in the

total sample and with age classes considered separately.

Inspection of mean plots of % HAV revealed considerable variability in sector proportions within rings and across age classes (Figure 6-16 a), as well as overlap in the distribution of % HAV among sectors within age classes (Figure 6-16 b). Several trends can be recognized. In the inner ring, lower mean % HAV was observed in sectors 3 - 6 (lateral to posteromedial) compared to other sectors in the DECID and M1 age classes. Older age classes showed considerable overlap among sectors, but there was a trend in the M3 and EPIPH age classes for % HAV to show an “M” shaped distribution; % HAV was reduced in sector 5 (posterior) and slightly higher in sectors 3 and 7 or 8 compared to adjacent sectors. In the mid-cortical ring, there was considerable variation and overlap in % HAV among sectors in the DECID and M1 age classes (except sector 5 in the M1 age class). In the M2 – EPIPH age classes, mean % HAV was usually considerably lower in sectors 5-6 (posterior and posteromedial) compared to sectors 1-4 (anterior to posterolateral) of the mid-cortical ring. Mean % HAV was increased to a variable extent in sectors 7-8 (medial and anteromedial). In the outer ring, mean % HAV showed a variable distribution across sectors in the DECID – M2 age classes. In the M3-EPIPH age classes, however, mean % HAV was greatest in sector 1 (anterior) and, although there was considerable overlap with adjacent sectors, % HAV was generally lowest in sector 5 (posterior).

Friedman’s ANOVA detected statistically-significant variability in % HAV among radial sectors in the total sample, and in the M2 (outer and mid-cortical rings), M3 and EPIPH age classes. (Table 6-22). Statistically-significant post-hoc comparisons among radial sectors at $p \leq 0.10$ were identified only in the total sample (all age classes

combined), and primarily reflected a high mean % HAV in sector 1 of the outer ring and sectors 1-3 in the mid-cortical ring.

Qualitative observations. Intracortical remodeling at the midshaft humerus was qualitatively similar to that observed at the midshaft femur. Secondary osteons were concentrated in older periosteal cortex and at the boundary between endosteal and periosteal cortex, where they invaded cortical tissues of endosteal origin. In the latter, they occurred in association with interstitial tissues having a circumferential or compacted coarse cancellous organization. (Figure 6-17 and Appendix G-2). Large resorption spaces were common in DECID individuals, and secondary osteons were often eccentric or irregular in shape, showing evidence of drift. (Figure 6-18 b).

P. troglodytes

Complete descriptive statistics for whole cross-section % HAV are summarized by age class in Appendix F (Table F-3 c). Tissue maps derived from all individuals in the sample are provided in Appendix G-3.

Whole cross-section % HAV. In the total sample with all age classes combined, HAV comprised a mean 54.0 % (range = 37.7 – 78.1, SD = 11.8610) of cortical area at the midshaft femur. Figure 6-18 depicts % HAV at the midshaft femur, plotted against cortical area and color-coded for age class. Due to small sample sizes and unknown sex, nonparametric statistical analysis was restricted to the total sample, with all age classes combined. Results of Spearman correlation analysis demonstrated no statistically-significant association of % HAV with age class or cortical area (CA) for % HAV.

However, a significant association was found for absolute area of HAV. (Table 6-23).

Regional variability in % HAV at the midshaft femur. Variability in % HAV among circumferential rings. Figure 6-19 depicts age class mean % HAV categorized by circumferential ring. % HAV was lower in the outer ring (mean = 29.5 % in the total sample) compared to the mid-cortical (mean = 65.7 % in the total sample) and inner (mean = 78.1 % in the total sample) rings across all age classes. (Also see Table 6-24). The inner ring showed evidence of an increase in % HAV in older age classes compared to young age classes. The outer ring showed considerable variation in % HAV in the M3 and EPIPH age classes.

Friedman's ANOVA revealed significant variation in % HAV among rings in the total sample. (Table 6-24). All post-hoc statistical contrasts among rings were statistically significant at $p \leq 0.05$ after Bonferroni correction.

Variability in % HAV among radial sectors. As described above, age class and circumferential ring were significant factors in explaining variation in % HAV. Thus, analysis of variation among radial sectors was undertaken for each ring separately, in the total sample and with age classes considered separately.

Inspection of mean plots of % HAV revealed several trends. (Figure 6-20). There was considerable overlap among sectors in % HAV in the inner ring, although slight sector differences were apparent in some age classes. Sector proportions in the mid-cortical and outer rings followed a generally similar pattern, with lower mean % HAV observed in sectors 6-8 (posteromedial, medial and anteromedial) compared to sectors 1-5. This pattern was most marked in the mid-cortical ring.

Friedman's ANOVA detected statistically-significant variability in % HAV among radial sectors in the total sample, with all age classes combined. (Table 6-25). Significant post-hoc contrasts at $p \leq 0.10$ after Bonferroni correction provide support for reduced % HAV in sectors 6-8 in the mid-cortical and outer rings described above.

Qualitative observations. Qualitative observations indicated that secondary osteons were commonly distributed in older periosteal cortex and at the boundary between endosteal and periosteal cortex, where they invaded cortical tissues of endosteal origin. (Figure 6-21 and Appendix G-3). As intracortical remodeling in this region was often dense, the precise location of the reversal between endosteal and periosteal formation was frequently difficult to reconstruct locally. Secondary osteons were often observed in regions of endosteal circumferential and compacted coarse cancellous bone. They were also observed throughout cortical regions of periosteal origin, showing a patterned regional distribution. In younger individuals, secondary osteons tended to be concentrated in older-deposited regions of the periosteal cortex, often in association with primary vascular interstitial bone. (Figures 6-21 b and c). In more recently-deposited regions of the periosteal cortex of older individuals, it was often associated with less vascular interstitial bone. (Figure 6-21 d). Intracortical remodeling of periosteal cortex was also more extensive posteriorly where it occurred in association with Sharpey fibers (Figure 6-21 e), and in older individuals, in the lateral and anterior cortices.

DISCUSSION

In the current study, ontogenetic variability in the proportion of secondary osteonal bone was examined at the midshaft femur and humerus in *C. aethiops* and *H. lar*

and at the midshaft femur in *P. troglodytes*. Results of this study 1) contribute new information concerning age-related patterns of intracortical remodeling in wild-collected nonhuman primates, and 2) contribute to our understanding of the manner in which regional variation in intracortical remodeling of primate limb diaphysis may reflect local dynamics of bone growth and mechanical loading history.

General patterns of intracortical remodeling: whole cross-section proportions.

When all age classes were combined, secondary osteonal bone was found to comprise a mean 7.7 % of cortical area at the midshaft femur and 21.2 % of cortical area at the midshaft humerus of *C. aethiops*, a mean 38.2 % of cortical area at the midshaft femur and 49.2 % of cortical area at the midshaft humerus of *H. lar*, and a mean 54.0 % of cortical area at the midshaft femur of *P. troglodytes*.

Intracortical remodeling of long bone diaphyses in *C. aethiops* has been quantitatively examined by Schaffler and Burr (1984) and Paine and Godfrey (Paine, 1994; Paine and Godfrey, 1997). In the former study, secondary osteonal bone accounted for 8.0 % of cortical area in the single adult individual included in their sample. This value falls entirely within the range of the current sample, and it is also consistent with adult values. Paine and colleague (Paine, 1994; Paine and Godfrey, 1997) reported mean percent areas of secondary osteonal bone of 20.9 % and 35.8 % at the midshaft femur and humerus, respectively, based on a sample of 19 adult vervet monkeys. These values are higher than reported here for the total sample, and for adults when considered separately.

Intracortical remodeling at the midshaft femur of *P. troglodytes* has been quantitatively examined in two previous investigations. In the single adult individual

examined by Schaffler and Burr (1984), *in tact* secondary osteons comprised 28.0 % of total cortical area at the midshaft femur. This value was almost identical to the proportion of secondary osteonal bone observed by them in a subadult individual. Mulhern and Ubelaker (2003) collected histological data from four circular fields (1.62 mm in diameter) positioned at the periosteal surface of the anterior, posterior, medial and lateral cortices at the midshaft femur. They reported a mean of 48.5 % (range = 16 – 85, SD = 21.48) percent lamellar (i.e., primary) bone at the midshaft femur in a sample of common chimpanzees ranging in age from 2.0 – 35.0 years.

Previous quantitative analyses of intracortical remodeling in *H. lar* are lacking. In his comparative monograph, Foote (1916) reported dense intracortical remodeling at the midshaft femur of a gibbon. However, Singh and colleagues (1974) reported that secondary osteons were rare in the femur of a white handed gibbon two years of age. Proportions of secondary osteonal bone at the midshaft femur and humerus of *H. lar* observed here are intermediate between those reported previously for other suspensory primates (*Pan troglodytes*, *Ateles* species) and humans (Mulhern and Ubelaker, 2003; Schaffler and Burr, 1984).

While results from the current study are generally consistent with earlier quantitative reports, differences between studies may be explained by several factors. First, examinations of bone histological variables necessarily rely on small and cross-sectional samples, and it is likely that some variation between studies reflects sampling limitations. Measurement techniques employed also likely contribute to variation among studies. For example, Schaffler and Burr (1984) did not include fragmentary osteons in their measure of secondary osteon area, in contrast to the current study. This may have

only a limit effect on reported results for *C. aethiops*, which undergoes relatively little intracortical remodeling. However, this method is likely to underestimate the extent of total intracortical remodeling where secondary osteons are more densely distributed, as is the case in *P. troglodytes*. Results obtained from the sampling method employed in Mulhern and Ubelaker's (2003) investigation of *P. troglodytes* are influenced by the extent to which intracortical remodeling at the midshaft femur shows a regional distribution. Variation among studies may also reflect population differences in intracortical remodeling. While humans show inter-population variability in secondary remodeling parameters (e.g., reviewed by Robling and Stout, 2000), nothing is known about other primates in this respect.

Another important factor which may contribute to variation between the current and previous studies may relate to the fact that primates examined in previous reports were obtained from captive settings, including zoos and laboratories (Kerley, 1966; Mulhern and Ubelaker, 2003; Paine, 1994; Paine and Godfrey, 1997; Schaffler and Burr, 1984). Although vervet monkeys examined by Paine and colleagues (Paine, 1994; Paine and Godfrey, 1997) were "housed in large indoor/outdoor cages that allowed considerable movement" (Paine, 1994: 47), the range of behaviors permitted in such settings may only approximate positional behavior repertoires in the wild. To my knowledge, there have been no quantitative studies of the influence of captivity (vs. wild settings) on parameters of intracortical remodeling in primates. Reduction in mechanical loading approximating conditions of disuse are associated with alterations in BMU activation frequency and a shift in resorption-formation balance towards net bone loss (e.g., Schock et al., 1975; Stout, 1982; Wronski and Morey, 1983), although the pattern

of bone loss may vary in different conditions of disuse (Stout, 1982). Whether this may account for the increased proportion of secondary osteonal bone in captive vervet monkeys studied by Paine and colleague (Paine, 1994; Paine and Godfrey, 1997) is a question that warrants further study.

Finally, age may account for some of the variability observed among adults in different studies. Chronological age was not reported for primates studied by Schaffler and Burr (1984), and vervet monkeys examined by Paine and colleague (Paine, 1994; Paine and Godfrey, 1997) ranged in age from 54 – 161 months (female mean = 100 months, SD = 30.0; male mean = 122 months, SD = 18.8). Although geriatric individuals were excluded from the current study, chronological age of adults examined here is unknown. The extent to which variation in the proportion of secondary osteonal bone among adult nonhuman primates is age-dependent is not well understood. In a study of intracortical remodeling at the midshaft of various long bones in adult saddle-back tamarins (*Saguinus fuscicollis*), Glassman (1985) reported that the accumulation of secondary osteons was most marked during later adulthood (7-15 years of age) compared to early and middle adulthood (1-7 years of age). Age-related variability in the extent of intracortical remodeling during postnatal ontogeny will be considered further below.

Does the percent area of secondary osteonal bone vary with respect to age?

As intracortical remodeling is a destructive process, characterization of ontogenetic variability in proportions of secondary osteonal bone provided an important context for investigation of primary bone tissue type proportions presented in Chapter 5 of this thesis. Results of the current study indicated that sex was not a significant factor

accounting for variation in the proportion of osteonal bone observed at the midshaft femur and humerus of *C. aethiops* and *H. lar*. Similar findings have been reported for rhesus macaques (*Macaca mulatta*) in the midshaft and proximal femur diaphysis (Burr, 1992; Burr et al., 1989; Havill, 2004). Findings concerning the extent to which the proportion of secondary osteonal bone varies with age during postnatal ontogeny are discussed below.

In *C. aethiops* examined here, results demonstrated a significant positive correlation between age class and percent area of secondary osteonal bone at the midshaft femur and humerus. Similar results were obtained when the absolute area of secondary osteonal bone was examined. However, age class explained only 38 % and 23 % of the variance in the proportion of intracortical remodeled bone at the midshaft femur and humerus, respectively, indicating that other factors must also be important.

In *H. lar*, age class was not a significant factor accounting for variation in the proportion of intracortical remodeling. However, absolute area of secondary osteonal bone did show a significant positive correlation with age class. This association was strengthened when cortical area was substituted for age class. These results indicate that, although the absolute area of secondary osteonal bone at the midshaft femur and humerus increased with age in *H. lar*, the proportion of secondary osteonal cortex relative to total cortical area did not. A similar pattern was also found for the midshaft femur of *P. troglodytes*.

The increase in absolute area of secondary osteonal bone with cortical area is consistent with scaling trends described by Paine and Godfrey (Paine, 1994; Paine and Godfrey, 1997). They reported that absolute area of secondary osteonal bone at the

midshaft femur and humerus scaled with isometry or positive allometry with respect to cortical area in galagonids and cercopithecids. The association between absolute area of secondary osteonal bone and age class reported here likely reflects the strong correlation between the latter and cortical area. (See Chapter 4).

Previous examinations of rhesus macaques and common chimpanzees found that age accounted for little, if any, of the variation observed in percent secondary osteonal bone, however (Burr, 1992; Burr et al., 1989; Havill, 2004; Mulhern and Ubelaker, 2003). Burr (1992) reported that while percent area of secondary osteonal bone at the midshaft femur increased slightly with age in growing rhesus macaques, age accounted for only 7 % of the variation. A study of the proximal femur of immature and adult rhesus macaques found no significant age-related increase in this measure (Havill, 2004). Further, no significant age-related variation in percent area of primary lamellar bone was observed at the midshafts of lower limb elements in common chimpanzees during postnatal ontogeny (Mulhern and Ubelaker, 2003). In fact, the proportion of primary cortex at the midshaft femur of a 35 year-old individual included in the latter study was well within the range of immature individuals.

The percent area of secondary osteonal bone only indirectly reflects variation in other remodeling parameters which may be important. Nonhuman primates do exhibit significant age-related variability in other parameters reflecting remodeling rates (Burr, 1992; Burr et al., 1989; Glassman, 1985; Havill, 2004; Kerley, 1966; Mulhern and Ubelaker, 2003). That age accounts for little of the variation observed in percent area of secondary osteonal bone during postnatal ontogeny reflects, at least in part, the influence of local bone growth dynamics, involving addition of new bone subperiosteally and

resorption of older tissues at the endosteal surface (Burr, 1992; Havill, 2004). This serves to remove secondary osteons, which are more commonly observed in inner cortical regions (discussed below), preventing their accumulation with age. This may be particularly true if the rate of accumulation of new secondary osteons slows significantly by the time skeletal maturity is reached, as has been shown for rhesus macaques (Burr, 1992; Burr et al., 1989; Havill, 2004).

Vervet monkeys examined here undergo less intracortical remodeling than gibbons and common chimpanzees and, in this respect, are more similar to rhesus macaques studied previously (Burr, 1992; Burr et al., 1989; Havill, 2004). However, it is not clear how similar vervet monkeys may be to macaques in other parameters of intracortical remodeling. A question that arises from the current study is, given that percent area of secondary osteonal bone shows no age-related variability in *H. lar* and *P. troglodytes*, why does this measure increase with age during postnatal ontogeny in *C. aethiops*, which undergoes less intracortical remodeling? This may reflect the smaller cortical area dimensions of *C. aethiops* compared to *H. lar* and *P. troglodytes*. (See Chapter 4 of this thesis; Figures 4-2 c, 4-4 c, and 4-6 b). The latter taxa deposit more bone subperiosteally at the midshaft femur and humerus during postnatal ontogeny and, in the case of the *P. troglodytes*, lose more bone area at the endosteal surface. Alternatively, in *H. lar* and *P. troglodytes*, which undergo more extensive intracortical remodeling, these observations may also reflect a pattern of recurrent intracortical remodeling within relatively localized cortical regions. The extent to which this variation may reflect differences in other parameters of intracortical remodeling warrants further study.

Does intracortical remodeling show a regional distribution within cross-sections?

While many researchers have made the observation that secondary intracortical remodeling shows a regional distribution within long bone cortices (Amprino and Godina, 1947; Burr, 1992; Currey, 1984, 2002; Enlow and Brown, 1956, 1957, 1958; Foote, 1916; Singh et al., 1974; Skedros et al., 2001; Smith, 1961), this variability has been the focus of few systematic studies in nonhuman primates (Bouvier and Hylander, 1981; Enlow, 1962b; Newell-Morris and Sirianni, 1982; Paine, 1994; Paine and Godfrey, 1997). Thus, the extent to which regional variation in intracortical remodeling of primate long bones reflects the influence of local conditions of bone growth (Enlow, 1962a, 1963, 1976) and mechanical loading history (e.g., Paine, 1994) is not well understood. The current study begins to address this issue by examining regional variability in the percent area of secondary osteonal bone across three circumferential rings and eight radial sectors at the midshaft femur and humerus.

Intracortical remodeling is most prevalent in inner and mid-cortical regions. Results of the current study are consistent with previous reports on bone histology in other vertebrates, including primates, which have indicated that intracortical remodeling in many (though not all) animals is more common in inner cortical regions compared to outer cortical regions near the periosteum (Bouvier and Hylander, 1981; Burr, 1992; Currey, 1984, 2002; Enlow and Brown, 1956, 1957, 1958; Singh et al., 1974; Skedros et al., 2001; Smith, 1961). Several factors have been proposed to explain this pattern, and are discussed below.

The influence of tissue age. Tissue age is suggested to be a particularly important factor influencing the distribution of intracortical remodeling within cross-sections, by virtue of its consequences for the accumulation of increased loading cycles and microdamage due to fatigue (Bouvier and Hylander, 1981; Martin et al., 1998). If intracortical remodeling functions in the repair of microdamage, as suggested by recent evidence (discussed above), older tissues may be preferentially targeted for intracortical remodeling if they approach the end of their fatigue lives earlier than more recently-deposited tissues. Intracortical remodeling may also function in the replacement of older cortical regions which have undergone osteocyte necrosis (Enlow, 1962a, 1976; Enlow and Hans, 1996). As tissues deposited at the periosteal surface are relocated to inner cortical regions during growth (e.g., Enlow, 1963), it was expected in the current study that cortical regions comprising the inner and mid-cortical rings would exhibit higher proportions of secondary osteonal bone than more recently-deposited tissues comprising the outer ring.

Results of the current study were generally consistent with this prediction. Within taxa, statistically-significant variation in the percent area of secondary osteonal bone was observed among circumferential rings in the total sample (all age classes combined) and when age classes were considered separately, with the exception of the *P. troglodytes* femur (for which sample size did not permit separate analyses by age class) and the *C. aethiops* humerus (M1-M3 age classes). Although post-hoc contrasts were not always significant when age classes were considered separately, mean proportions were usually higher in the inner and mid-cortical rings compared to the outer ring. Exceptions to this pattern were the midshaft humerus of *C. aethiops* (M1 age class) and the midshaft

humerus of *H. lar* (DECID age class).

However, no consistent pattern characterized intracortical remodeling in the inner versus mid-cortical rings across all taxa and elements. The midshaft femur of *C. aethiops*, which undergoes the least intracortical remodeling of all taxa examined in the current study, showed consistently higher proportions of secondary osteonal bone in the inner ring compared to the mid-cortical ring. In contrast, other skeletal sites and taxa showed a more complicated pattern, with considerable overlap or age-related variability in the relative proportions of secondary osteonal bone in the inner and mid-cortical rings.

In part, this observation reflects a trend discussed previously (Smith, 1961), namely that intracortical remodeling concentrated in inner cortical regions is more typical of taxa and elements characterized by low remodeling rates, such as the midshaft femur of *C. aethiops* examined here. Where remodeling rates are high, however, secondary osteons may eventually be distributed throughout the thickness of the cortex, leaving only surface layers of primary bone *in tact* endosteally and periosteally. In the current study, *H. lar* and *P. troglodytes*, which undergo more extensive intracortical remodeling, were characterized by more overlap in the proportion of secondary osteonal bone in the mid-cortical and inner rings. Although intracortical remodeling was extensive in these taxa during later ontogeny, the majority of recently-deposited bone in the outer ring was unremodeled even in the oldest (EIPPH) age class. Only in two older *P. troglodytes* individuals (belonging to the M3 and EIPPH age classes, respectively) did secondary osteonal bone comprise the majority of cortical area in the outer ring.

The segmentation analysis utilized in the current study did not permit the discrimination of bone regions of endosteal and periosteal origin in quantitative analyses

of spatial variation in intracortical remodeling. However, qualitative assessment (also see Chapter 5) indicated that secondary osteons in the inner and mid-cortical circumferential rings were distributed in both endosteal and periosteal cortical regions, and the extent to which endosteal tissues were represented across rings varied according to taxon, age and bone element examined. The significance of developmental origin for interpreting regional patterns of intracortical remodeling is considered further below.

The influence of developmental origin: prevalence of secondary osteons in cortical regions of endosteal origin. Qualitative assessment indicated that secondary osteons were concentrated in older cortical regions of periosteal origin *and* cortical regions of endosteal origin. In fact, in many cases, intracortical remodeling appeared to be preferentially distributed along the boundary between periosteal and endosteal cortices. A similar distribution of intracortical remodeling in endosteal regions has been described by previous investigators (Amprino and Godina, 1947; Enlow, 1962a, 1963; Enlow and Brown, 1956, 1957, 1958; Foote, 1916; Pfeiffer et al., 1995; Skedros et al., 2001).

The significance of tissue age for explaining this pattern is complicated by the fact that deposition of bone at the endosteal surface is not temporally related to deposition of bone at the periosteal surface in a straightforward manner. Depending on the skeletal site, endosteal cortex may be deposited during *earlier* or *later* growth stages relative to tissue comprising the periosteal cortex (Enlow, 1962b, 1963). In the absence of marked cortical drift (i.e., when evidence of differential growth in opposing cortices is clear, as in individuals depicted in Figure 6-10 a), distinguishing between these two possibilities may be difficult. [For example, see Figure 6-7 a. Endosteal bone (depicted in orange) in M2 -

EIPH individuals shown here may be of more recent depositional origin than the majority of the periosteal cortex, but this cannot be verified.] Therefore, while secondary osteons in regions of endosteal origin were frequently more prevalent among older endosteal tissues, tissue age alone may not account for the apparent overall prevalence of secondary osteons in endosteal cortical regions.

Secondary osteons were often located in older endosteal regions showing a compacted coarse cancellous organization in interstitial areas, where they were recognized as such from their enclosure by a cement line border and other details described above. (For example, see Figure 6-7 e). This microstructural pattern was originally described by Enlow (Enlow, 1962a, 1963, 1976), who observed that secondary osteons show a predictable distribution in regions of developing long bones undergoing endosteal (i.e., inward) growth. Compacted coarse cancellous tissue is formed when endosteal growth takes place within a region originally comprised of coarse cancellous bone. This coarse cancellous tissue is “compacted” as a result of lamellar bone deposition in the available spaces between cancellous trabeculae, giving it a characteristically convoluted appearance in cross-section. As Enlow described, regions undergoing endosteal growth and cancellous compaction are often subject to extensive remodeling, which may involve reversals in growth direction as cancellous trabeculae are partially resorbed, rebuilt, and later in-filled. Thus, endosteal regions undergoing cancellous compaction may be inherently more likely to form secondary osteons, particularly during this process of reconstruction, in regions where a marrow sinusoid is enclosed by concentric lamellae.

Endosteal compacted coarse cancellous bone is often characteristic of bone

regions undergoing metaphyseal remodeling (see Chapter 2, Figure 2-2). As cortical levels are displaced further from the metaphysis with continued linear bone growth, coarse cancellous trabeculae become less numerous and newly-deposited endosteal bone is characterized by a circumferential organization. As this latter tissue is often subject to less extensive intracortical remodeling, zones of secondary osteonal bone are relocated towards mid-cortical regions. According to Enlow (Enlow, 1962a, 1963, 1976), this constitutes a basic microstructural pattern observed across vertebrates.

Several observations of the current study are consistent with this pattern. Evidence that the distribution of secondary osteons was influenced by metaphyseal growth remodeling was observed at the midshaft femur and humerus of *H. lar*. Metaphyseal remodeling at these skeletal sites was evidenced by several microstructural features observed in the DECID and M1 age classes, and described in Chapter 5 of this thesis. In the current study, quantitative results demonstrated higher proportions of secondary osteonal bone in the mid-cortical ring compared to the inner ring at the midshaft femur and humerus in the DECID and M1 age classes. The reverse was true in older age classes at the midshaft femur, while the midshaft humerus was characterized by considerable overlap among the mid-cortical and inner rings during later ontogeny. Qualitative observations revealed that secondary osteons were commonly concentrated at the boundary between endosteal and periosteal cortex. In regions of endosteal origin, interstitial areas frequently showed a compacted coarse cancellous arrangement in the DECID and M1 age classes, particularly at the midshaft humerus. As a result of more recent deposition of endosteal circumferential bone, which showed less extensive intracortical remodeling, zones of more densely-distributed secondary osteons were

relocated to mid-cortical regions during early ontogeny as part of the growth remodeling process (thus, the higher mid-cortical proportions). As dimensions of the medullary canal expanded during later ontogeny and bone was removed from the endosteal surface, intracortical remodeling was more commonly observed in cortical regions of periosteal origin.

In other taxa, secondary osteons were distributed in regions of endosteal growth associated with a pattern of cortical drift. This was most obvious at the midshaft humerus of *C. aethiops*, which was characterized by a prominent pattern of cortical drift in a predominantly posterior – posteromedial direction. Observations described in Chapter 5 suggest that drift was accomplished by more rapid periosteal deposition in posterior and posteromedial cortices, and a combination of endosteal deposition and periosteal resorption in the anterolateral and adjacent cortices. Consequently, zones of secondary osteonal bone, concentrated in earlier-deposited tissues of endosteal and periosteal origin in anterior and adjacent cortices, were apparently relocated towards more peripheral regions (i.e., represented by the mid-cortical and outer circumferential rings). More recently-deposited endosteal and periosteal tissue showed less extensive intracortical remodeling. The significance of cortical drift for interpreting variation in proportions of secondary osteonal bone across radial sectors at other skeletal sites examined here is discussed in a following section below.

While intracortical remodeling was commonly found in association with compacted coarse cancellous bone, it was also observed in regions of endosteal circumferential bone. Further, secondary intracortical remodeling of endosteal regions was often particularly extensive in *H. lar* and *P. troglodytes*. While this may reflect

factors associated with developmental origin, the possibility remains that other factors may also influence subsequent intracortical remodeling of endosteal cortex during later growth stages. Possible mechanical influences on the distribution of intracortical remodeling in inner cortical regions are considered below.

The mechanical significance of intracortical remodeling of inner and mid-cortical regions. Results of the current study suggest that an important factor influencing the distribution of intracortical remodeling in inner cortical regions is tissue age. Prior studies have reached similar conclusions, and the significance of tissue age for the fatigue properties of bone was discussed above. However, if intracortical remodeling functions in the repair of microdamage due to fatigue, tissue age probably exerts a combined influence on its distribution along with other aspects of the mechanical strain environment. For instance, evidence indicates that loading at higher cyclic strain magnitudes is associated with increased microdamage production and reduced fatigue life (Burr et al., 1985; Carter and Caler, 1985; Carter et al., 1981; Schaffler et al., 1989). Therefore, one might expect to find increased proportions of secondary osteonal bone in the outermost cortex, where the highest strain magnitudes are experienced in long bones subjected to bending and torsion (Biewener, 1992; Ruff and Hayes, 1983; Wainwright et al., 1976). During growth, however, tissues deposited at the periosteal surface are sequentially relocated to inner cortical regions (Enlow, 1962b, 1963), and thus, their exposure to the surface strain environment may be temporary. Accumulation of loading cycles at high *in vivo* strain magnitudes may therefore serve as a more important mechanical stimulus for intracortical remodeling in the outermost cortex as subperiosteal growth slows in adulthood.

This explanation would not account for the prevalence of intracortical remodeling in endosteal regions, however. At many midshaft skeletal sites, bone tissues deposited at the endosteal surface are never exposed to strain environments at the outer bone surface, nor are they necessarily of older depositional history. (It is noted that the midshaft humerus of *C. aethiops* is an exception, as the anterior periosteal surface undergoes a resorptive phase during development. See Chapter 5.) According to Frost's mechanostat paradigm (Frost, 1986, 1990, 2001, 2003), the prevalence of intracortical remodeling in inner endosteal regions may reflect a history of low *in vivo* strains in cortical regions adjacent to the neutral axis of bending (e.g., as also discussed by Skedros et al., 1994; Skedros et al., 2001). However, observations reported here are not entirely consistent with this explanation either; among endosteal tissues, intracortical remodeling at some skeletal sites (e.g., at the midshaft humerus of *H. lar* during early ontogeny) is more extensive in mid-cortical compared to inner cortical regions. If intracortical remodeling occurs in response to low mechanical strain environments in accordance with the mechanostat (i.e., below the MESr), it should be accompanied by an increase in the initiation of new BMU remodeling events and increased porosity (indicating net bone loss). This would be a subject for further quantitative study.

Alternatively, intracortical remodeling in bone regions of endosteal origin may serve to improve the mechanical properties of bone, particularly in regions previously occupied by compacted coarse cancellous tissue, which is characteristically more disordered in its microstructural organization than other tissue types. This hypothesis was suggested by Currey (2002) based on observations that compacted coarse cancellous tissue is characterized by reduced impact strength compared to secondary osteonal bone.

However, if intracortical remodeling of compacted coarse cancellous regions improves bone strength, this would appear to be more important in regions of high mechanical strains. Future research is clearly needed to understand the mechanical properties of endosteal bone, and the significance of *in vivo* strains in inner cortical regions as a potential stimulus for intracortical remodeling of long bone diaphyses (see discussion by Skedros et al., 2001).

Intracortical remodeling shows a patterned distribution across radial sectors. Many recent efforts in the study of intracortical remodeling have been based on the premise that it serves a mechanical function, acting in concert with growth remodeling at endosteal and periosteal surfaces to maintain *in vivo* strains within appropriate limits with respect to the current loading environment and by repairing fatigue-induced microdamage (Martin et al., 1998). Accordingly, it is generally accepted that the regional distribution of intracortical remodeling within single cross-sections reflects a history of loading-induced *in vivo* strains and / or microdamage accumulation. As the material properties and fatigue behavior of bone vary considerably in tension and compression (Carter et al., 1981; Currey, 2002; Griffin et al., 1997; Martin et al., 1998; Patten et al., 1996), bending loads are likely to be particularly important determinants of regional variability in intracortical remodeling within single cross-sections (Skedros et al., 1994; Skedros et al., 1996).

In the current study, variation in the percent area of secondary osteonal bone was examined across radial sectors at the midshaft femur and humerus, representing eight anatomically-defined cortical regions. As circumferential ring was a significant factor

accounting for variation in % HAV, analysis of variation among radial sectors was undertaken for each ring separately. Results indicated that in all taxa, the midshaft femur and humerus was characterized by statistically-significant variation in the percent area of secondary osteonal bone among radial sectors. (Post-hoc contrasts were largely non-significant when age classes were considered separately, however, likely due in part to small sample sizes.) As *in vivo* strains have only been characterized for the midshaft humerus during brachiation in *H. lar* among the taxa examined here, the mechanical significance of this regional variability is considered within the context of results from analysis of cross-sectional geometry presented in Chapter 4 of this thesis. Specifically, sectors exhibiting the highest proportions of secondary osteonal bone were considered in relation to the orientation of greatest structural rigidity to bending (i.e., the *maximum second moment of area*, or $I_{\max}$). Findings are summarized and discussed by taxon below.

C. aethiops. At the midshaft femur, the proportion of secondary osteonal bone was significantly higher in the posterior sector compared to other sectors in the total sample, and this pattern was most marked in the outer circumferential ring. Increased intracortical remodeling in the posterior cortex characterized all age classes when considered separately, although this pattern was most marked during later ontogeny. In the DECID and M1 age classes, intracortical remodeling was also increased in the anterior and adjacent sectors, and this pattern was most pronounced in the inner followed by mid-cortical rings.

At the midshaft humerus, the posterior and adjacent sectors were characterized by significantly reduced proportions of secondary osteonal bone compared to other sectors

in total sample. This general pattern characterized all age classes and circumferential rings when considered separately. In the mid-cortical and outer rings, the proportion of secondary osteonal bone was greatest in the anterolateral and adjacent sectors of the DECID age class. Increased proportions were also observed in the anteromedial and, to a lesser extent, medial sectors in older age classes.

These results are consistent with the findings of Paine and colleague (Paine, 1994; Paine and Godfrey, 1997), who examined variation in the percent area of secondary osteonal bone across four quadrants at the midshaft femur and humerus. As in the current study, Paine (Paine, 1994) also observed increased intracortical remodeling in the posterior cortex at the midshaft femur of adult *C. aethiops* and other Old world monkeys. He interpreted this pattern to reflect regionally high compressive loads, as inferred from antero-posterior diaphyseal curvature.

At the midshaft femur, increased intracortical remodeling in the posterior cortex is broadly consistent with structural proportions reported in Chapter 4 of this thesis. Although the midshaft femur showed a predominantly anteromedial-posterolateral orientation of $I_{\max}$ across age classes, this orientation shifted towards the anteroposterior plane during later ontogeny. This pattern was also associated with an age-related increase in relative anteroposterior bending rigidity. Qualitative observations indicate that these structural proportions were accomplished in part by an overall pattern of posterior cortical drift in the youngest age classes. (Also see Chapter 5). Posterior cortical drift during early ontogeny was also coincident with increased intracortical remodeling of older-deposited periosteal tissues of the anterior and adjacent radial sectors, as was observed in the current study. Interestingly, qualitative observations

revealed that older age classes were characterized by more variable drift patterns, and intracortical remodeling which was largely confined (with the exception of the posterior cortex) to regions of endosteal compacted coarse cancellous bone.

Observations at the midshaft humerus are also generally consistent with Paine (Paine, 1994). He observed the highest proportions of secondary osteonal bone in the anterior and lateral quadrants in *C. aethiops* and other Old World monkeys. For the most part, this distribution of intracortical remodeling is in accordance with cross-sectional geometric properties of the midshaft humerus, which indicated a predominantly anterolateral – posteromedial plane of greatest bending rigidity ($I_{\max}$) and an overall increase in relative medio-lateral bending strength during ontogeny. (Chapter 4). Cross-sectional proportions deviated substantially from circularity, reflecting a significant structural adaptation for unilateral bending in the anterolateral – posteromedial plane.

As discussed above, structural properties and intracortical remodeling at the midshaft humerus were associated with cortical drift in a predominantly posteromedial or posterior direction during postnatal ontogeny. This was accomplished by more rapid periosteal deposition in the posterior and posteromedial cortex, and a combination of endosteal deposition and periosteal resorption in the anterolateral, anterior and adjacent cortices. (See also Chapter 6). A similar pattern was also noted at the midshaft humerus of neonatal pig-tailed macaques (Newell-Morris and Sirianni, 1982). In many older individuals examined here, proportions of secondary osteonal bone increased in regions undergoing endosteal growth in the anteromedial and medial sectors, which appeared to be associated with a slight lateral shift in the direction of cortical drift.

Opposing cortices in the anterolateral – posteromedial plane at the midshaft

humerus exhibited a very different pattern, as the posterior and adjacent sectors were characterized by relatively little intracortical remodeling across all age classes in the current study. This observation reflects the more recent depositional origin of periosteal tissues in the posterior and adjacent sectors. Alternatively, if the midshaft humerus does experience bending in an anterolateral – posteromedial plane, as suggested by cross-sectional geometry, this difference in the proportion of secondary osteonal bone may also reflect an influence of strain mode on initiation of intracortical remodeling.

H. lar. The midshaft femur and humerus of *H. lar* showed a more variable distribution of intracortical remodeling across radial sectors between age classes, compared to the pattern observed in *C. aethiops*. There was also frequently more overlap in proportions of secondary osteonal bone across radial sectors within age classes.

At the midshaft femur of *H. lar*, statistically-significant variation in the proportion of secondary osteonal bone among sectors was identified for the mid-cortical and outer rings in the total sample. The mid-cortical ring exhibited the highest mean proportions in the posterior, anterior and anteromedial sectors. This distribution was also evident, to a lesser extent, in the outer ring, where the largest mean proportion was observed in the posterior sector. This pattern characterized older age classes when considered separately. In the DECID age class, however, significant variation among sectors was identified in the inner and outer rings. The inner ring showed a preferential distribution of secondary osteonal bone in lateral (in a clockwise direction) to posteromedial sectors, while the outer ring showed a generally similar distribution to that described above for the mid-cortical ring.

At the midshaft humerus, statistically-significant variation in the proportion of

secondary osteonal bone among sectors was identified for all rings in the total sample, although there was considerable overlap in proportions among all sectors in the inner ring. Mean proportions were highest in the anterior sector compared to other sectors of the mid-cortical and outer rings. Mean proportions were lowest in the posterior and posteromedial sectors of the mid-cortical ring. The outer ring showed a similar trend, although there was substantial overlap with adjacent sectors. This general pattern also characterized the M3 and EIPPH age classes when considered separately, where slightly reduced proportions of secondary osteonal bone were also observed in the posterior sector of the inner ring. The pattern of intracortical remodeling across radial sectors within rings was more variable in the DECID and M1 age classes.

As reported in Chapter 4 of this thesis, analysis of cross-sectional geometry found that the midshaft femur and humerus of *H. lar* both exhibited a fairly circular cross-section overall, with slight but significant structural reinforcement in the medio-lateral plane. Other studies of cross-sectional geometry have reported similar results (Ruff and Runestad, 1992; Schaffler et al., 1985). The orientation of $I_{\max}$ showed considerable variation among individuals compared to the pattern observed in *C. aethiops*, particularly at the midshaft femur. Results reported in Chapter 5 also indicated that, while many individuals exhibited evidence of cortical drift at the midshaft femur and humerus, particularly during early ontogeny, the pattern of drift was variable both between age classes and among individuals. In light of these results and in the absence of *in vivo* strain data, observations of the midshaft femur in the current study are difficult to explain. Increased intracortical remodeling in the posterior cortex may reflect the influence of other factors, such as local sites of muscle attachment (discussed below).

At the midshaft humerus, the distribution of secondary osteonal bone across radial sectors can be considered in light of available *in vivo* strain data and cross-sectional geometry. *In vivo* strain analysis by Swartz and colleagues (1989) demonstrated that the midshaft humerus of gibbons during brachiation was loaded predominantly in axial tension, combined with torsion and anteroposterior bending. Peak strains at the anterior and posterior surfaces were tensile (at magnitudes of 419 $\mu\epsilon$ and 1,492 $\mu\epsilon$, respectively), but strain magnitudes were significantly larger on the posterior surface. In the current study, proportions of secondary osteonal bone were generally *lower* in the posterior sector compared to anterior sectors (except in the M1 age class), which is not in accordance with the expectation that intracortical remodeling should be distributed in regions of high strains (e.g., Bouvier and Hylander, 1996; Lanyon and Baggott, 1976; Lanyon et al., 1979; Lieberman et al., 2003). However, this distribution is generally consistent with predictions of the mechanostat paradigm (Frost, 1986, 1990, 2001, 2003), in which increases in mechanical strain magnitude above approximately 400 $\mu\epsilon$ are expected to coincide with a decrease in the initiation of intracortical remodeling.

Slightly increased structural rigidity in the mediolateral plane, as reported in Chapter 4 of this thesis and by Schaffler and colleagues (1985), is not in agreement with *in vivo* strain distributions (Swartz et al., 1989). However, the midshaft humerus also exhibits a fairly circular cross-section overall. The distribution of intracortical remodeling showed no clear relationship to structural proportions indicating medio-lateral bending, although overlap among sectors in the medial, anterior and lateral cortices may reflect the influence of predominantly axial tensile loading experienced at this skeletal site. The lack of clear correspondence between bone strain data, structural

and microstructural characteristics may reflect the difficulty in fully characterizing *in vivo* loading regimes. This may be particularly true with arboreal positional behaviors, which are suggested to impose more variably-oriented bending loads on supporting limb elements as individuals navigate three-dimensionally complex substrates (e.g., Carlson, 2005).

P. troglodytes. At the midshaft femur, a patterned distribution of intracortical remodeling across radial sectors was most marked in the mid-cortical and outer rings, and was broadly similar (with some variation) across age classes when considered separately. In the mid-cortical ring, the mean proportion of secondary osteonal bone was substantially higher in sectors distributed, in a clockwise direction, from the anterior to the posterior cortex (i.e., across the lateral half of the cortex) compared to all other regions. The lowest mean proportion was observed in the medial sector. A similar pattern was observed in the outer ring, although mean proportions were slightly higher in the posterolateral and/or posterior sectors compared to anterolateral and adjacent sectors.

As reported in Chapter 4 of this thesis, analysis of cross-sectional geometry indicated that the midshaft femur of *P. troglodytes* exhibited a fairly circular cross-section overall. The orientation of $I_{\max}$ was roughly coincident with the antero-posterior anatomical plane in the DECID age class, while in older age classes it was observed to vary between a more or less medio-lateral and an anteromedial - posterolateral orientation. This change was associated with an increase in relative mediolateral bending rigidity in the M1 and older age classes. Ohman (1993) and Carlson (2002) reported a predominantly medio-lateral orientation of $I_{\max}$ at the midshaft femur in adult common chimpanzees, though considerable variability was observed in this parameter and in the

distribution of cortical thickness (for the latter, see Ohman, 1993). Together, these observations indicate bending in a predominantly mediolateral plane, although the relatively circular cross-section and variation in the orientation of $I_{\max}$ may indicate the influence of multidirectional bending loads (Carlson, 2005).

The prevailing pattern of increased intracortical remodeling in the lateral half of the cortex at the midshaft femur observed here may reflect a pattern of posterior – medial cortical drift (i.e., with secondary osteons concentrated in older cortical regions), observed in several individuals of the M1-M2 age classes. However, evidence of drift was less apparent in other age classes, as described in Chapter 5 of this thesis. Many individuals showed a clear pattern of increased intracortical remodeling in the lateral half of the cortex, which may reflect medio-lateral bending loads as suggested by structural proportions. It is interesting to note that, even in the two older individuals in which secondary osteonal bone comprised greater than 70% of total cortical area, the medial and posteromedial sectors showed substantially less intracortical remodeling than other cortical regions. Extensive intracortical remodeling of the posterior cortex may reflect the influence of local muscle attachments at the linea aspera, as discussed below. However, an increased proportion of secondary osteonal bone in the anterior sector is not consistent with either of these explanations.

The influence of local muscle attachments. In all taxa examined, the proportion of secondary osteonal bone was high in the posterior sector of the midshaft femur relative to other radial sectors. Qualitative assessment revealed that secondary osteons in this region were often observed in association with Sharpey's fibers. Sharpey's fibers are extrinsic collagen fiber bundles, continuous with the fibrous attachment of soft tissue

structures to the bone surface. They provide anchorage for local muscle attachments (Boyde, 1972; Enlow, 1962a). A similar association between secondary osteons and Sharpey's fibers was also observed in anterior, medial and lateral cortical regions of the humeral cortex of *C. aethiops* and *H. lar*, although Sharpey's fibers were more variably observed at the midshaft humerus compared to the midshaft femur.

The prevalence of intracortical remodeling at sites of muscle attachment suggests that mechanical strains due to muscle pull may have an important influence on the distribution of intracortical remodeling within long bone cross-sections observed here. This pattern was particularly prominent across taxa in the region of the linea aspera, a prominent muscle attachment site along the posterior aspect of the femoral diaphysis (Aiello and Dean, 1990; Berringer et al., 1968; Sigmon and Farslow, 1986). The local influence of muscle pull on *in vivo* bone strains is not well understood, but attachment sites for large muscles would presumably experience locally high tensile strains (e.g., as suggested by the distribution of preferred collagen fiber orientation, Goldman et al., 2003).

However, intracortical remodeling at sites of muscle attachments may also play an important role in the growth process, as suggested by Enlow and colleague (Enlow, 1962a, 1963; Hoyte and Enlow, 1966). Bone resorption and formation associated with the process of intracortical remodeling may serve to release and then re-establish collagen fiber linkages between muscle and bone, allowing for the sequential relocation of muscle attachments across bone surfaces during growth, as bones expand in overall size. Such a mechanism would be particularly important on resorptive surfaces, providing a means for maintaining muscle attachments on surfaces which themselves are in the process of being

removed. This may contribute to the increased proportions of secondary osteonal bone observed in the anterior and adjacent cortical regions of the midshaft humerus in *C. aethiops*, which appears to undergo a resorptive phase during early ontogeny (see Chapter 5). Although, local strains arising from muscle pull may also play a role at this skeletal site (Aiello and Dean, 1990; Berringer et al., 1968; Miller, 1932).

The influence of strain mode on distributions of secondary osteonal bone. The manner in which secondary intracortical remodeling may be influenced by strain mode is not entirely understood. Differential activation of intracortical remodeling in compression and tension cortices may be determined in part by differences in strain magnitudes associated with these loading modes (Martin and Burr, 1989; Skedros et al., 2001). However, material properties and fatigue behavior of bone vary considerably in tension and compression (Carter et al., 1981; Currey, 2002; Griffin et al., 1997; Martin et al., 1998; Pattin et al., 1996), and strain mode may exert an influence on intracortical remodeling independently of strain magnitude.

If initiation of microdamage is more likely under tensile loading than compressive loading due to bone's reduced material strength in tension, tension may be more effective at initiating intracortical remodeling than compression at similar strain magnitudes (Frost, 1986; Martin et al., 1998). This may explain the increased proportion of secondary osteonal bone observed at the midshaft humerus of *H. lar*. As discussed in the above section, *in vivo* strain analysis of the midshaft humerus of gibbons during brachiation has demonstrated that the humerus is loaded predominantly in axial tension (Swartz et al., 1989). However, it is difficult to reconcile this explanation with the observation that the proportion of secondary osteonal bone is lower in the posterior cortex, where tensile

strains recorded by Swartz and colleagues (Swartz et al., 1989) were the highest.

It is unclear how regional variation in the proportion of secondary osteonal bone observed at other skeletal sites examined here may reflect loading in compression or tension. On the basis of diaphyseal curvature at the midshaft femur of *C. aethiops*, Paine (Paine, 1994) reasoned that maximum compressive strains should characterize the posterior concave surface, while maximum tensile strains should characterize the anterior convex surface. At the midshaft humerus, he reasoned that posterior and medial surfaces should experience maximum compressive strains, while maximum tensile strains should characterize anterior and lateral surfaces. According to these predictions then, results reported here and by Paine (Paine, 1994; Paine and Godfrey, 1997) would suggest that intracortical remodeling of the posterior cortex at the midshaft femur is distributed in regions of compressive strains, while intracortical remodeling in the anterior and lateral cortices at the midshaft humerus is distributed in regions of tensile strains.

However, as *in vivo* strains have not been characterized at the midshaft femur or humerus of any Old World monkey quadruped, Paine's predictions can not be verified. Diaphyseal curvature in many animals does appear to be designed to accentuate bending for the purpose of increasing load predictability (Biewener, 1991; Biewener and Taylor, 1986; Biewener et al., 1988; Lanyon and Rubin, 1984; Rubin, 1984). However, forces due to muscle action may have a significant influence on bending loads at midshaft (Currey, 2002), and presumably on strains experienced at local muscle attachment sites. A similar problem complicates interpretation of the midshaft femur of *P. troglodytes* and the midshaft humerus of *H. lar*. Future studies of the mechanical significance of intracortical remodeling would benefit from examination of the distribution of preferred

collagen fiber orientation, as a source of information about the prevailing strain mode conditions under which intracortical remodeling was initiated (e.g., Ascenzi and Bonucci, 1967; Ascenzi and Bonucci, 1968, 1972; Ascenzi et al., 1973; Simkin and Robin, 1974).

Interpreting skeletal function from intracortical remodeling: the influence of local

growth dynamics. A focus of comparative studies of intracortical remodeling in primate bone has been the manner in which its taxonomic and/or intra-skeletal distribution reflects skeletal function (Paine, 1994; Paine and Godfrey, 1997; Schaffler and Burr, 1984). While most investigators agree that intracortical remodeling has mechanical significance, the influence of local circumstances of bone growth on its distribution has also been recognized (Bouvier and Hylander, 1981, 1996; Bromage and Boyde, n.d.; Enlow, 1962a, 1976; Enlow and Hans, 1996; Newell-Morris and Sirianni, 1982). Observations of the current study suggest that the distribution of intracortical remodeling within and among cross-sections may be influenced by both mechanical and developmental factors, and at times, these may be difficult to tease apart. Interpretations of skeletal function based on the distribution of intracortical remodeling should consider these factors whenever possible.

At many skeletal sites and taxa examined, the distribution of intracortical remodeling across radial sectors was broadly consistent with cross-sectional geometric properties described in Chapter 4. However, observations also suggested that regional variability in intracortical remodeling was strongly influenced by patterns of remodeling at periosteal and endosteal surfaces during growth. Not only did intracortical remodeling show a preferential distribution in inner and mid-cortical rings (i.e., in older cortical

regions), but those radial sectors characterized by the highest proportions of secondary osteonal bone were also often comprised of periosteal cortical regions interpreted to be of older depositional history. This was most evident in skeletal sites undergoing marked cortical drift, including the *C. aethiops* midshaft humerus, the anterior and adjacent sectors of the *C. aethiops* midshaft femur during early ontogeny, and the lateral cortex of the *P. troglodytes* midshaft femur in the M1-M2 age classes. In such cases, the extent to which regional variability in intracortical remodeling may be explained by tissue age alone, versus some aspect of the strain stimulus itself (i.e., strain mode), is difficult to determine, particularly in the absence of available *in vivo* strain data. Local dynamics of bone growth may have other implications for interpretations of skeletal function from secondary remodeling. As a result of the relocation of bone regions during the growth remodeling process, secondary osteons may find themselves in strain environments which differ considerably from those in which they were originally formed. An understanding of developmental history is necessary in order to determine whether observed secondary osteon distributions reflect bone adaptation to past or current loading conditions (Bouvier and Hylander, 1996; Bromage, 1992; Bromage and Boyde, n.d.; McMahon et al., 1995; Skedros et al., 2001).

Qualitative observations also indicated that local circumstances of bone growth remodeling may influence secondary osteon distributions in ways that are not related in an obvious manner to tissue age or mechanical strain history, in accordance with earlier proposals by Enlow (Enlow, 1962a, 1963, 1976; Hoyte and Enlow, 1966). First, developmental origin (e.g., primary tissue type), particularly relating to endosteal growth and compaction of coarse cancellous bone, appeared to have an important influence on

the distribution of secondary osteons in growing bone. This point was clearly illustrated in the example provided by *H. lar*, in which the prevalence of intracortical remodeling in mid-cortical regions of the midshaft femur and humerus was associated with a history of metaphyseal remodeling during earlier growth stages, at section levels that were once located more proximally or distally along the long bone shaft. Secondly, secondary osteons were commonly observed in association with Sharpey's fibers at muscle attachment sites. While this pattern may have mechanical significance, as discussed above, intracortical remodeling may also provide a mechanism for maintaining muscle attachments during growth, particularly on resorptive bone surfaces. This may contribute to explanations for the prevalence of remodeling observed at the midshaft humerus of *C. aethiops*. Results of this study suggest that teasing apart developmental versus mechanical factors influencing the taxonomic and intra-skeletal distribution of secondary intracortical remodeling should be an important component of future research efforts.

SUMMARY

This study aimed to: 1) determine whether proportions of secondary osteonal bone at the midshaft femur and humerus during postnatal ontogeny vary in an age-related manner within taxa; and 2) determine whether proportions of secondary osteonal bone show a patterned regional distribution within cross-sections, as observed across circumferential rings and radial sectors. As intracortical remodeling is a partially destructive process, characterization of ontogenetic variability in the proportion and distribution of secondary osteonal bone provides an important context for interpretations of primary bone microstructural variation presented in Chapter 6 of this thesis. Further,

as few studies have examined both ontogenetic and regional variation in intracortical remodeling in primates, results of this study also contribute to our understanding of the manner in which regional variation in intracortical remodeling may reflect the influence of skeletal function and local dynamics of bone growth.

First, results indicated that while the absolute area of secondary osteonal bone at the midshaft femur and humerus increased with age in all taxa, the proportion of remodeled cortex relative to total cortical area showed a significant age trend only in *C. aethiops*. In the latter taxon, a weak positive association between age class and percent area of secondary osteonal bone was observed. Secondly, results also demonstrated significant regional variability in the proportion of secondary osteonal bone within cross sections. With a few exceptions, proportions of secondary osteonal bone were highest in the inner and mid-cortical circumferential rings, indicating preferential intracortical remodeling of older cortical regions. Further, at many skeletal sites and taxa examined, the distribution of intracortical remodeling across radial sectors was broadly consistent with cross-sectional geometric properties (Chapter 4), namely the orientation of $I_{\max}$. There was less correspondence between structural properties and the distribution of intracortical remodeling in *H. lar*. At the midshaft femur of all taxa, the proportion of secondary osteonal bone was high in the posterior cortex relative to other radial sectors, indicating that local strains due to muscle force may have an important influence on the distribution of intracortical remodeling.

Qualitative observations also suggested that the distribution of intracortical remodeling across both circumferential rings and radial sectors was strongly influenced by local dynamics of bone remodeling at periosteal and endosteal surfaces during growth.

In addition to the preferential distribution of intracortical remodeling in inner and mid-cortical rings noted above, proportions of secondary osteonal bone were also often highest in those radial sectors comprised of periosteal cortical regions interpreted to be of older depositional history. This was most evident in skeletal sites undergoing marked cortical drift. Secondly, observations also indicated that regional variability in intracortical remodeling within diaphyseal cross-sections may be influenced by other factors not related in an obvious manner to mechanical strain history or tissue age. These factors include: 1) developmental origin, particularly endosteal growth and compaction of coarse cancellous bone, and 2) intracortical remodeling for the purpose of maintaining muscle attachments during growth, particularly on resorptive bone surfaces. These observations suggest that teasing apart developmental versus mechanical factors influencing the taxonomic and intra-skeletal distribution of secondary intracortical remodeling should be an important component of future research efforts.

TABLE 6-1. Biological age classes.

Age Class	Alveolar emergence/epiphyseal union criteria
DECID	alveolar emergence of one or more deciduous teeth
M1	alveolar emergence of one or more permanent 1st molars
M2	alveolar emergence of one or more permanent 2nd molars
M3	alveolar emergence of one or more permanent 3rd molars
EPIPH	fully erupted permanent dentition, long bone epiphyseal union complete

TABLE 6-2. Sample representation across biological age classes for analysis of secondary intracortical remodeling (femur/humerus).

Sex	Total N	DECID.	M1	M2	M3	EPIPH	Immature (unknown) ¹
<i>Chlorocebus aethiops</i>							
F	14/13	1/1	4/5 ²	2/1	4/3	3/3	0/0
M	16/17	4/4	1/1	4/5	3/3	3/3	1/1 ³
<i>Hylobates lar</i>							
F	13/12	2/2	2/2	2/1	2/2	3/3	2/2 ⁴
M	15/14	2/2	1/1	4/4	5/4	3/3	0/0
<i>Pan troglodytes</i>							
F	5	1	0	1	3	0	0
M	2	0	0	1	0	1	0
unk.	5	1	1	2 ⁵	0	1	0

¹ Includes individuals lacking associated dental emergence data.

² SLW 21 humerus - Anterior orientation lost during processing. This specimen was excluded from analysis of variation among radial sectors.

³ SLW 101 – Assigned to M3 age class for analysis, based on diaphyseal length and epiphyseal union.

⁴ SLW 10 & 35 – Assigned to M3 age class for analysis, based on diaphyseal length and epiphyseal union.

⁵ MNH 35526 femur and humerus – Questionable association with skull having only permanent M1s erupted and unerupted M2s; reassigned to M2 age class based on diaphyseal length.

TABLE 6-3. Nonparametric Spearman correlation of % HAV and absolute HAV area with age class and cortical area in *C. aethiops*. Significant results in bold ($p \leq 0.05$).

Element	Variable	Age Class				Cortical Area			
		N	R	t(N-2)	p	N	R	t(N-2)	p
Femur	%HAV	30	0.617476	4.153853	0.0003	30	0.664961	4.711135	0.0001
Femur	HAV_Ar	30	0.764852	6.282484	0.0000	30	0.837152	8.098792	0.0000
Humerus	%HAV	30	0.477567	2.876236	0.0076	30	0.421135	2.456934	0.0205
Humerus	HAV_Ar	30	0.826602	7.771977	0.0000	30	0.858954	8.876361	0.0000

TABLE 6-4. Kruskal-Wallis and Median tests: Whole section % HAV among AGE CLASSES in *C. aethiops*.
Significant contrasts in bold ($p \leq 0.05$). Mann-Whitney post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Element	df	total N	Kruskal-Wallis		Median test		Significant post-hocs ($p'=0.005$)	Significant post-hocs ($p'=0.010$)
			H	p	Chi-Square	p		
Femur	4	30	12.55054	0.0137	7.033333	0.1341	DE*M3	DE*M1, DE*EP
Humerus	4	30	10.32670	0.0353	10.60952	0.0313		M2*EP

¹ Age class abbreviations: DECID (DE); M1 (M1); M2 (M2); M3 (M3); EPIPH (EP).

TABLE 6-5. Wilcoxon matched pairs tests: % HAV between ELEMENTS in *C. aethiops*. Significant contrasts in bold ($p \leq 0.05$).

Wilcoxon Matched Pairs					
Age class	Variable	N	T	Z	p
Total sample	% HAV	28	1.00	4.599828	0.00000
DECID	% HAV	5	0	2.0225996	0.04312
M1	% HAV	5	1	1.7529196	0.07962
M2	% HAV	5	0	2.0225996	0.04312
M3	% HAV	7	0	2.3664319	0.01796
EPIPH	% HAV	6	0	2.2013982	0.02771

TABLE 6-6. Mann-Whitney and Kolmogorov-Smirnov tests: % HAV between SEXES in *C. aethiops* (M3 - EPIPH sub-sample). Post-hoc contrasts are not statistically significant at $p \leq 0.05$.

sample). Post hoc contrasts are not statistically significant at p<0.05.														
Mann-Whitney								Kolmogorov-Smirnov						
Element	N _F	N _M	Rank Sum F	Rank Sum M	U	Z	p	Max Neg Diff	Max Pos Diff	p	Mean F	Mean M	S.D. F	S.D. M
Femur	14	16	221.00	244.00	108.00	0.16628	0.86794	-0.1875	0.25	> 0.10	7.43073	7.88139	3.39829	6.88272
Humerus	13	17	238.00	227.00	74.00	1.52759	0.12662	-0.0588	0.39819	> 0.10	24.0549	19.0533	7.23947	8.82118

TABLE 6-7. Friedman's ANOVA tests of whole section % HAV among RINGS, in the total sample and with age classes considered separately: *C. aethiops* femur. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Age class	N	OUTER RING		MIDDLE RING		INNER RING		Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.0167)	Post-hocs (p'=0.0333)
		Mean	S.D.	Mean	S.D.	Mean	S.D.					
TOTAL	30	1.43	1.7151	7.11	7.0563	17.09	11.3656	53.6870	0.8948	0.00000	O*M, O*I, M*I	
DECID	5	0.00		0.61	0.4893	0.61	0.4893	6.5000	0.6500	0.03878		
M1	5	0.10	0.2177	4.44	5.8925	16.61	10.7793	10.0000	1.0000	0.00674		
M2	6	0.79	1.3254	5.60	3.2537	16.74	10.1973	11.5652	0.9638	0.00308		O*I, M*I
M3	8	2.53	1.6083	10.01	6.4515	20.23	13.1800	14.2500	0.8906	0.00080	O*M, O*I	
EPIPH	6	2.88	1.6890	12.37	9.6612	23.23	9.4892	12.0000	1.0000	0.00248		O*M, O*I, M*I

¹ Ring abbreviations: Outer (O); Mid-cortical (M); Inner (I).

TABLE 6-8. Kruskal-Wallis and Median tests of % HAV among AGE CLASSES, with rings considered separately: *C. aethiops* femur. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Ring	df	total N	Kruskal-Wallis		Median test		Significant post-hocs ($p'=0.005$) ¹	Significant post-hocs ($p'=0.010$) ¹
			H	p	Chi-Square	p		
Outer	4	30	17.51871	0.0015	18.0000	0.0012	DE*M3	DE*EP, M1*EP
Middle	4	30	13.22251	0.0102	10.1333	0.0382		DE*EP, M1*EP
Inner	4	30	8.675699	0.0697	9.0333	0.0603		

¹ Age class abbreviations: DECID (DE); M1 (M1); M2 (M2); M3 (M3); EPIPH (EP).

TABLE 6-9. Friedman's ANOVA tests of % HAV among RADIAL SECTORS, with age classes and rings considered separately: *C. aethiops* femur. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Ring	Chi Sq.	Coef. Concord	p	Post-hocs ($p'=0.00179$)	Post-hocs ($p'=0.00357$)
<i>TOTAL SAMPLE (N = 30)</i>					
Outer	82.7603	0.3941	0.00000	All sector 5 contrasts	
Middle	51.8559	0.2469	0.00000	3*5, 4*5, 5*6-8	1*6, 2*5
Inner	36.4841	0.1737	0.00001	4*5, 5*6-7	3*5
<i>DECID. (N = 5)</i>					
Middle	7.6627	0.2189	0.36327		
Inner	8.7650	0.2504	0.26998		
<i>M1 (N=5)</i>					
Outer	7.0000	0.2000	0.42888		
Middle	9.4348	0.2696	0.22293		
Inner	8.9502	0.2557	0.25625		
<i>M2 (N=6)</i>					
Outer	28.0000	0.6667	0.00022		
Middle	8.4924	0.2022	0.29119		
Inner	7.3326	0.1746	0.39509		
<i>M3 (N=8)</i>					
Outer	23.3869	0.4176	0.00146		
Middle	22.6203	0.4039	0.00198		
Inner	8.7607	0.1564	0.27030		
<i>EPIPH. (N=6)</i>					
Outer	38.1111	0.9074	0.00000		
Middle	17.7877	0.4235	0.01297		
Inner	21.2333	0.5056	0.00344		

TABLE 6-10. Friedman's ANOVA tests of whole section % HAV among RINGS, in the total sample and with age classes considered separately: *C. aethiops* humerus. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Age class	N	OUTER RING		MIDDLE RING		INNER RING		Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.0167)	Post-hocs (p'=0.0333)
		Mean	S.D.	Mean	S.D.	Mean	S.D.					
TOTAL	30	15.09	7.2910	27.60	12.0698	22.07	13.2089	24.06667	0.40111	0.00001	O*M, M*I	O*I
DECID	5	4.14	6.7527	15.49	11.5023	16.61	8.4451	7.6000	0.7600	0.02237		
M1	6	21.18	4.9481	30.09	15.9031	19.94	11.1685	5.3333	0.4444	0.06948		
M2	6	16.61	6.2754	24.98	6.1559	16.83	12.0688	4.0000	0.3333	0.13534		
M3	7	15.93	5.2158	28.97	11.9717	25.82	20.0343	5.4286	0.3878	0.06625		
EPIPH	6	15.63	2.5885	36.23	4.6624	29.62	6.8309	10.3333	0.8611	0.00570		O*M, O*I

¹ Ring abbreviations: Outer (O); Mid-cortical (M); Inner (I).

TABLE 6-11. Results of Kruskal-Wallis and Median tests of % area of HAV among age classes, with rings considered separately: *C. aethiops* humerus. Significant contrasts in bold ($p < 0.05$). Post-hoc contrasts were not significant at $p < 0.10$ after Bonferroni correction.

Ring	df	total N	Kruskal-Wallis		Median test	
			H	p	Chi-Square	p
Outer	4	30	13.0048	0.0113	8.4762	0.0756
Middle	4	30	8.7438	0.0678	10.6095	0.0313
Inner	4	30	4.9584	0.2916	5.2762	0.2601

TABLE 6-12. Friedman's ANOVA tests of % HAV among RADIAL SECTORS, with age classes and rings considered separately: *C. aethiops* humerus. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Ring	Chi Sq.	Coef. Concord	p	Post-hocs ($p'=0.00179$)	Post-hocs ($p'=0.00357$)
<i>TOTAL SAMPLE (N = 28)</i>					
Outer	133.3560	0.6569	0.00000	All sector 1 contrasts, 2*4-6, 2*8, 3*4-6, 4*7-8, 5*7-8, 6*7-8.	
Middle	138.0711	0.6802	0.00000	1*4-7, 2*4-6, 3*4-7, 4*7- 8, 5*7-8, 6*7-8, 7*8.	2*7
Inner	86.6902	0.42705	0.00000	1*4-6, 2*3-6, 3*4-7, 4*7- 8, 5*7-8, 6*8.	
<i>DECID. (N = 5)</i>					
Outer	18.9163	0.5405	0.00846		
Middle	27.9588	0.7988	0.00022		
Inner	22.1377	0.6325	0.00241		
<i>M1 (N=5)</i>					
Outer	31.2583	0.8931	0.00006		
Middle	29.2555	0.8359	0.00013		
Inner	22.3898	0.6397	0.00218		
<i>M2 (N=6)</i>					
Outer	33.0742	0.7875	0.00003		
Middle	29.9636	0.7134	0.00010		
Inner	22.1816	0.5281	0.00236		
<i>M3 (N=7)</i>					
Outer	37.9500	0.7745	0.00000		
Middle	30.5944	0.6244	0.00007		
Inner	17.5363	0.3579	0.01425		
<i>EPIPH. (N=6)</i>					
Outer	34.7083	0.8264	0.00001		
Middle	34.8722	0.8303	0.00001		
Inner	31.4088	0.7478	0.00005		

TABLE 6-13. Nonparametric Spearman correlation of % HAV and absolute HAV area with age class and cortical area in *H. lar*. Significant results in bold ($p \leq 0.05$).

Element	Variable	Age Class				Cortical Area			
		N	R	t(N-2)	p	N	R	t(N-2)	p
Femur	%HAV	28	-0.01352	-0.06894	0.9456	28	0.089765	0.45957	0.6496
Femur	HAV_Ar	28	0.849449	8.208461	0.0000	28	0.931582	13.06668	0.0000
Humerus	%HAV	26	0.181916	0.906324	0.3738	26	0.240342	1.212985	0.2369
Humerus	HAV_Ar	26	0.752948	5.605218	0.0000	26	0.872821	8.761409	0.0000

TABLE 6-14. Kruskal-Wallis and Median tests: Whole section % HAV among AGE CLASSES in *H. lar*.

Element	df	total N	Kruskal-Wallis		Median test	
			H	p	Chi-Square	p
Femur	4	28	3.978243	0.4090	1.77778	0.7765
Humerus	4	26	2.714245	0.6067	4.2	0.3796

TABLE 6-15. Wilcoxon matched pairs tests: % HAV between ELEMENTS in *H. lar*. Significant contrasts in bold ($p \leq 0.05$).

Wilcoxon Matched Pairs					
Age class	Variable	N	T	Z	p
Total sample	% HAV	26	12.00	4.152569	0.00003
DECID	% HAV	4	4	0.3651484	0.71500
M1	% HAV	3	1	1.069045	0.28505
M2	% HAV	5	0	2.0225996	0.04312
M3	% HAV	8	0	2.5205042	0.01172
EPIPH	% HAV	6	0	2.2013982	0.02771

TABLE 6-16. Results of Mann-Whitney and Kolmogorov-Smirnov tests of whole section % HAV between SEXES in *H. lar*.
Contrasts were not significant at $p \leq 0.05$.

Contrasts were not significant at p<0.05.														
		Mann-Whitney						Kolmogorov-Smirnov						
Element	N _F	N _M	Rank Sum	Rank Sum	U	Z	p	Max Neg Diff	Max Pos Diff	p	Mean	Mean	S.D.	S.D.
			F	M							F	M	F	M
Femur	13	15	160.00	246.00	69.00	-1.3129	0.18923	-0.3692	0.06667	> 0.10	35.72	40.32	7.5291	8.65537
Humerus	12	14	161.00	190.00	83.00	-0.0514	0.95898	-0.2024	0.16667	> 0.10	48.48	49.86	7.69239	10.0425

TABLE 6-17. Friedman's ANOVA tests of whole section % HAV among RINGS in the total sample and with age classes considered separately: *H. lar* femur. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts¹ reported here are significant at the $p < 0.05$ and $p < 0.10$ levels after Bonferroni correction.

Age class	N	OUTER RING		MIDDLE RING		INNER RING		Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.0167)	Post-hocs (p'=0.0333)
		Mean	S.D.	Mean	S.D.	Mean	S.D.					
TOTAL	28	9.04	6.4489	52.42	18.0054	63.56	11.3968	44.5714	0.7959	0.00000	O*M, O*I, M*I	
DECID	4	13.54	7.0453	71.15	20.1650	49.55	10.9587	8.0000	1.0000	0.01832		
M1	3	7.48	1.2931	68.69	12.7408	55.21	4.2401	6.0000	1.0000	0.04979		
M2	6	4.73	4.6929	42.19	11.6255	65.45	13.2950	12.0000	1.0000	0.00248		O*M, O*I, M*I
M3	9	9.11	6.7273	47.39	18.8693	66.09	6.8073	16.2222	0.9012	0.00030	O*M, O*I	M*I
EPIPH	6	11.00	7.4255	49.59	10.8011	71.37	9.1285	12.0000	1.0000	0.00248		O*M, O*I, M*I

¹ Ring abbreviations: Outer (O); Mid-cortical (M); Inner (I).

TABLE 6-18. Kruskal-Wallis and Median tests of % area of HAV among AGE CLASSES, with rings considered separately: *H. lar* femur.
Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts were not significant at the $p \leq 0.10$ level after Bonferroni correction.

Ring	df	total N	Kruskal-Wallis		Median test	
			H	p	Chi-Square	p
Outer	4	28	4.6260	0.3279	2.1111	0.7153
Middle	4	28	8.9044	0.0635	6.7778	0.1481
Inner	4	28	11.2886	0.0235	10.6667	0.0306

TABLE 6-19. Results of Friedman's ANOVA tests of % HAV among radial sectors, with age classes and rings considered separately: *H. lar* femur.
Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Ring	Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.00179)	Post-hocs (p'=0.00357)
<i>TOTAL SAMPLE (N = 27)</i>					
Outer	51.5066	0.2725	0.00000	1*3, 2*3, 2*5, 3*5, 3*8, 4*5, 5*6-7	1*4-5, 3*6, 7*8
Middle	47.6723	0.2522	0.00000	1*3, 2*3, 3*5, 3*8, 4*5, 4*8, 5*6, 6*8	7*8
Inner	6.5677	0.03475	0.47523		
<i>DECID. (N = 4)</i>					
Outer	14.6707	0.5240	0.04046		
Middle	1.5000	0.0536	0.98231		
Inner	18.4090	0.6575	0.01026		
<i>M1 (N=3)</i>					
Outer	15.3529	0.7311	0.03173		
Middle	12.6667	0.6032	0.08066		
Inner	13.4444	0.6402	0.06200		
<i>M2 (N=6)</i>					
Outer	13.5263	0.3221	0.06028		
Middle	14.9324	0.3555	0.03688		
Inner	8.2222	0.1958	0.31341		
<i>M3 (N=8)</i>					
Outer	18.6398	0.3329	0.00940		
Middle	27.3333	0.4881	0.00029		
Inner	17.5000	0.3125	0.01444		
<i>EPIPH. (N=6)</i>					
Outer	23.6175	0.5623	0.00133		
Middle	22.2222	0.5291	0.00233		
Inner	9.8889	0.2355	0.19496		

TABLE 6-20. Friedman's ANOVA tests of whole section % HAV among RINGS in the total sample and with age classes considered separately: *H. lar* humerus. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts¹ reported here were significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Age class	N	OUTER RING		MIDDLE RING		INNER RING		Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.0167)	Post-hocs (p'=0.0333)
		Mean	S.D.	Mean	S.D.	Mean	S.D.					
TOTAL	26	24.08	14.8220	66.86	13.3155	63.60	21.1264	28.6154	0.5503	0.00000	O&M, O&I	
DECID	4	44.66	17.4709	61.29	14.6135	28.34	15.7950	6.5000	0.8125	0.03878		
M1	3	26.83	3.1005	68.87	14.9477	49.30	5.5101	6.0000	1.0000	0.04979		
M2	5	20.80	16.6998	64.30	17.2531	75.10	10.1311	8.4000	0.8400	0.01500		
M3	8	21.70	9.9940	69.48	12.8672	72.57	16.3420	9.2500	0.5781	0.00980	O*M	O*I
EPIPH	6	14.88	9.1444	68.21	12.3315	72.72	11.6484	9.3333	0.7778	0.00940		O*M, O*I

¹ Ring abbreviations: Outer (O); Mid-cortical (M); Inner (I).

TABLE 6-21. Results of Kruskal-Wallis and Median tests of % area of HAV among age classes, with rings considered separately: *H. lar* humerus. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts were not significant at the $p \leq 0.10$ level after Bonferroni correction.

Ring	df	total N	Kruskal-Wallis		Median test	
			H	p	Chi-Square	p
Outer	4	26	8.7449	0.0678	10.3667	0.0347
Middle	4	26	0.6077	0.9622	2.7000	0.6092
Inner	4	26	13.9781	0.0074	10.8000	0.0289

TABLE 6-22. Friedman's ANOVA tests of % HAV among RADIAL SECTORS, with age classes and rings considered separately: *H. lar* humerus. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts reported here are significant at the $p \leq 0.05$ and $p \leq 0.10$ levels after Bonferroni correction.

Ring	Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.00179)	Post-hocs (p'=0.00357)
<i>TOTAL SAMPLE (N = 26)</i>					
Outer	40.0991	0.2203	0.00000	1*6-8	
Middle	43.8205	0.2408	0.00000	1*5-6, 1*8, 2*6, 3*6	1*7
Inner	19.1119	0.10501	0.00785		6*7
<i>DECID. (N = 4)</i>					
Outer	10.9167	0.3899	0.14230		
Middle	4.2500	0.1518	0.75057		
Inner	6.6627	0.2380	0.46483		
<i>M1 (N=3)</i>					
Outer	5.9402	0.2829	0.54675		
Middle	3.3333	0.1587	0.85255		
Inner	8.5556	0.4074	0.28616		
<i>M2 (N=5)</i>					
Outer	16.2554	0.4644	0.02289		
Middle	16.1333	0.4610	0.02393		
Inner	1.5333	0.0438	0.98113		
<i>M3 (N=8)</i>					
Outer	32.5797	0.5818	0.00003		
Middle	31.5833	0.5640	0.00005		
Inner	25.5000	0.4554	0.00062		
<i>EPIPH. (N=6)</i>					
Outer	18.4950	0.4404	0.00993		
Middle	17.1111	0.4074	0.01670		
Inner	20.0000	0.4762	0.00557		

TABLE 6-23. Nonparametric Spearman correlation of % HAV and absolute HAV area with age class and cortical area in *P. troglodytes*. Significant results in bold ($p \leq 0.05$).

Element	Variable	Age Class				Cortical Area			
		N	R	t(N-2)	p	N	R	t(N-2)	p
Femur	%HAV	12	0.010796	0.034141	0.9734	12	-0.16783	-0.53837	0.6021
Femur	HAV_Ar	12	0.935639	8.382736	0.0000	12	0.881119	5.892098	0.0002

TABLE 6-24. Friedman's ANOVA tests of whole section % HAV among RINGS in the total sample and with age classes considered separately: *P. troglodytes* femur. Significant contrasts in bold ($p \leq 0.05$). Post-hoc contrasts¹ reported here are significant at the $p \leq 0.05$ level after Bonferroni correction.

Age class	N	OUTER RING		MIDDLE RING		INNER RING		Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.0167)
		Mean	S.D.	Mean	S.D.	Mean	S.D.				
TOTAL	12	29.54	18.1584	65.69	12.2757	78.07	11.2170	19.5000	0.8125	0.00006	O&M, O&I, M&I
DECID	2	32.20	0.3630	77.41	2.3886	66.91	0.6441				
M1	1	15.42		57.78		56.40					
M2	4	22.83	4.7683	65.22	11.0993	79.55	9.2323				
M3	3	37.02	23.4580	66.64	14.8993	88.45	5.0520				
EPIPH	2	36.15	42.5332	57.47	19.0287	81.52	1.5550				

¹ Ring abbreviations: Outer (O); Mid-cortical (M); Inner (I).

TABLE 6-25. Results of Friedman's ANOVA tests of % HAV among radial sectors in the total sample with age classes combined: *P. troglodytes* femur. Significant contrasts in bold ($p \leq 0.05$). Significant contrasts in bold ($p < 0.05$). Post-hoc contrasts reported here are significant at the $p < 0.10$ level after Bonferroni correction.

Ring	Chi Sq.	Coef. Concord	p	Post-hocs (p'=0.00179)	Post-hocs (p'=0.00357)
<i>TOTAL SAMPLE (N = 12)</i>					
Outer	63.8847	0.7605	0.00000		1*6-8, 2*7-8, 3*4, 3*7, 4*6-8, 5*6-8
Middle	62.3333	0.7421	0.00000		1*6-8, 2*6-8, 3*6-8, 4*6-8, 5*6-8, 7*8
Inner	15.0556	0.17923	0.03530		5*7

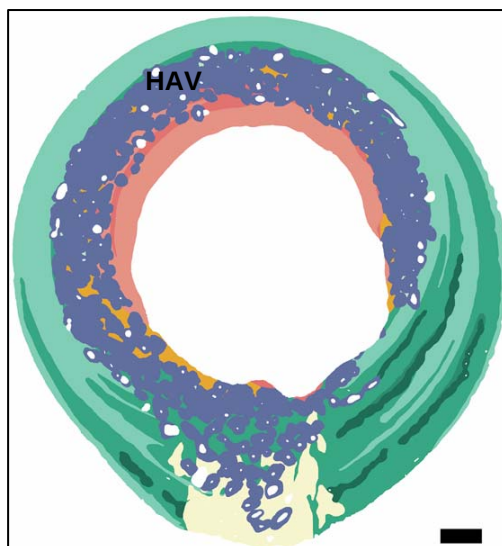
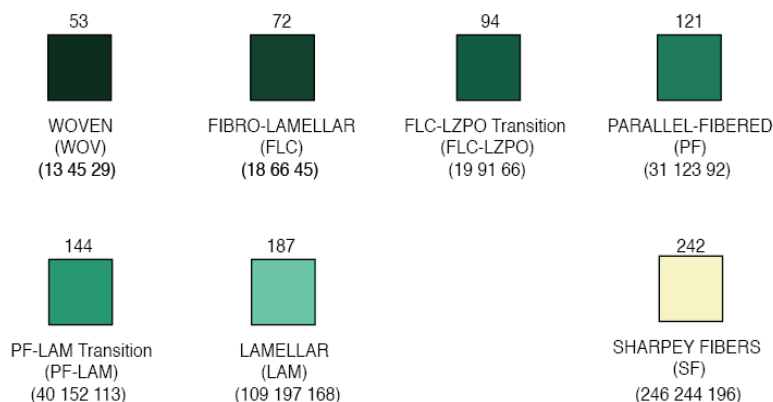


FIGURE 6-1. Tissue type maps. Areas representing secondary osteons (HAV) are color-coded for quantitative analysis.

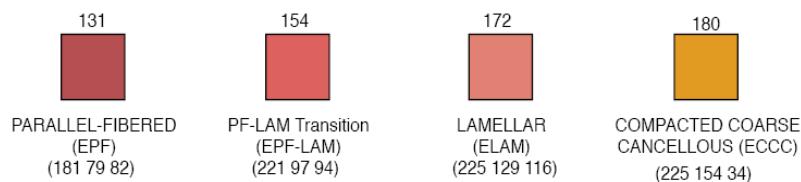
Left: Tissue type map derived from the midshaft femur of *H. lar* (MCZ 41502).

Below: Tissue type color key. Secondary osteons are represented in purple. Mapping of primary tissue types is explained in Chapter 5. Numbers specify gray-scale values (above) and RGB color values (below), as explained in the text.

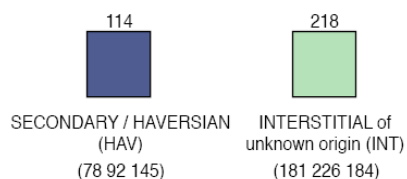
PERIOSTEAL ORIGIN



ENDOSTEAL ORIGIN



OTHER BONE TISSUES



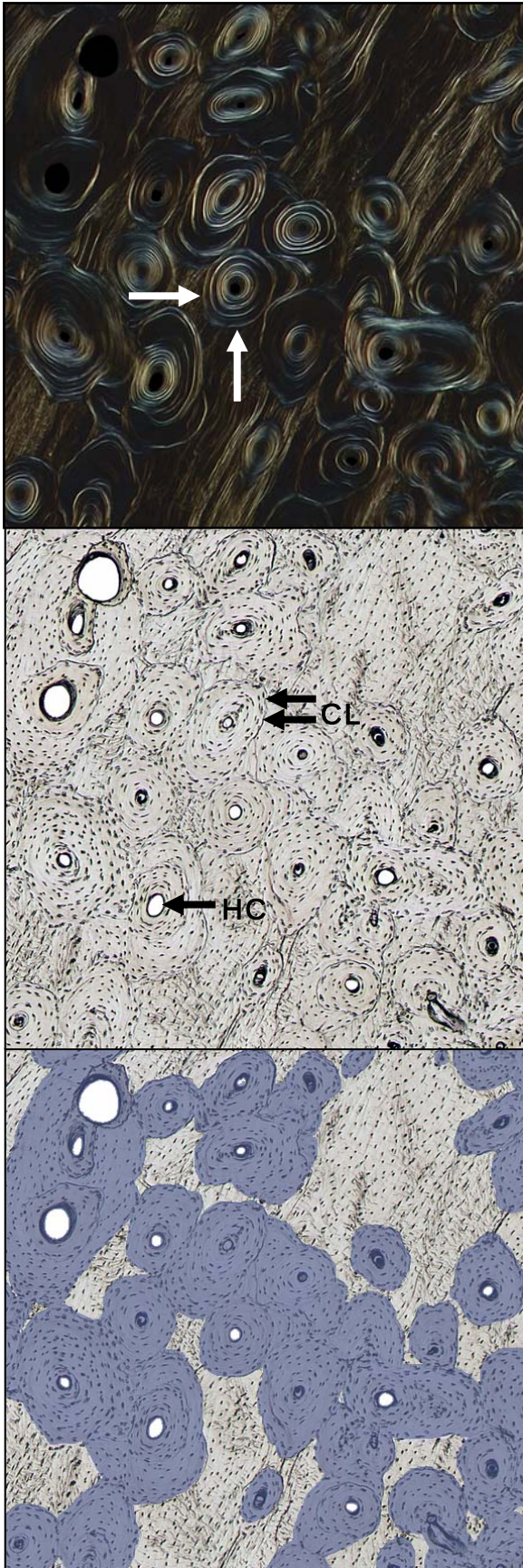


FIGURE 6-2. Imaging of secondary osteonal bone.

Top: CPL image, showing the collagen fiber organization of bone. Arrows point to centripetally-arranged lamellae (appearing bright) around the central Haversian canal of a secondary osteon.

Middle: LM image, showing Haversian canals (HC) and cement lines (CL).

Bottom: Tissue type map overlay. Bone areas comprised of secondary osteons are mapped in purple.

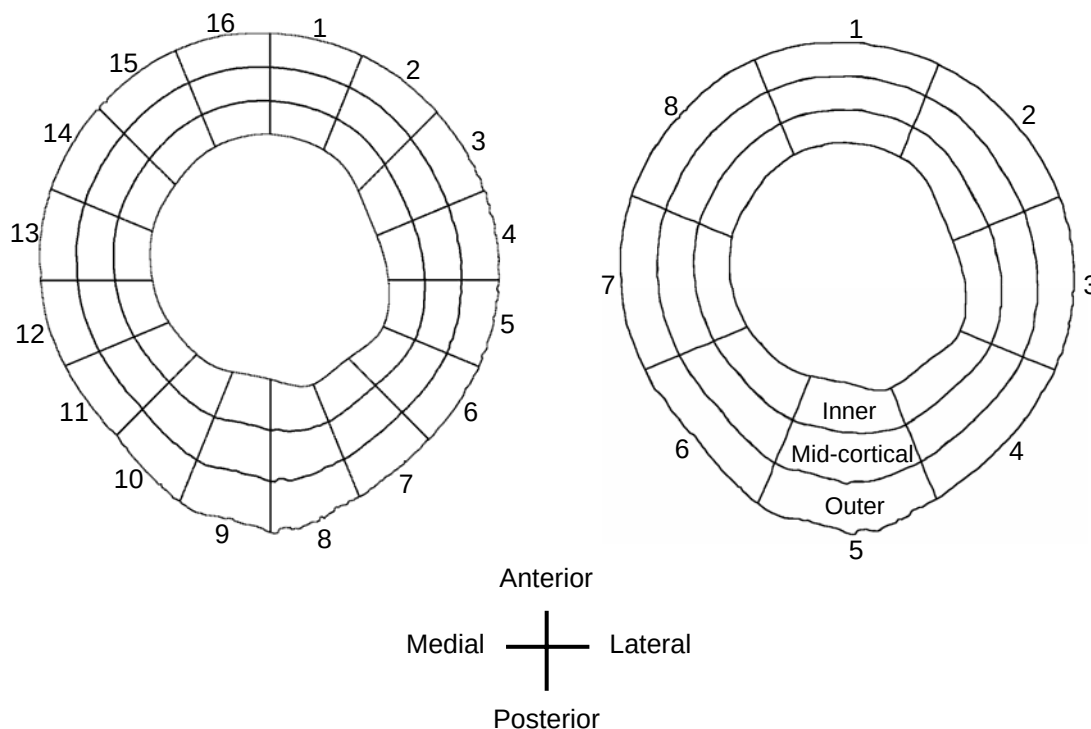


FIGURE 6-3. Bone segmentation analysis. *Left:* A customized macro divides each bone cross-section into 3 circumferential rings (labeled 'Inner', 'Mid-cortical', and 'Outer') and 16 radial sectors, resulting in 48 bone segments. Pixel dimensions for tissue types corresponding to different gray-level values are extracted for each bone segment in Optimas Image Analysis software. *Right:* Data for 16 radial sectors are collapsed into 8 radial sectors (multiplied by 3 rings), as shown here.

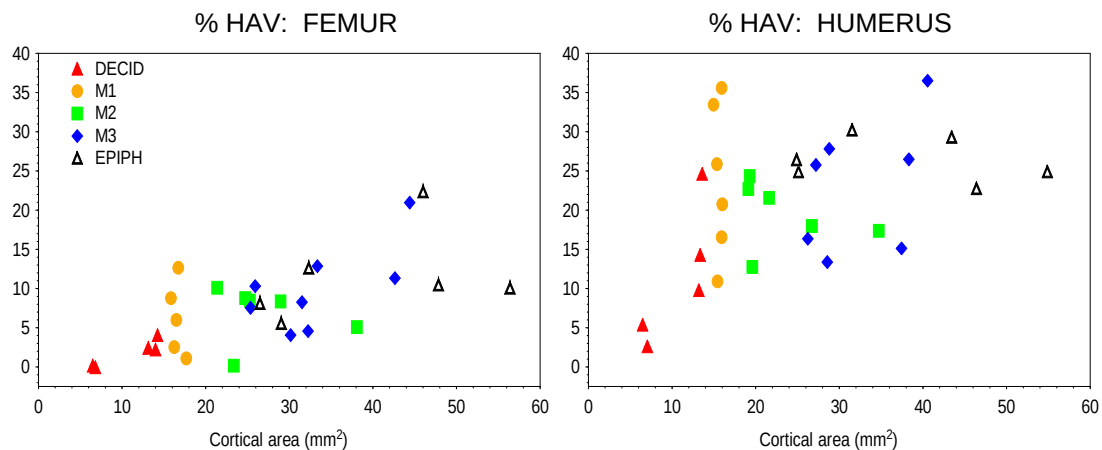


FIGURE 6-4 a. Scatterplots of whole-section % HAV by cortical area, categorized by age class: *C. aethiops* midshaft femur and humerus.

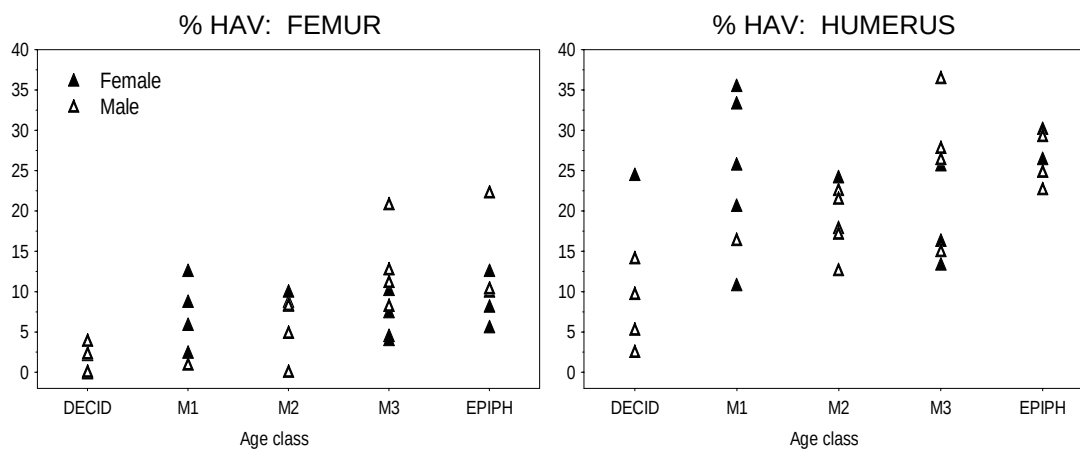


FIGURE 6-4 b. Scatterplots of whole-section % HAV by age class, categorized by sex: *C. aethiops* midshaft femur and humerus.

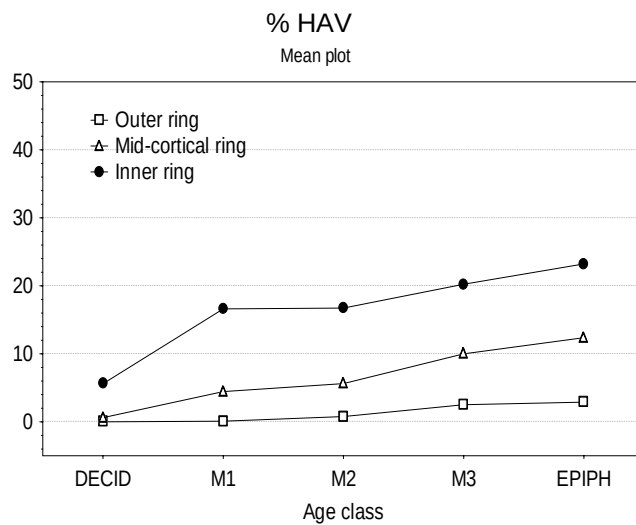


FIGURE 6-5 a. Plot of age class mean % HAV, with rings considered separately: *C. aethiops* midshaft femur.

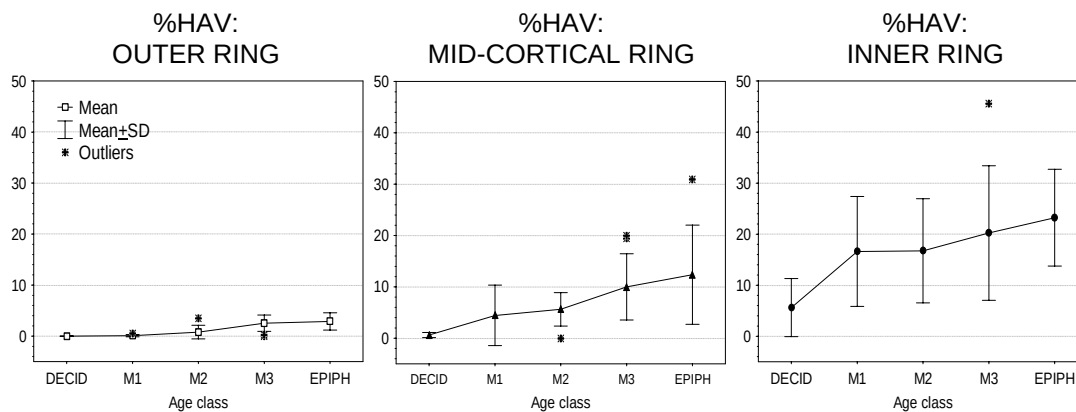


FIGURE 6-5 b. Plots of variation in % HAV across age class, with rings considered separately: *C. aethiops* midshaft femur.

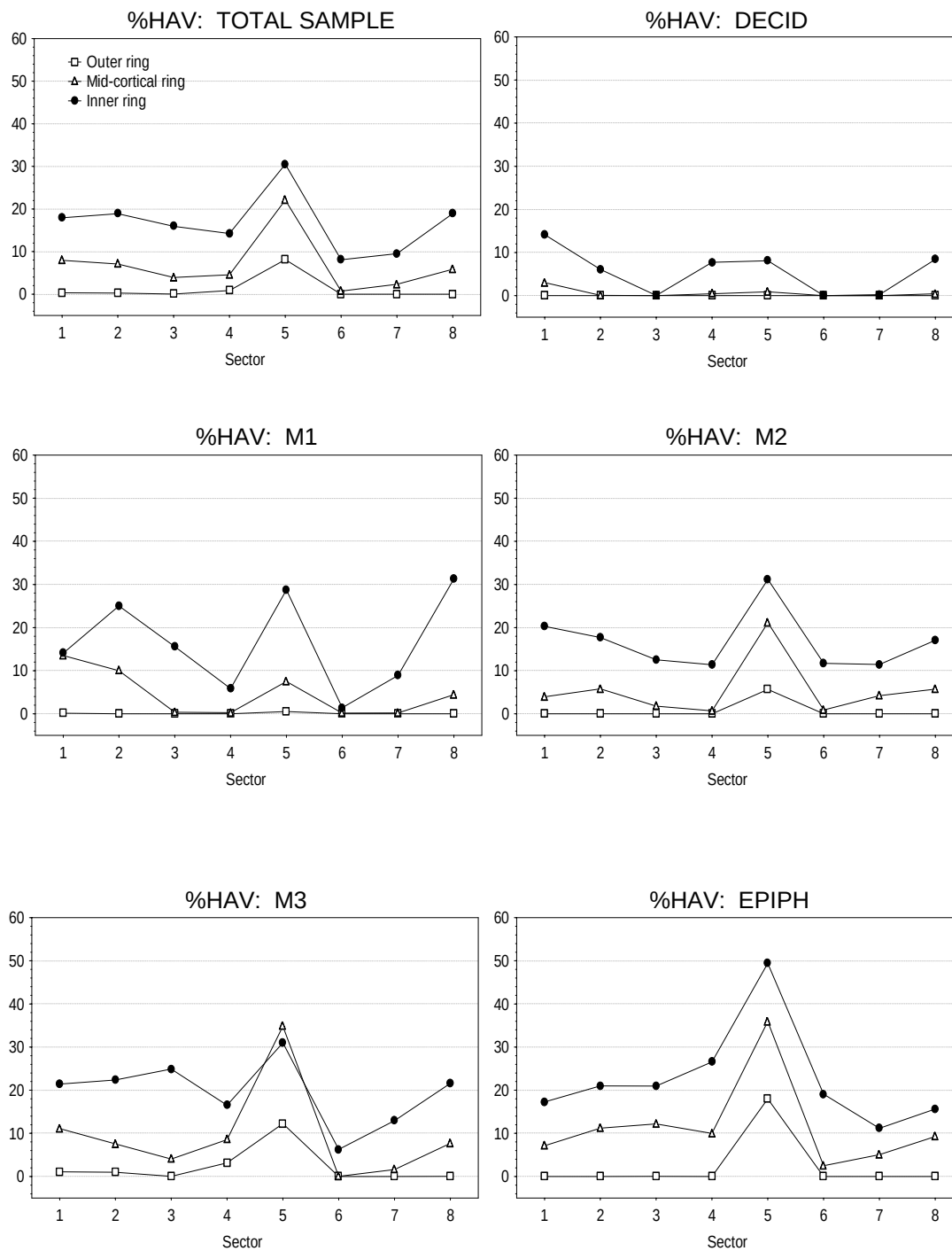


FIGURE 6-6 a. Plots of mean % HAV across radial sectors by ring, with age classes combined and considered separately: *C. aethiops* midshaft femur.

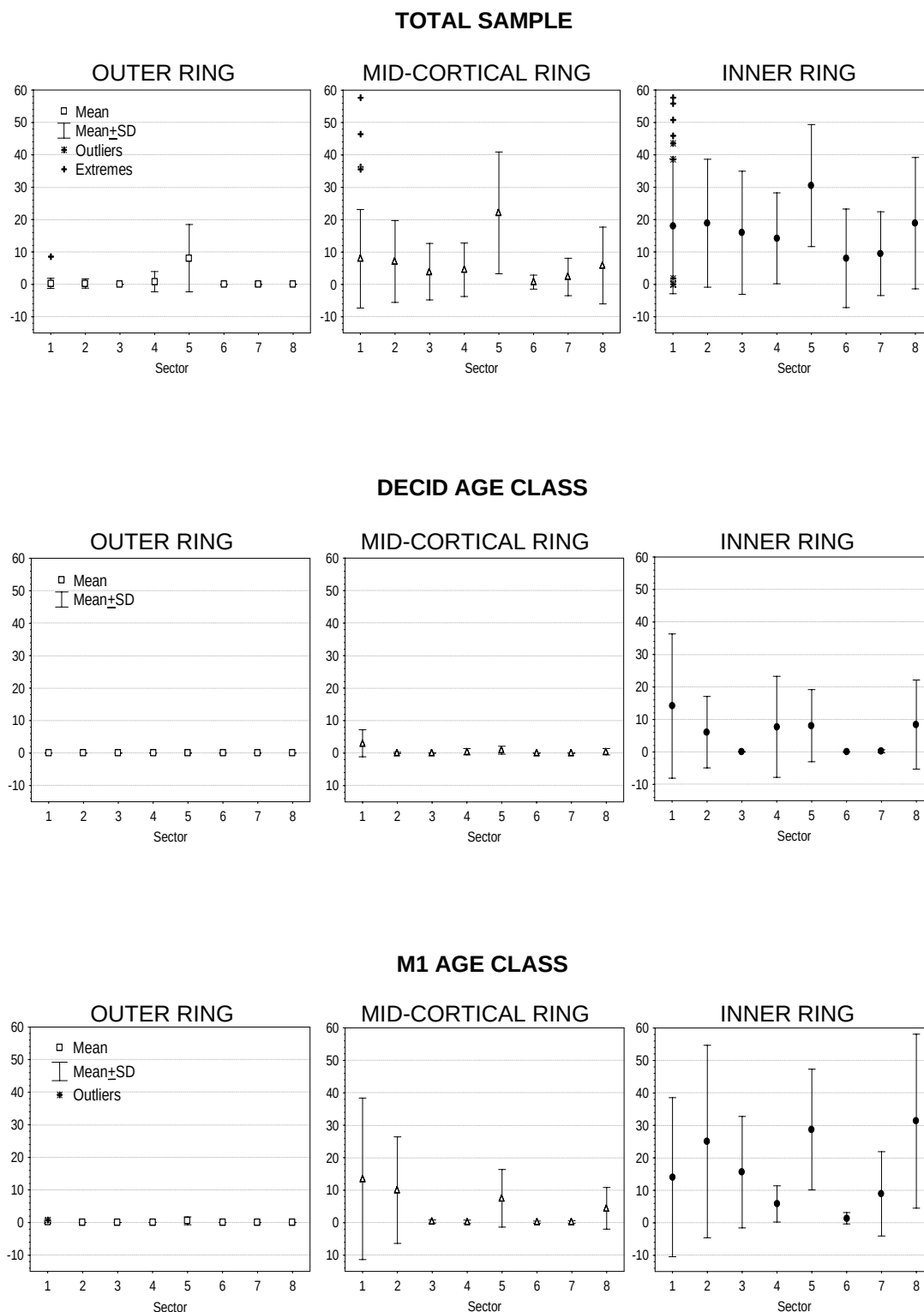


FIGURE 6-6 b. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *C. aethiops* midshaft femur.

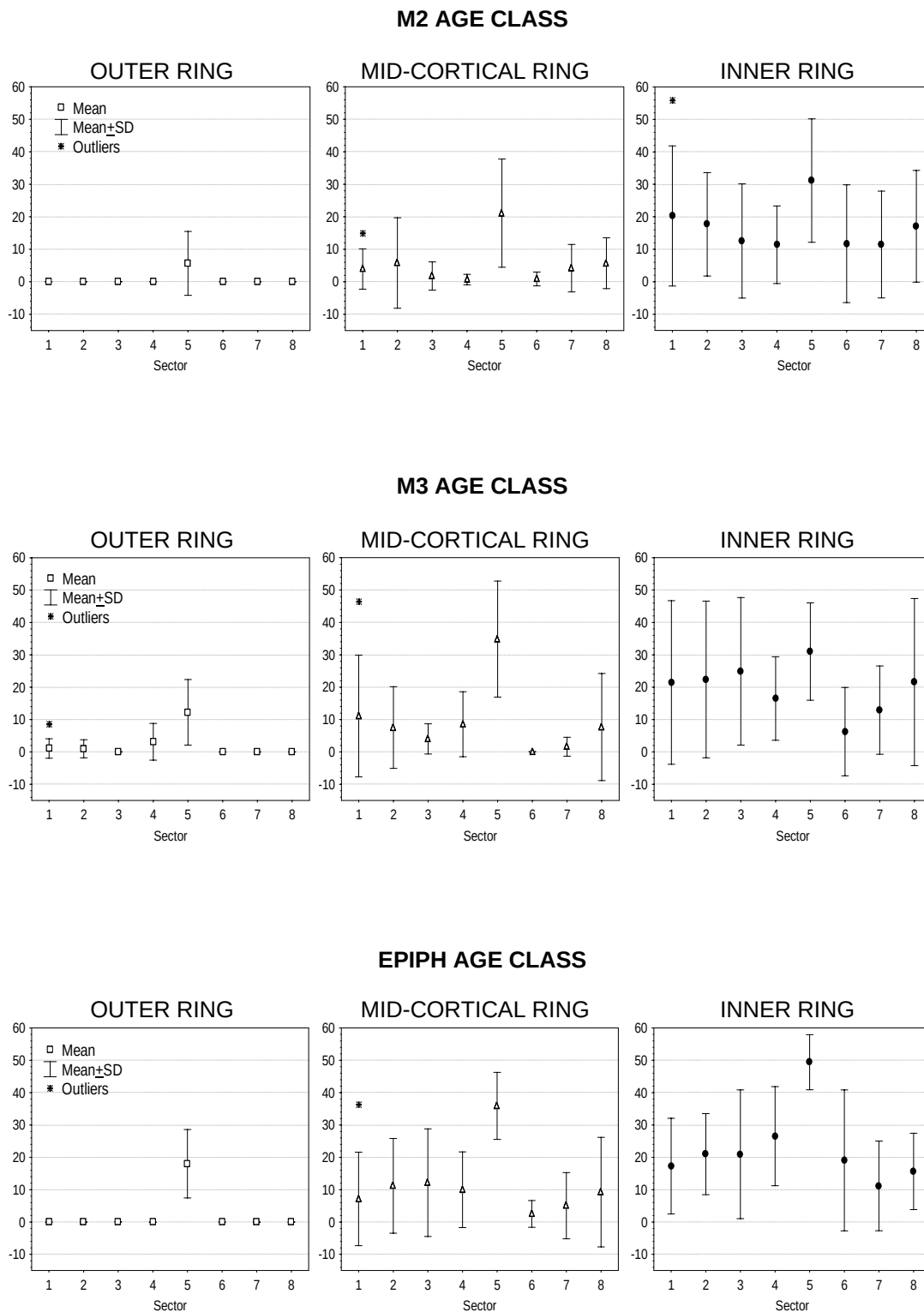
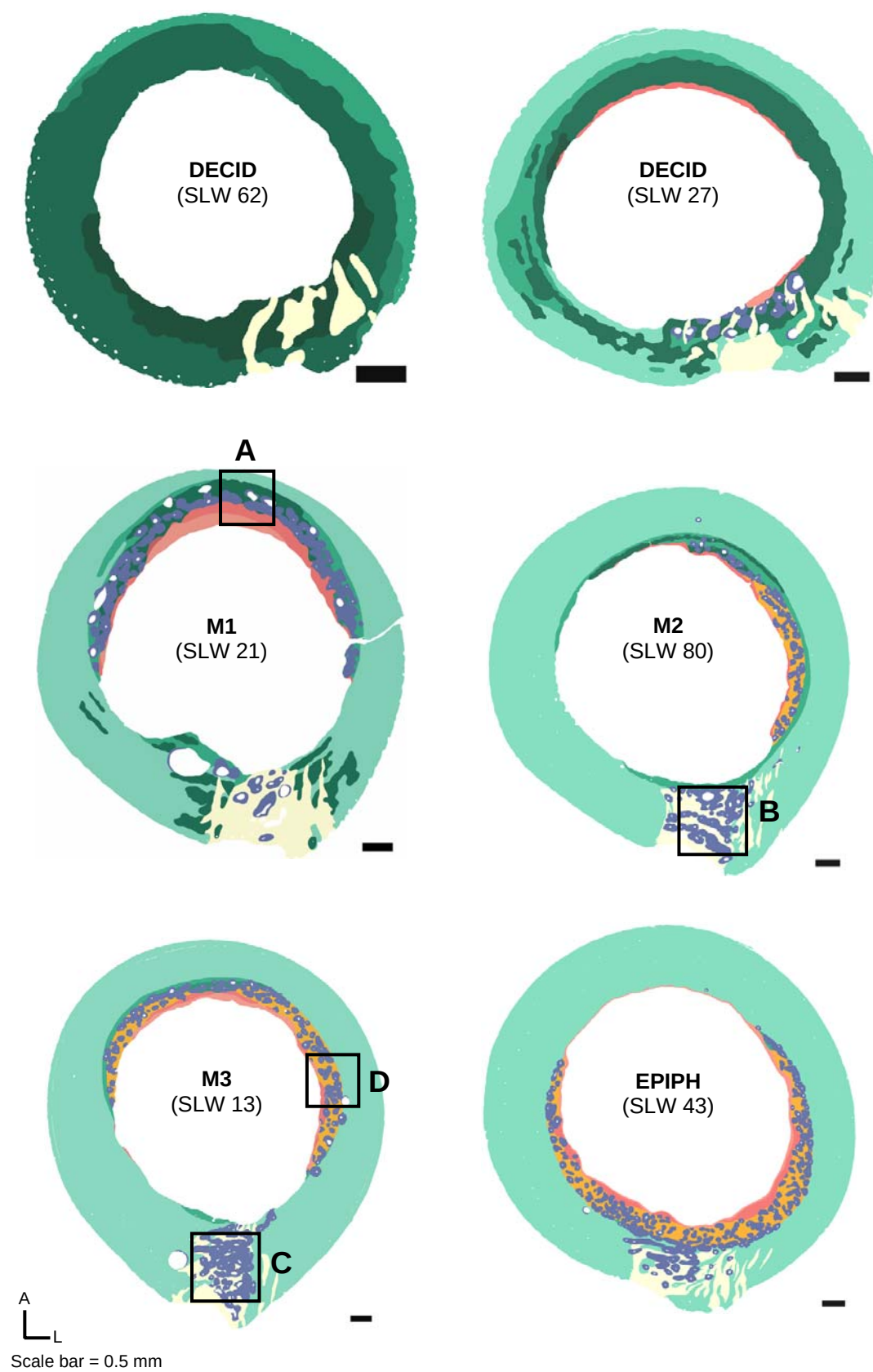


FIGURE 6-6 b cont.. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *C. aethiops* midshaft femur.

FIGURE 6-7 a. Intracortical remodeling at the midshaft femur of *C. aethiops*: Example tissue maps by age class. Two DECID individuals are shown here, to represent the range of variation observed among individuals in this age class. Bone regions occupied by secondary osteonal bone are mapped in purple, as depicted in Figure 6-1. Other bone tissue types represented here were recognized on the basis of microstructural features described in Chapter 5 of this thesis. Insets show locations of FIGURES 6-7 a-d.



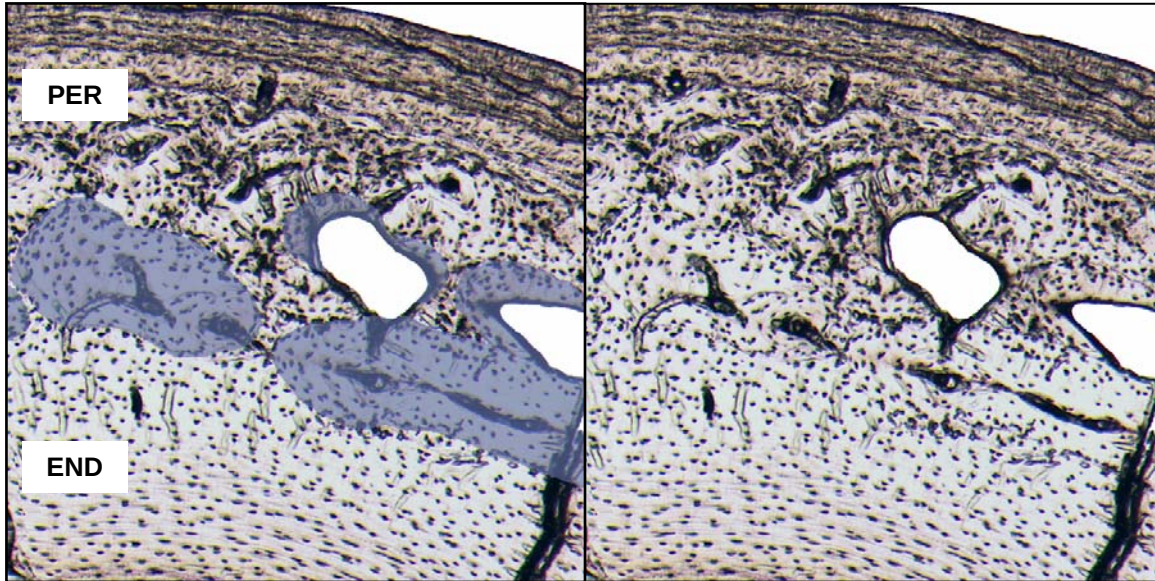


FIGURE 6-7 b. Intracortical remodeling in the *C. aethiops* midshaft femur (SLW 21, M1 age class): Secondary osteons in older periosteal regions of the anterior cortex. *Left:* LM image with color overlay, showing the distribution of secondary osteons. *Right:* LM image. Secondary osteons (mapped in purple on the left) are found at the boundary between endosteal (END) and periosteal (PER) cortex. (END cortex shown here is comprised of circumferential lamellar bone). Intracortical remodeling in the periosteal cortex is concentrated in regions of primary vascular tissue.

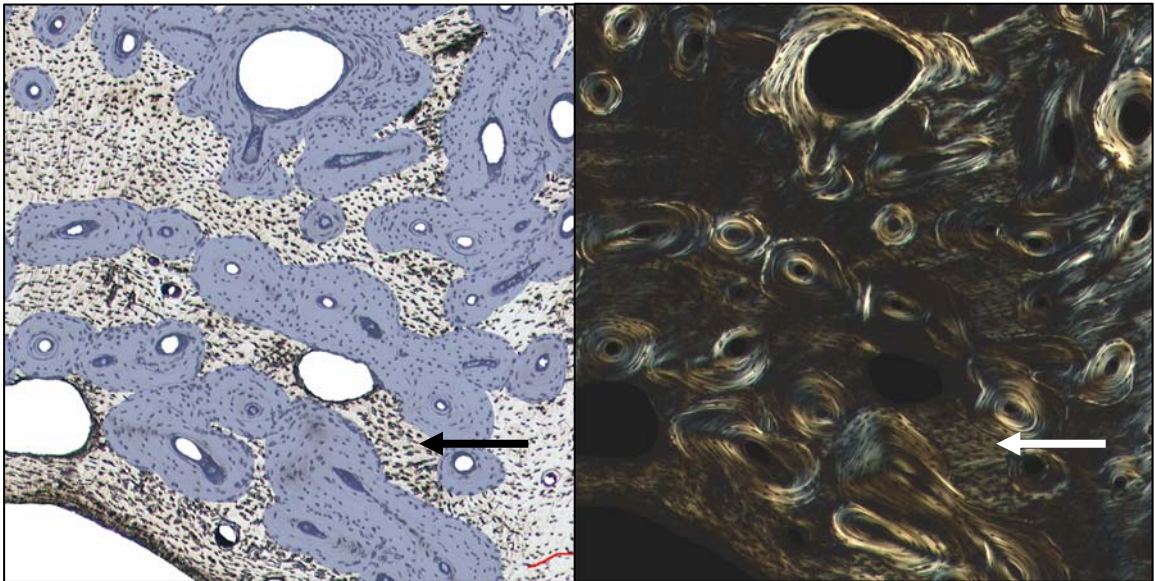


FIGURE 6-7 c. Intracortical remodeling in the *C. aethiops* midshaft femur (SLW 80, M2 age class): Secondary osteons and Sharpey's fibers in the posterior cortex. *Left:* LM image with color overlay, showing the distribution of secondary osteons. *Right:* CPL image. Secondary osteons invade more recently-deposited periosteal tissues in the posterior cortex, where they are also associated with Sharpey's fibers (arrows; appearing bright in CPL).

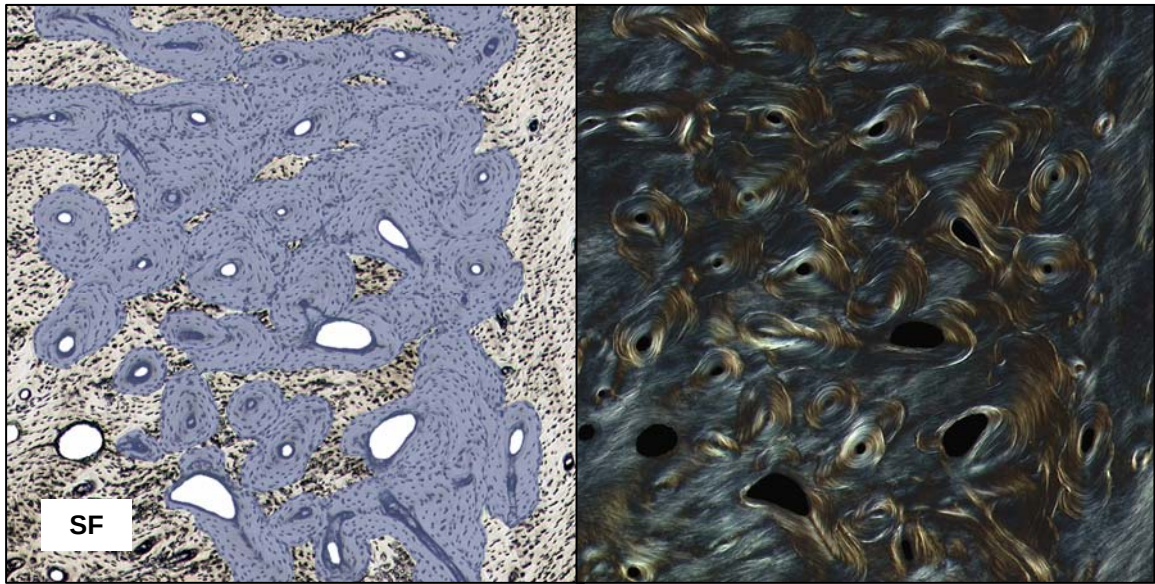


FIGURE 6-7 d. Intracortical remodeling in the midshaft femur of *C. aethiops* (SLW 13, M3 age class): Secondary osteons and Sharpey's fibers in the posterior cortex. *Left:* LM image. *Right:* CPL image. Secondary osteons invade more recently-deposited periosteal tissues, and are associated with Sharpey's fibers (SF).

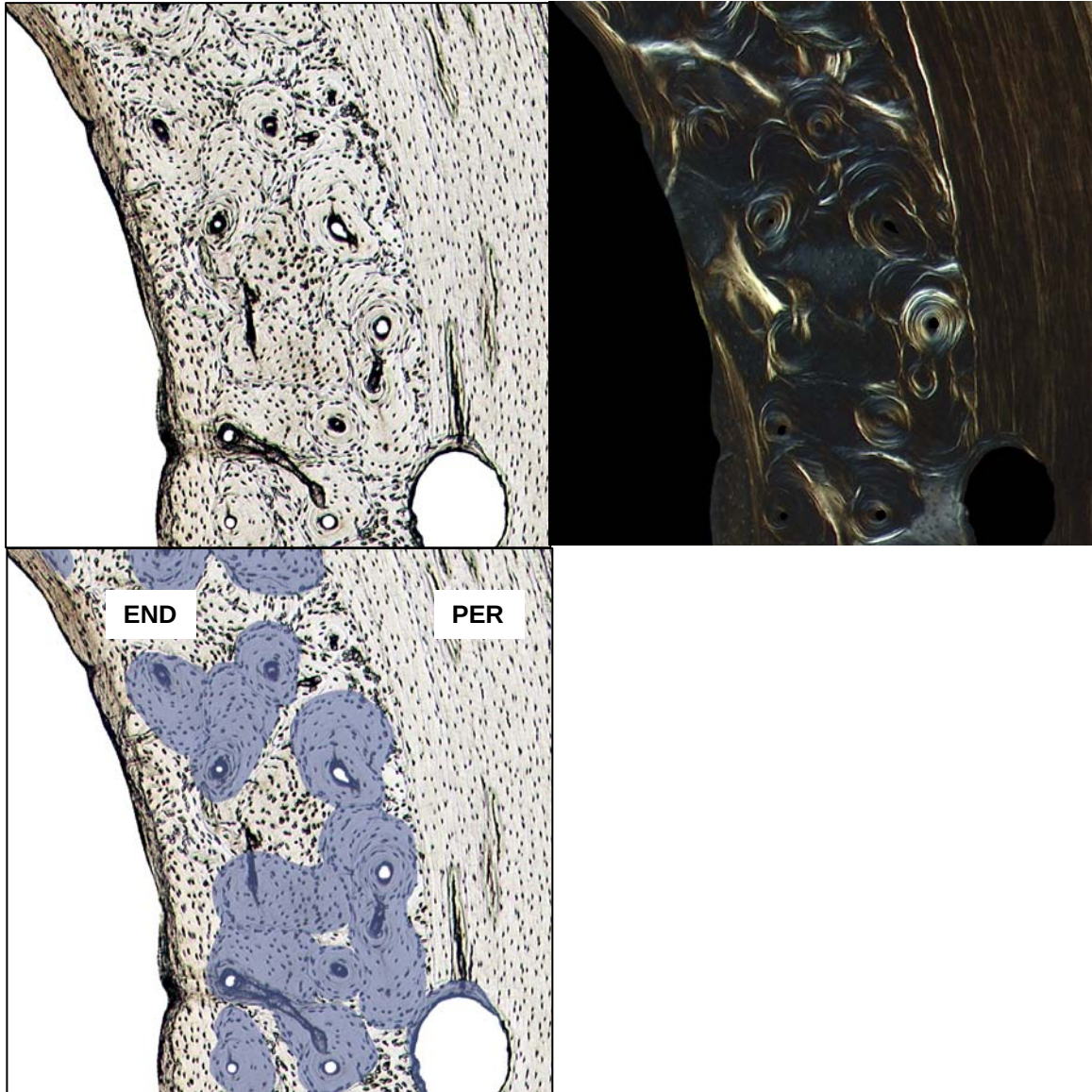


FIGURE 6-7 e. Intracortical remodeling in the midshaft femur of *C. aethiops* (SLW 13, M3 age class): Secondary osteons in regions of endosteal compacted coarse cancellous bone in the lateral cortex. *Top left:* LM image. *Top right:* CPL image. *Bottom left:* LM image with color overlay, showing the distribution of secondary osteons in this region. Secondary osteons are concentrated in endosteal (END) cortex, where they are associated with interstitial tissues having a compacted coarse cancellous organization. (Note the convoluted appearance of interstitial bone.) Secondary osteons are recognized in such regions on the basis of their cement line border and the presence of a Haversian canal. (Avascular tissue to the right of this image is primary periosteal lamellar bone; PER.)

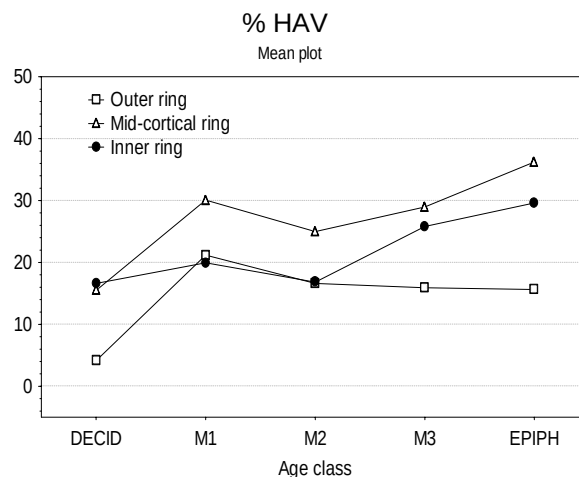


FIGURE 6-8 a. Plot of age class mean % HAV, with rings considered separately: *C. aethiops* midshaft humerus.

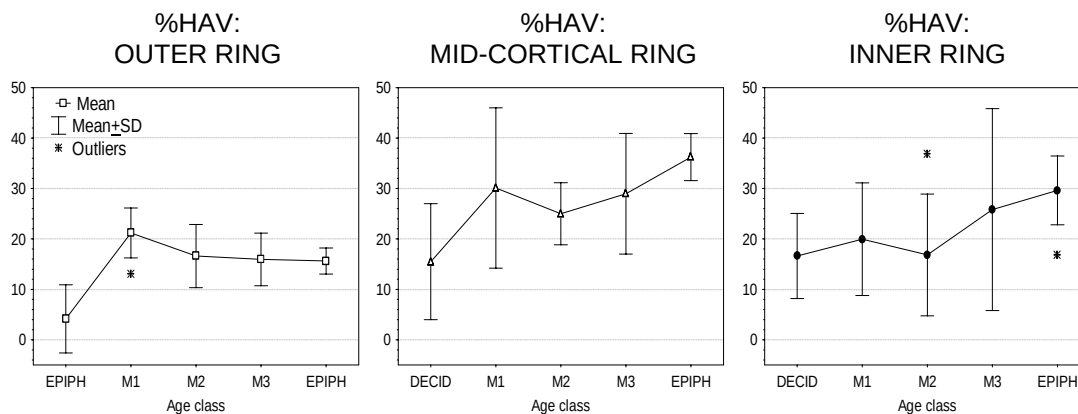


FIGURE 6-8 b. Plots of variation in % HAV across age classes, with rings considered separately: *C. aethiops* midshaft humerus.

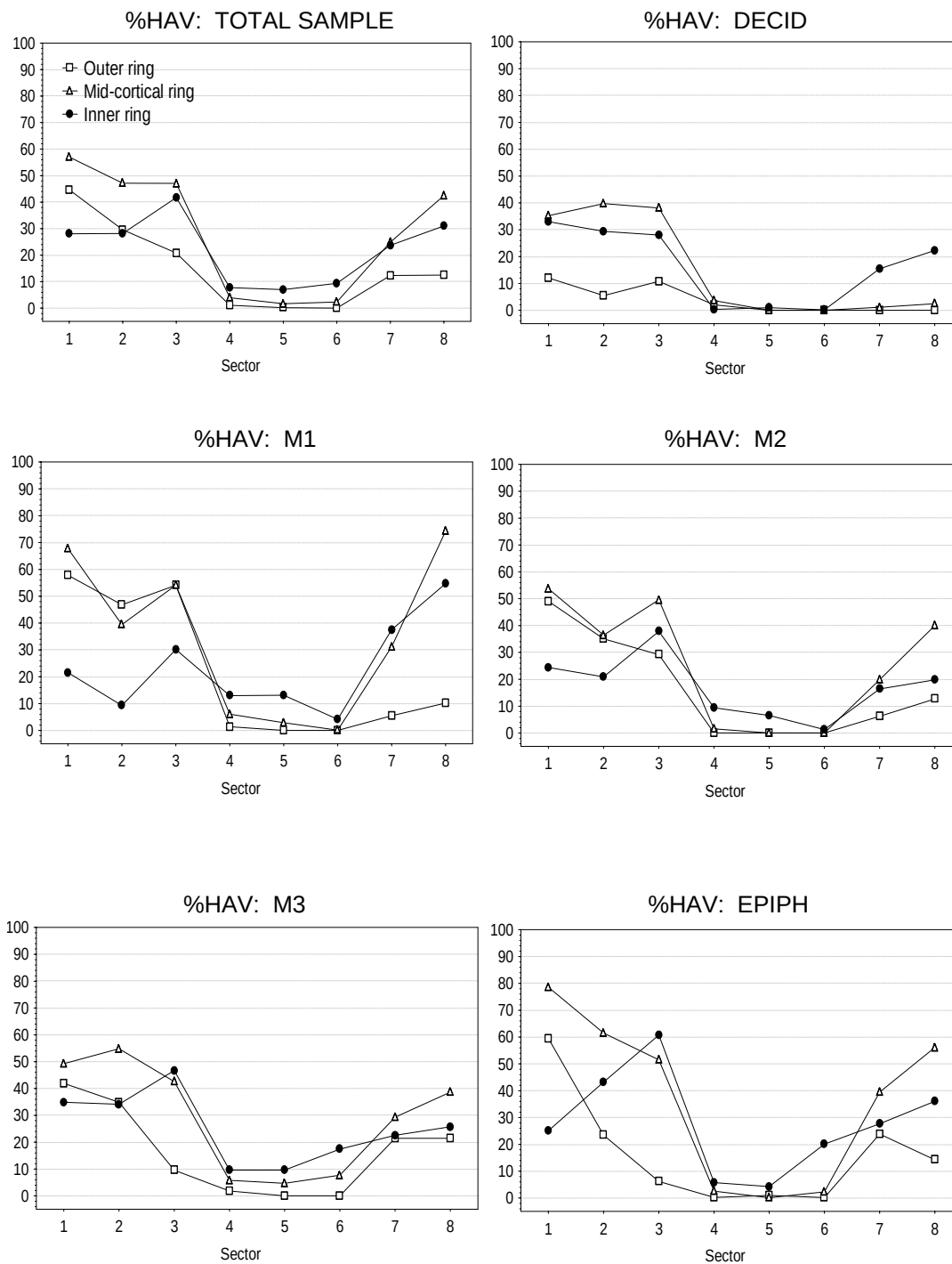


FIGURE 6-9 a. Plots of mean % HAV across radial sectors by ring, with age classes combined and considered separately: *C. aethiops* midshaft humerus.

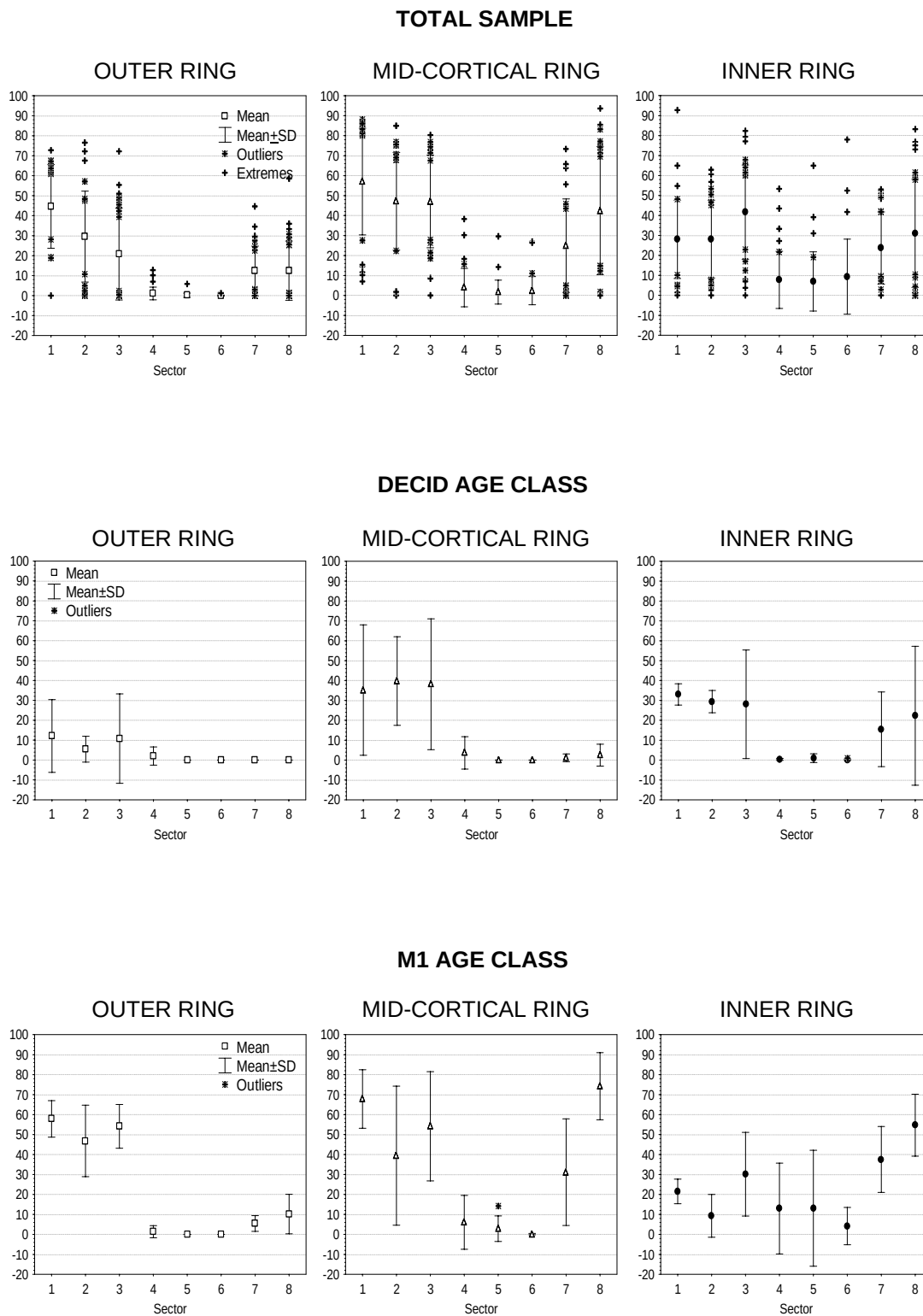


FIGURE 6-9 b. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *C. aethiops* midshaft humerus.

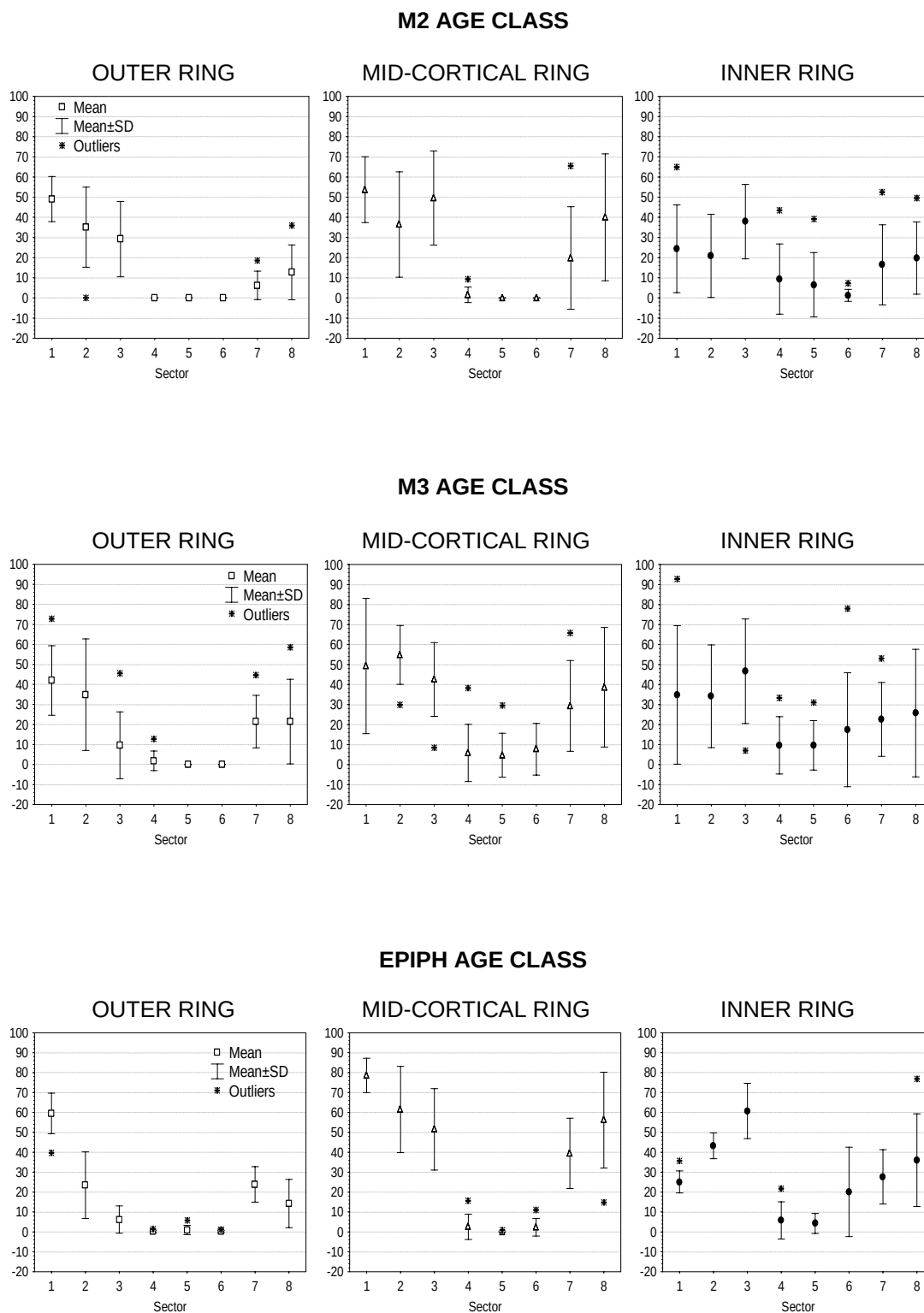
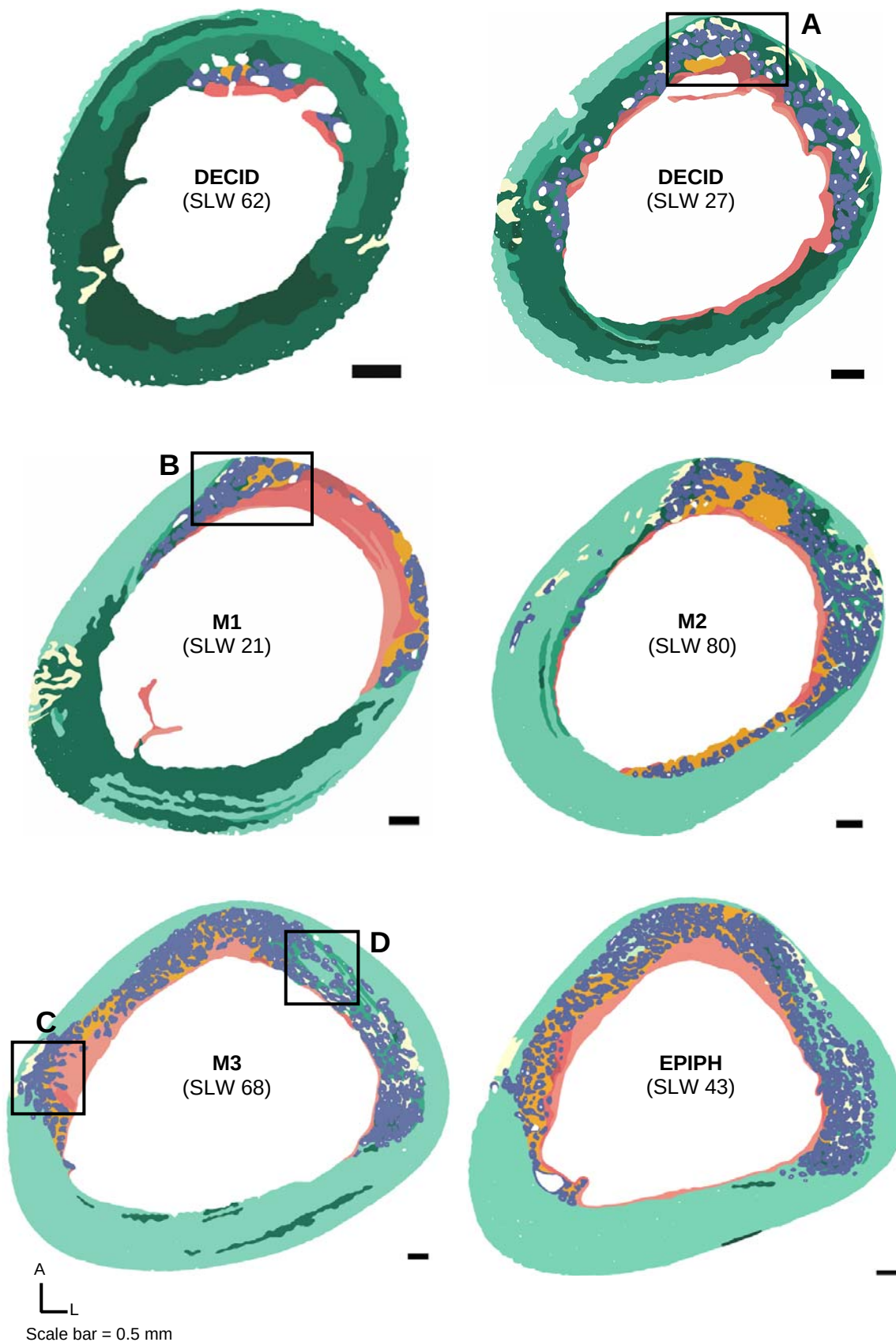


FIGURE 6-9 b cont.. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *C. aethiops* midshaft humerus.

FIGURE 6-10 a. Intracortical remodeling at the midshaft humerus of *C. aethiops*: Example tissue maps by age class. Two DECID individuals are shown here, to represent the range of variation observed among individuals in this age class. Bone regions occupied by secondary osteonal bone are mapped in purple, as depicted in Figure 6-1. Other bone tissue types represented here were recognized on the basis of microstructural features described in Chapter 5 of this thesis. Insets show locations of FIGURES 6-10 a-d.



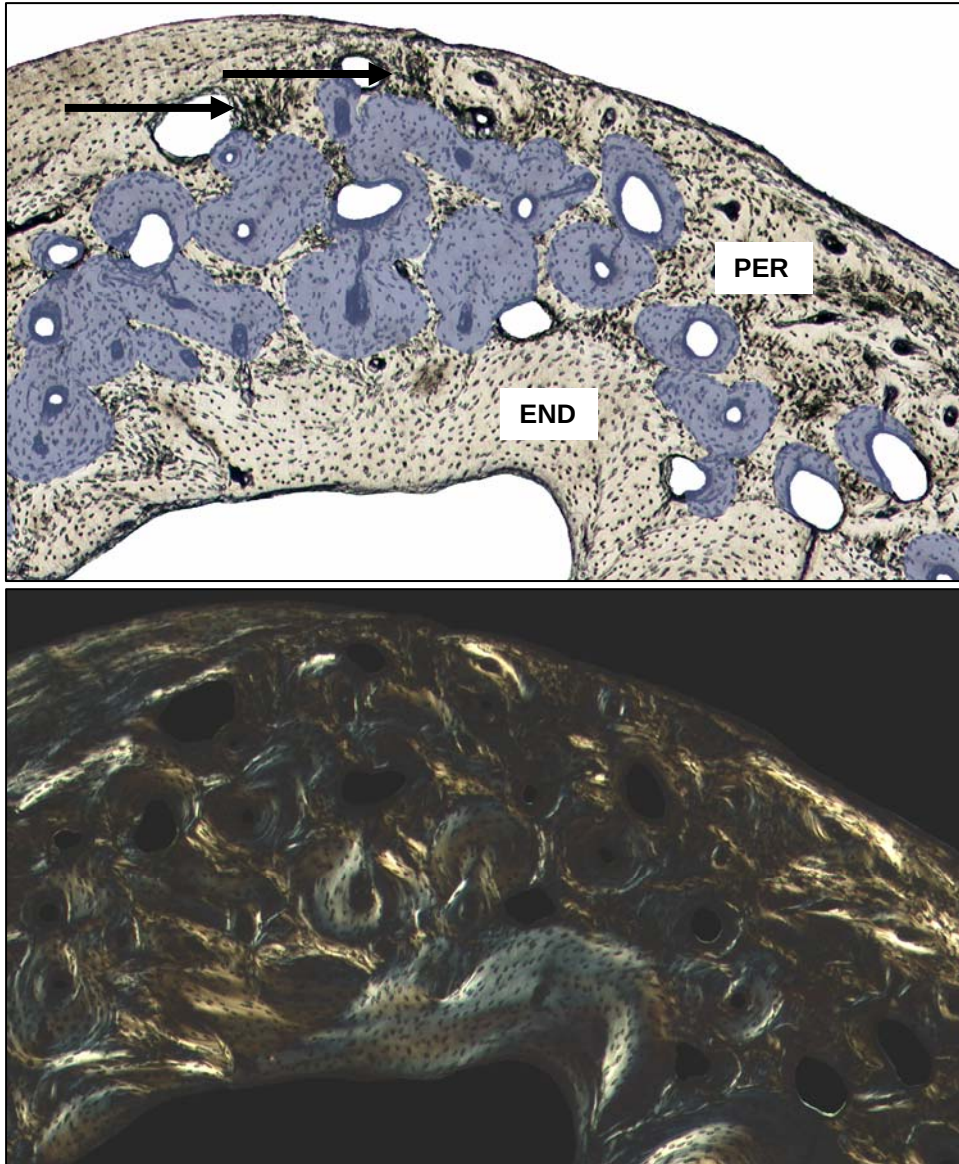


FIGURE 6-10 b. Intracortical remodeling in the midshaft humerus of *C. aethiops* (SLW 27, DECID age class): Secondary osteons, primary vascular bone, and Sharpey's fibers of the anterior cortex. *Top*: LM image. *Bottom*: CPL image. Secondary osteons (mapped in purple) are found in older periosteal (PER) cortex in this region, where they are associated with primary vascular interstitial tissues and Sharpey's fibers (arrows).

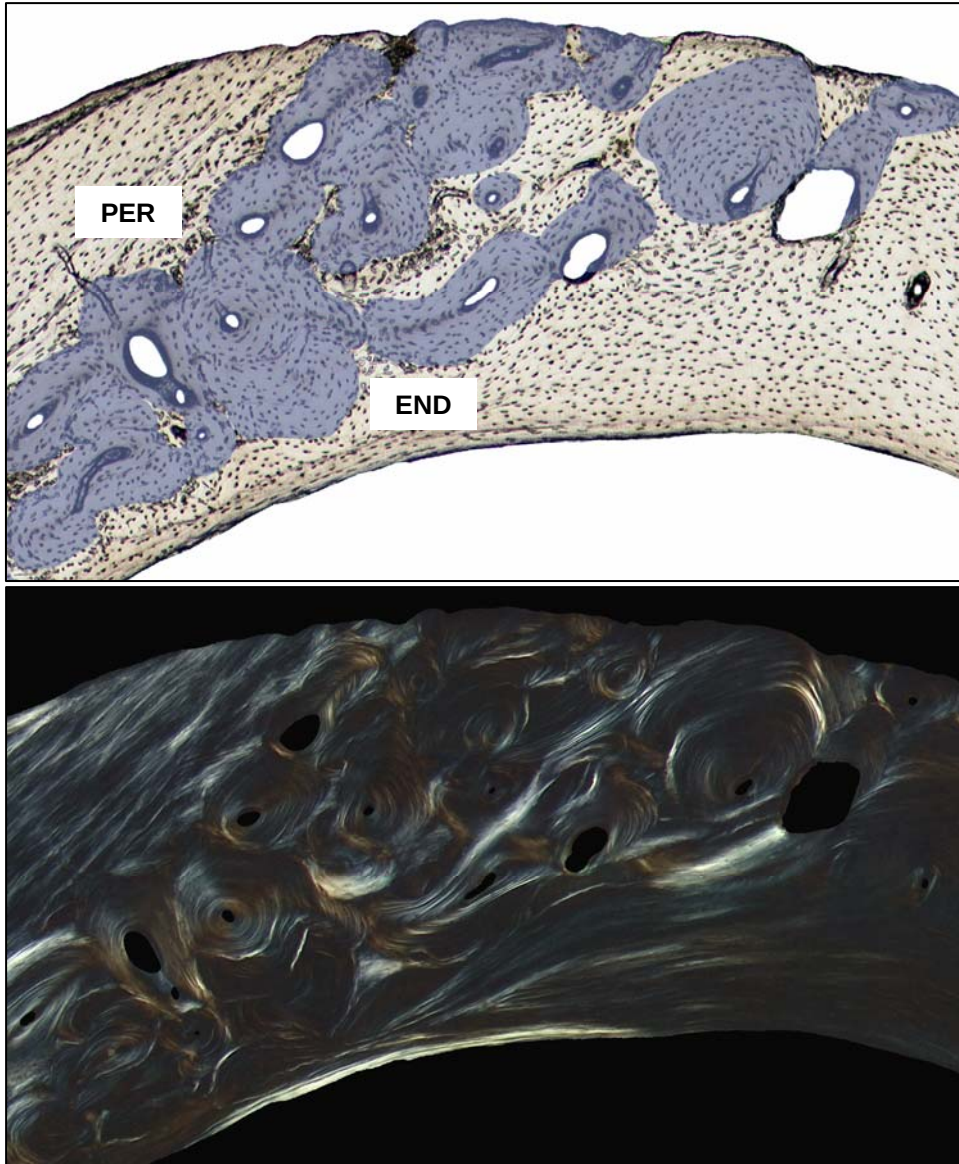


FIGURE 6-10 c. Intracortical remodeling in the midshaft femur of *C. aethiops* (SLW 21, M1 age class): Secondary osteons at the endosteal-periosteal boundary in the anterior cortex. *Top*: LM image. *Bottom*: CPL image. Secondary osteons (mapped in purple) are concentrated at the boundary between endosteal (END) and periosteal (PER) cortex. In the former, they are commonly associated with interstitial tissues having a compacted coarse cancellous organization.

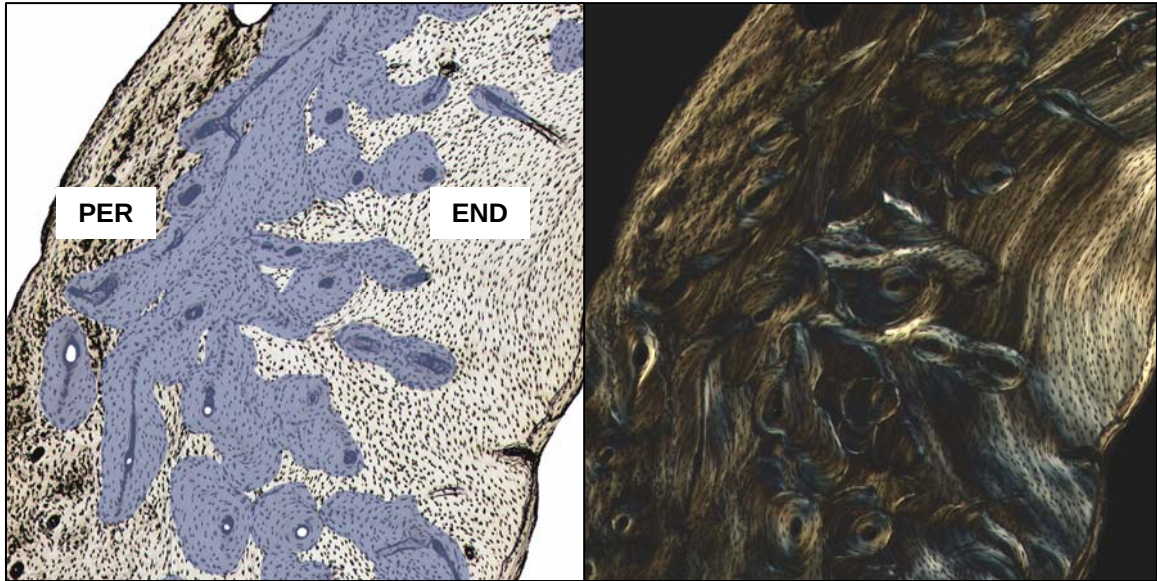


FIGURE 6-10 d. Intracortical remodeling in the midshaft humerus of *C. aethiops* (SLW 68, M3 age class): Secondary osteons at the endosteal – periosteal boundary in the lateral cortex. Left: LM image. Right: CPL image. The distribution of secondary osteons (mapped in purple) in this region is similar to that described in FIGURE 6-10 b. Endosteal circumferential lamellar bone is evident to the right of this section (END).

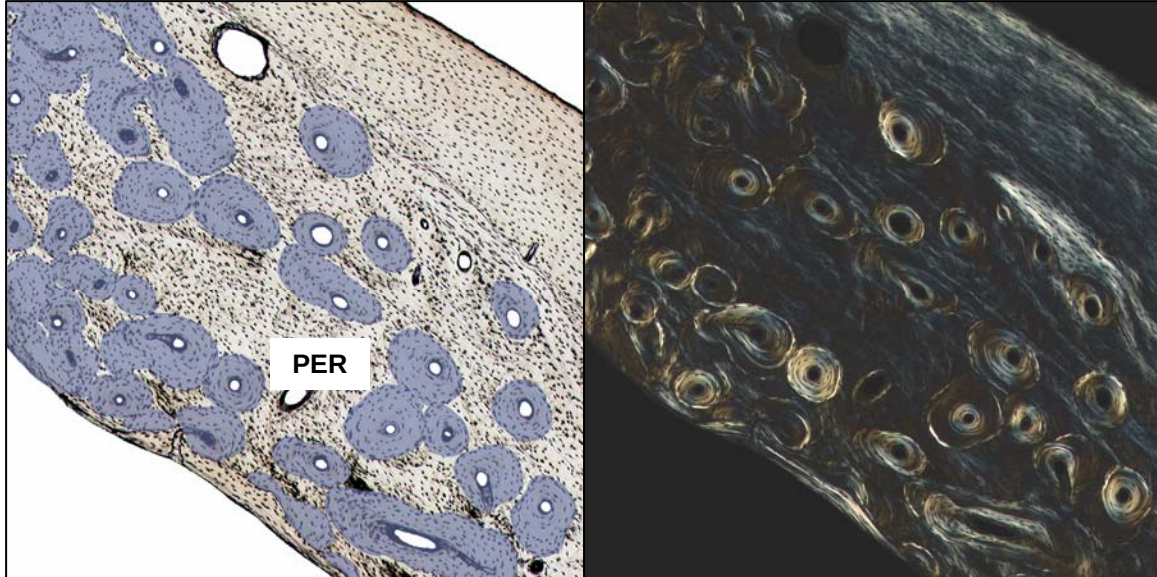


FIGURE 6-10 e. Intracortical remodeling in the midshaft humerus of *C. aethiops* (SLW 68, M3 age class): Secondary osteons in inner periosteal regions of the anterolateral cortex. Left: LM image. Right: CPL image. In the anterolateral cortex, secondary osteons (mapped in purple) are distributed at the endosteal – periosteal boundary, and scattered throughout the inner periosteal (PER) cortex where they are associated with less vascular interstitial bone. Intracortical remodeling of anterolateral periosteal cortex may be extensive in individuals of the M2 – EPIPH age classes.

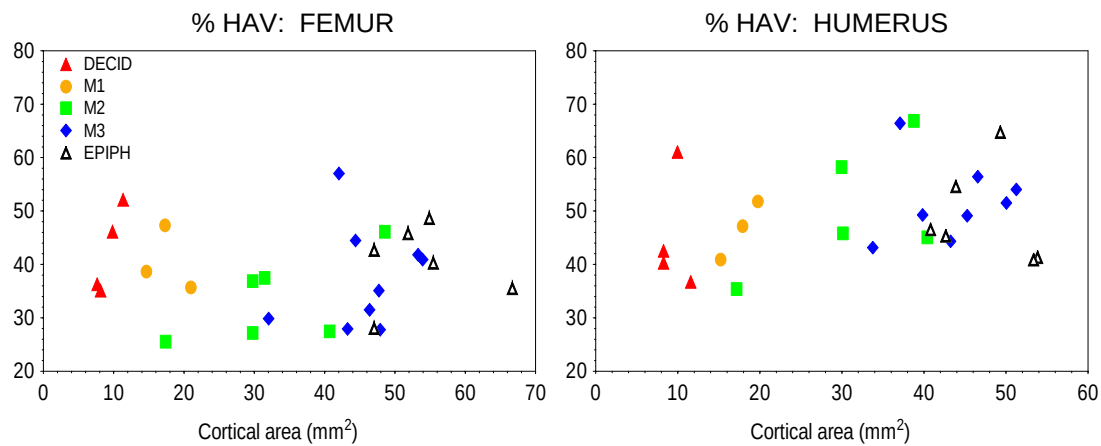


FIGURE 6-11 a. Scatterplots of whole-section % HAV by cortical area, categorized by age class: *H. lar* midshaft femur and humerus.

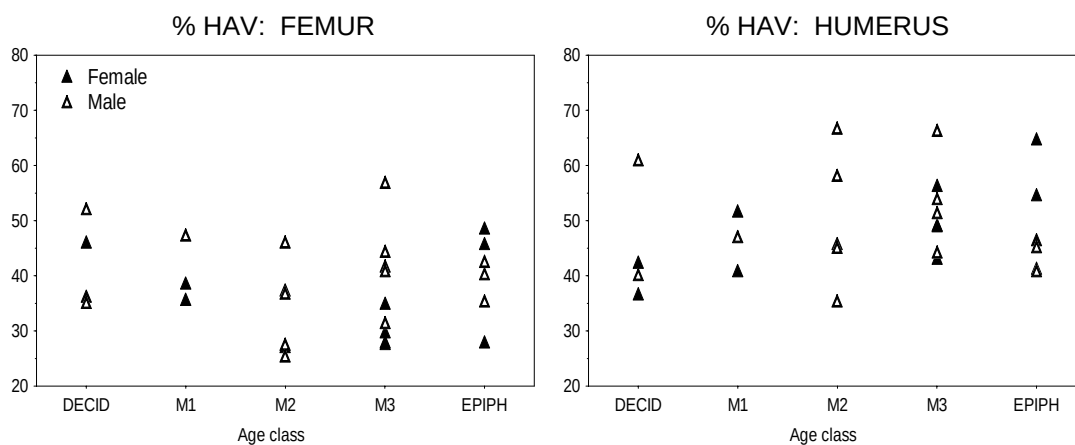


FIGURE 6-11 b. Scatterplots of whole-section % HAV by age class, categorized by sex: *H. lar* midshaft femur and humerus.

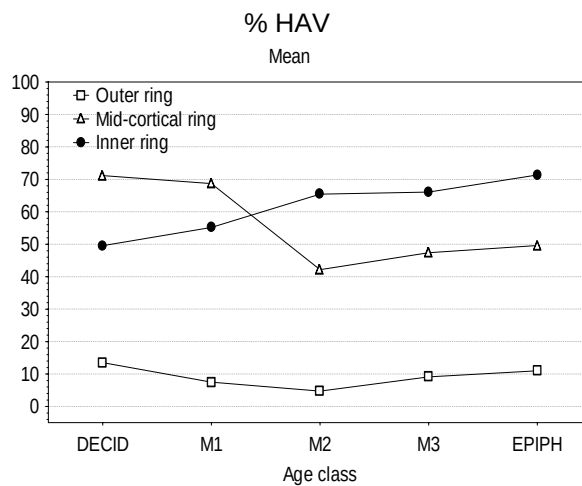


FIGURE 6-12 a. Plot of age class mean % HAV, with rings considered separately: *H. lar* midshaft femur.

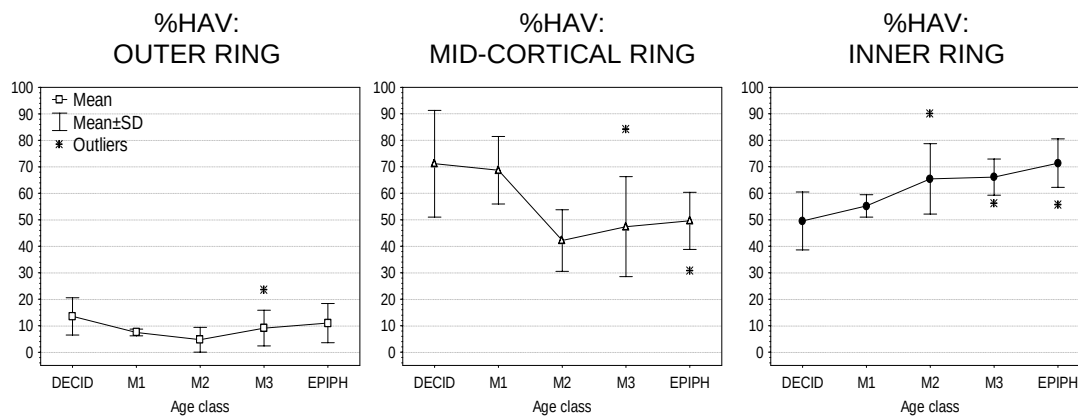


FIGURE 6-12 b. Plots of variation in % HAV across age classes, with rings considered separately: *H. lar* midshaft femur.

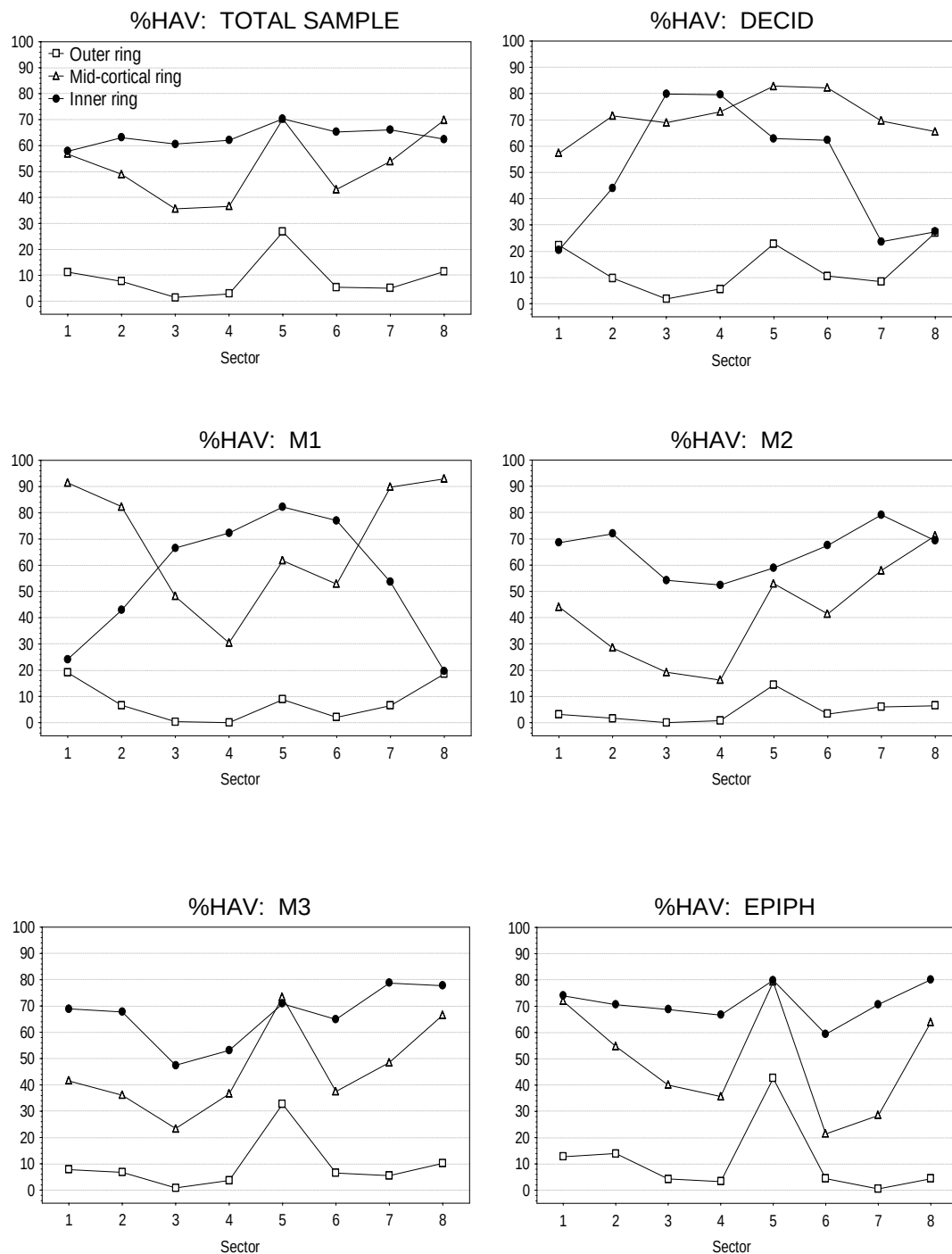


FIGURE 6-13 a. Plots of mean % HAV across radial sectors by ring, with age classes combined and considered separately: *H. lar* midshaft femur.

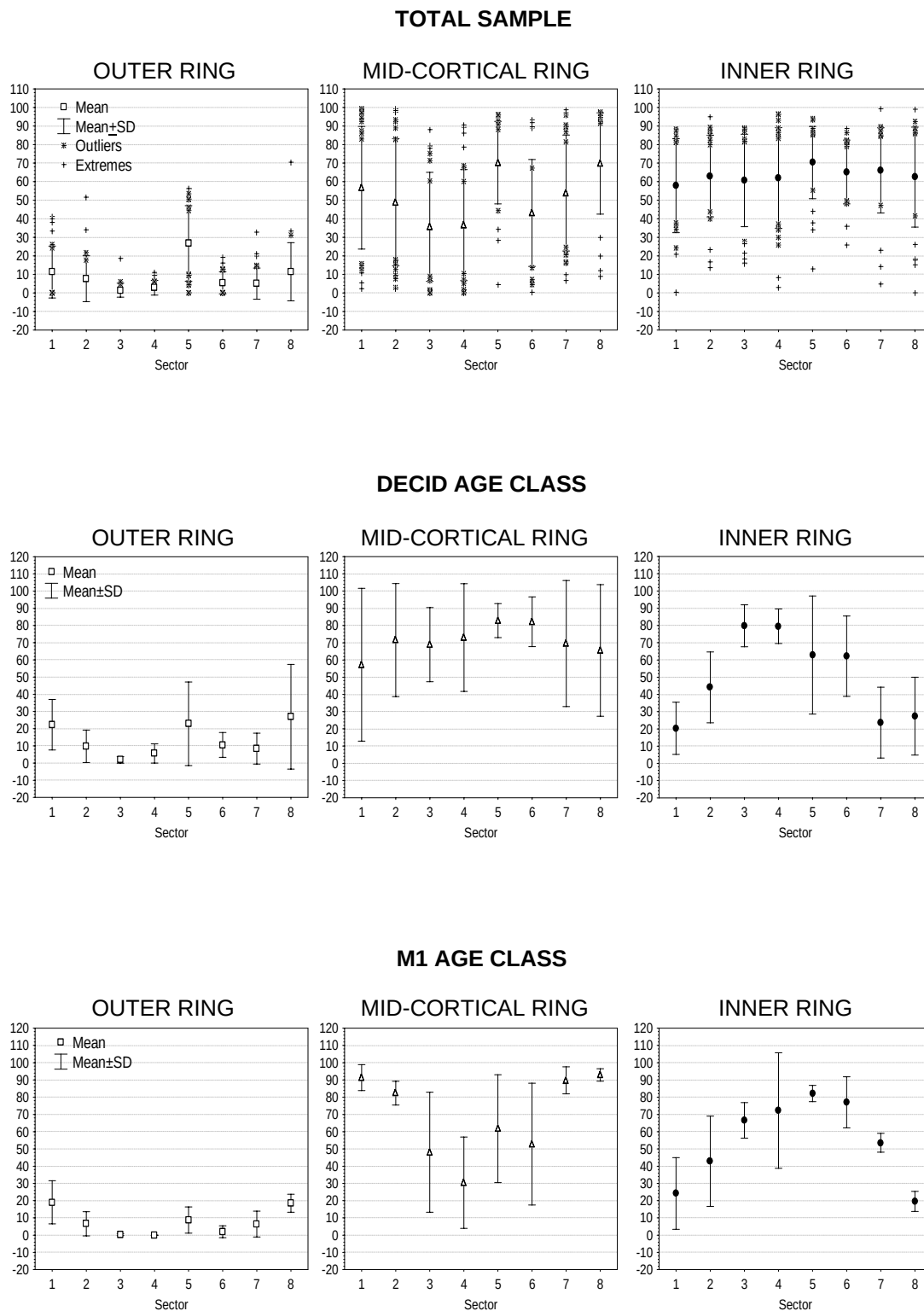


FIGURE 6-13 b. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *H. lar* midshaft femur.

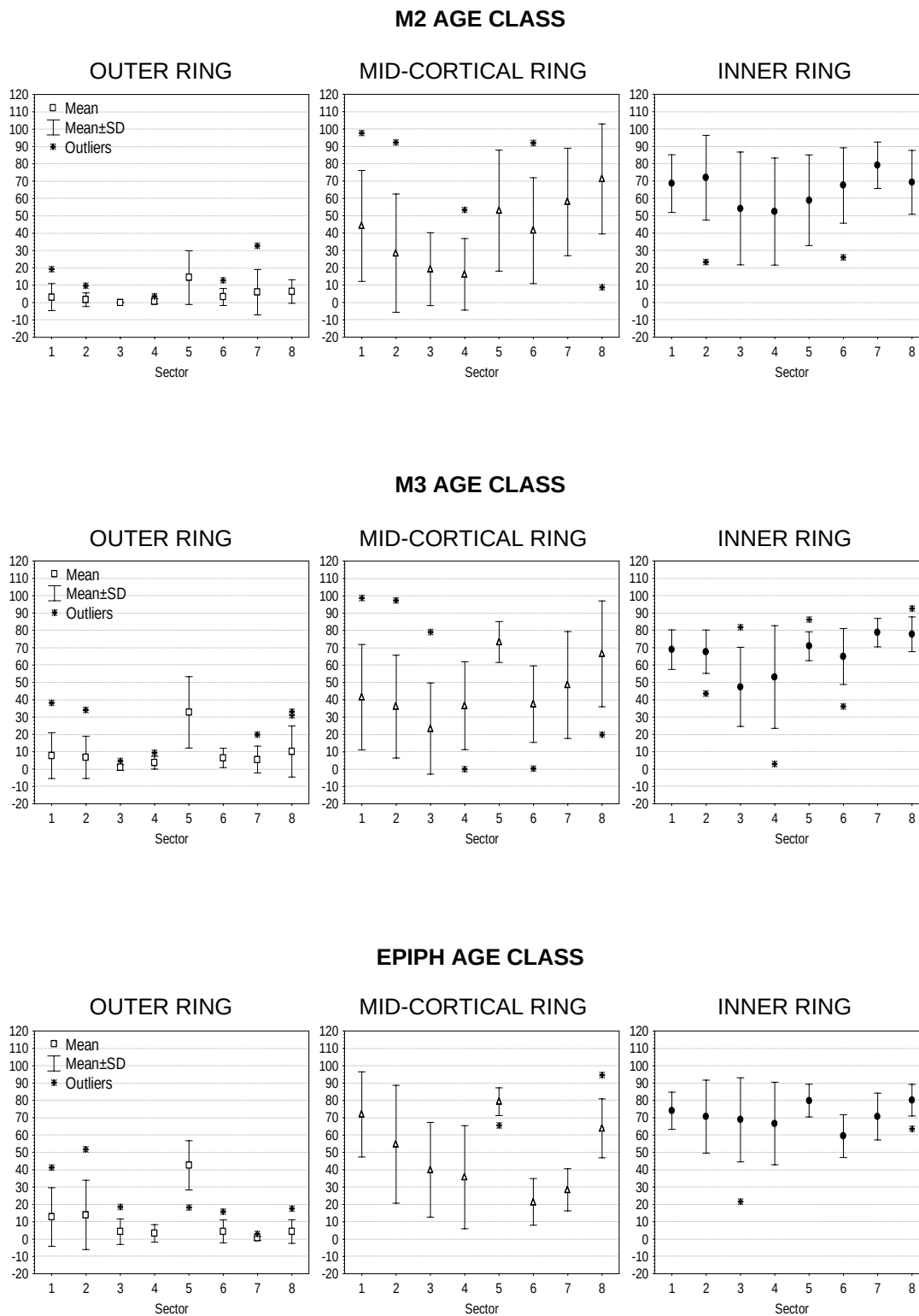
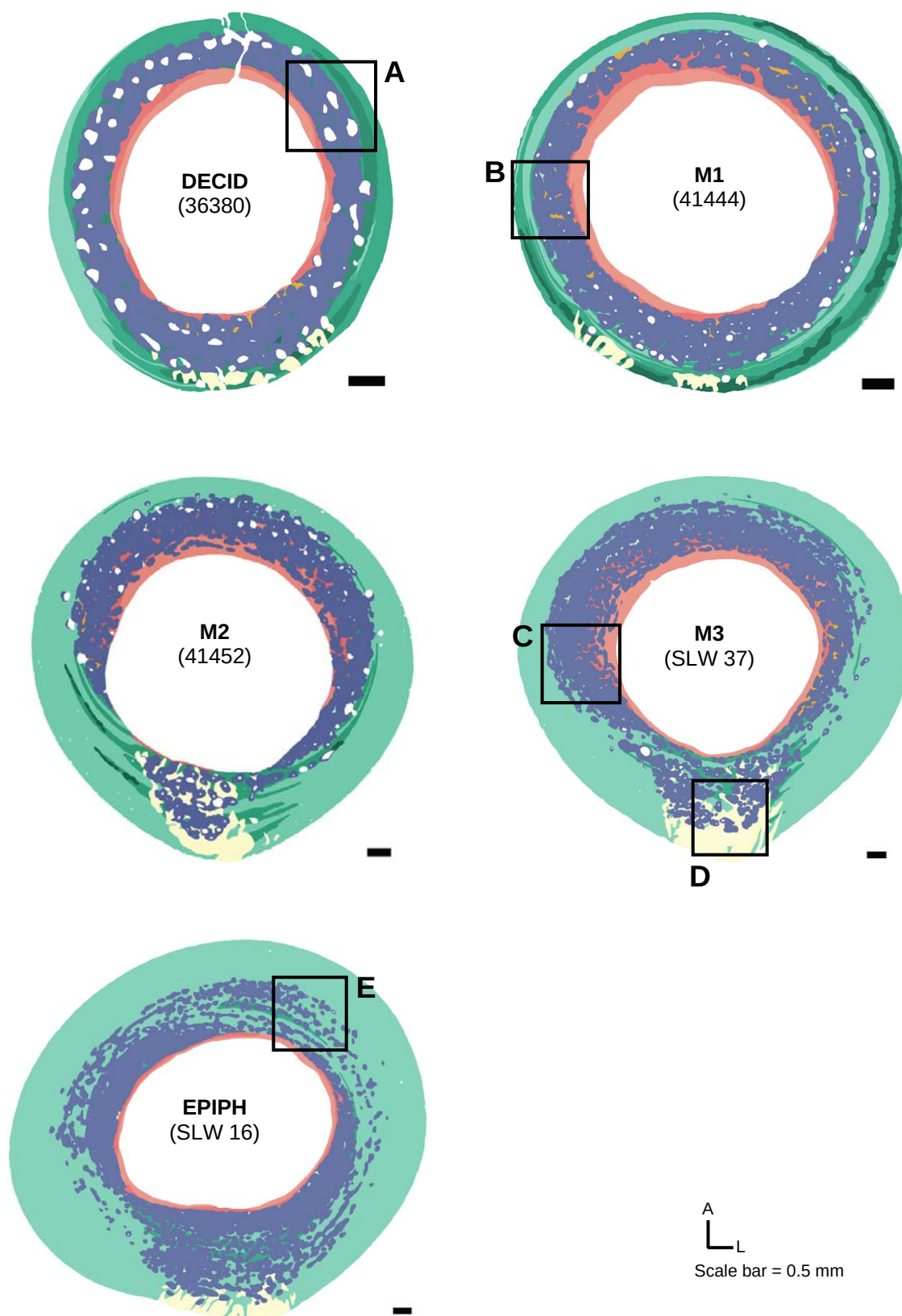


FIGURE 6-13 b cont.. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *H. lar* midshaft femur.

FIGURE 6-14 a. Intracortical remodeling at the midshaft femur of *H. lar*: Example tissue maps by age class. Bone regions occupied by secondary osteonal bone are mapped in purple, as depicted in Figure 6-1. Other bone tissue types represented here were recognized on the basis of microstructural features described in Chapter 5 of this thesis. Insets show locations of FIGURES 6-14 a-e.



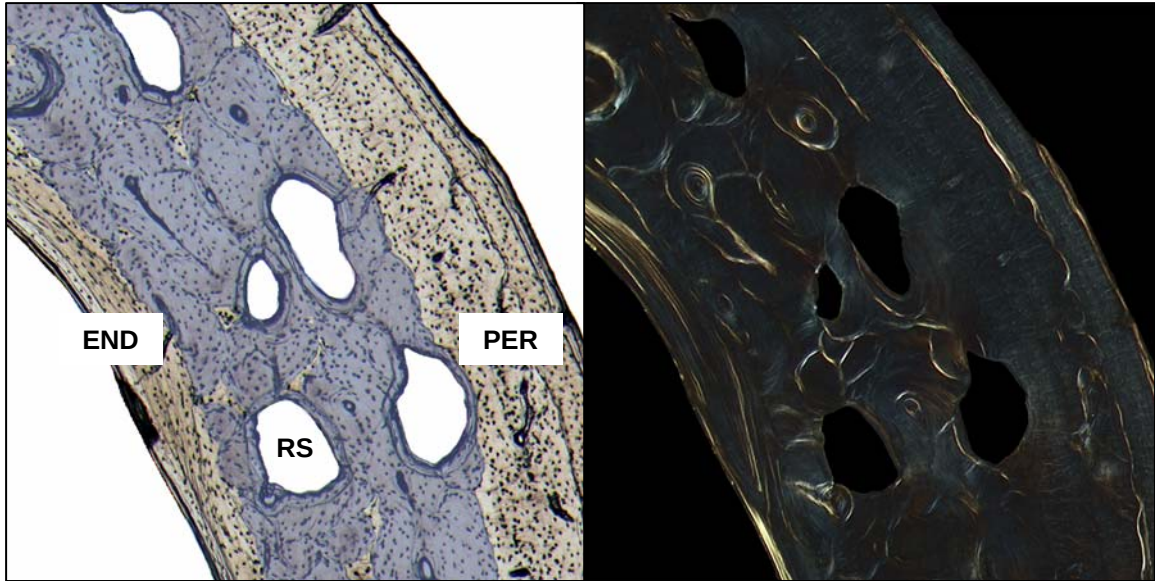


FIGURE 6-14 b. Intracortical remodeling in the midshaft femur of *H. lar* (MCZ 36380, DECID age class): Secondary osteons occupy a mid-cortical zone in the anterolateral cortex. Left: LM image. Right: CPL image. Secondary osteons (mapped in purple) are densely distributed at the boundary between endosteal (END) and periosteal (PER) cortex, occupying a mid-cortical zone. Large resorption spaces (RS) are common, and secondary osteons are often irregular in shape and show evidence of drift. (Primary bone and secondary osteons commonly appear dark in CPL, particularly in younger individuals.)

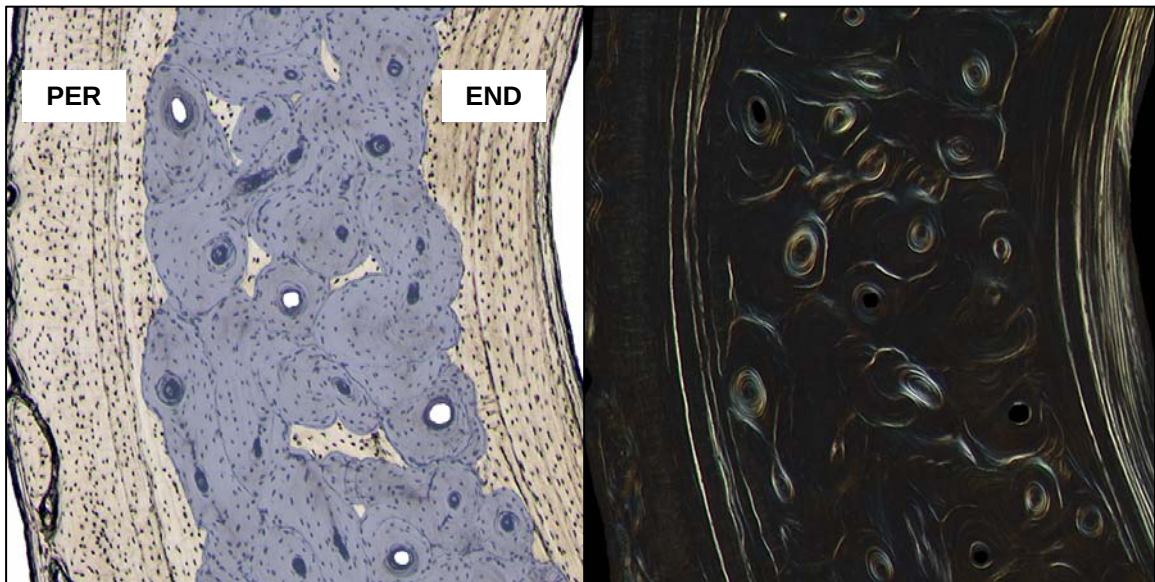


FIGURE 6-14 c. Intracortical remodeling in the midshaft femur of *H. lar* (MCZ 41444, M1 age class): Secondary osteons occupy a mid-cortical zone in the medial cortex. Left: LM image. Right: CPL image. Secondary osteons (mapped in purple) are concentrated at the boundary between endosteal (END) and periosteal (PER) cortex. They are densely-distributed in older regions of the endosteal cortex. In this image, secondary osteons occupy a mid-cortical zone. More recently-deposited endosteal bone is circumferential lamellar in organization and is not secondarily remodeled.

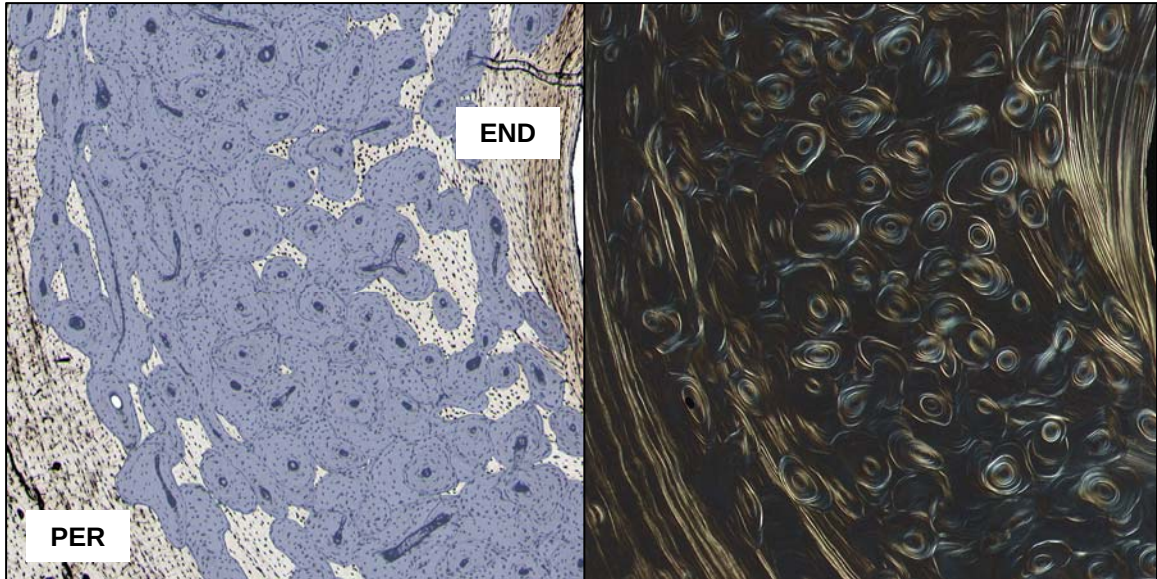


FIGURE 6-14 d. Intracortical remodeling in the midshaft femur of *H. lar* (SLW 37, M3 age class): Secondary osteons at the endosteal-periosteal boundary in the medial cortex. Left: LM image. Right: CPL image. Secondary osteons (mapped in purple) are densely distributed at the boundary between endosteal (END) and periosteal (PER) cortex, invading older tissues of periosteal and endosteal origin. Primary endosteal and periosteal bone is predominantly circumferential lamellar in its organization.

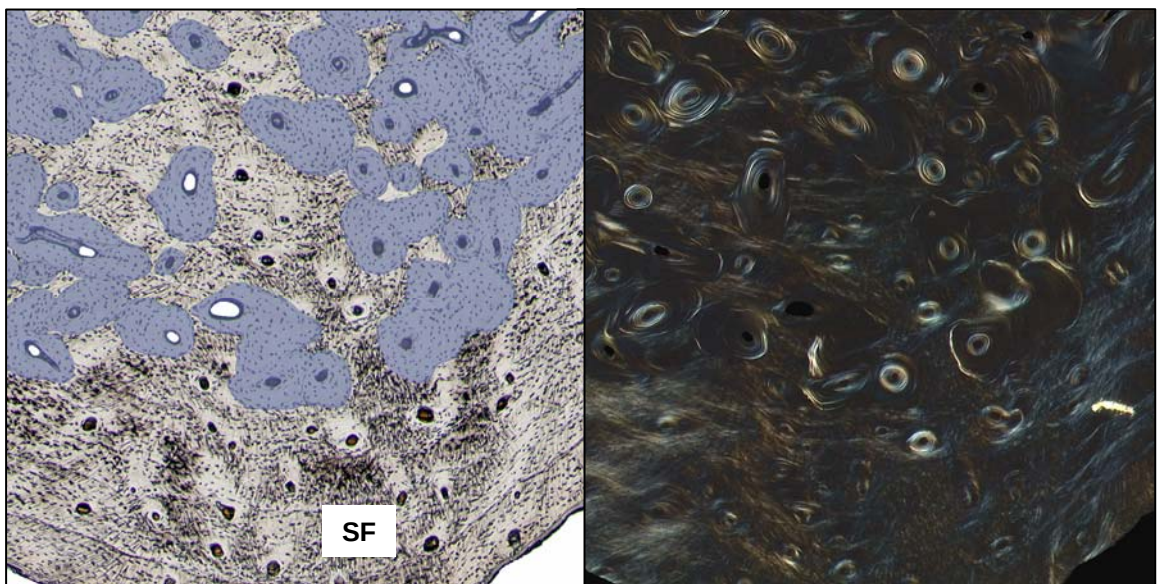


FIGURE 6-14 e. Intracortical remodeling in the midshaft femur of *H. lar* (SLW 37, M3 age class): Secondary osteons and Sharpey's fibers in the posterior cortex. Left: LM image. Right: CPL image. Secondary osteons (mapped in purple) invade more recently-deposited periosteal tissues here, and are associated with Sharpey's fibers (SF).

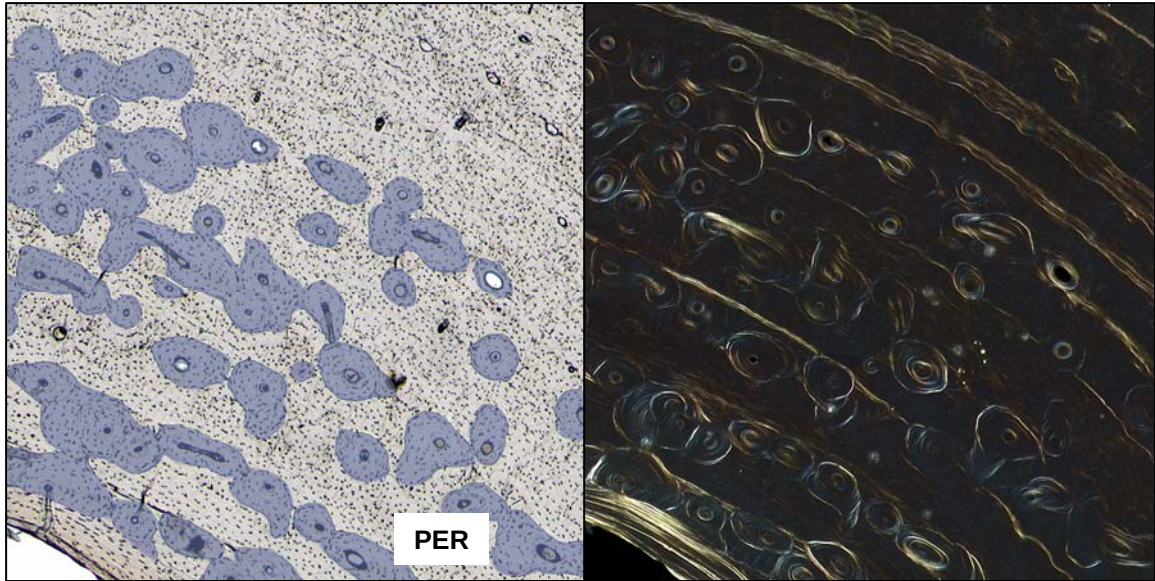


FIGURE 6-14 f. Intracortical remodeling in the midshaft femur of *H. lar* (SLW 16, EPIPH age class): Secondary osteons in inner periosteal regions of the anterolateral cortex. *Left:* LM image. *Right:* CPL image. In this region, secondary osteons (mapped in purple) are scattered throughout the inner periosteal (PER) cortex, where they are associated with less vascular interstitial bone.

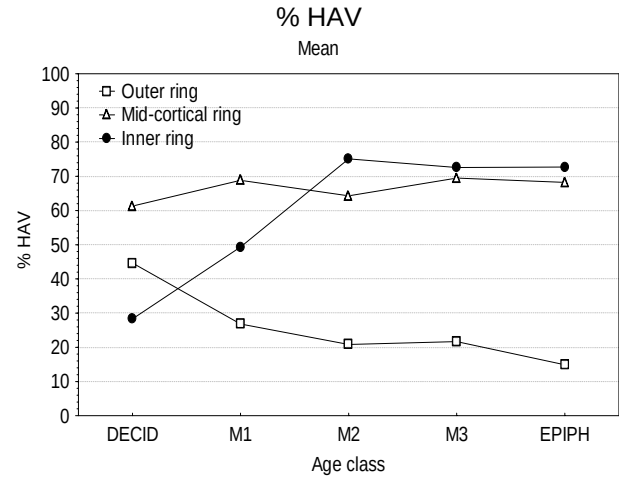


FIGURE 6-15 a. Plot of age class mean % HAV, with rings considered separately: *H. lar* midshaft humerus.

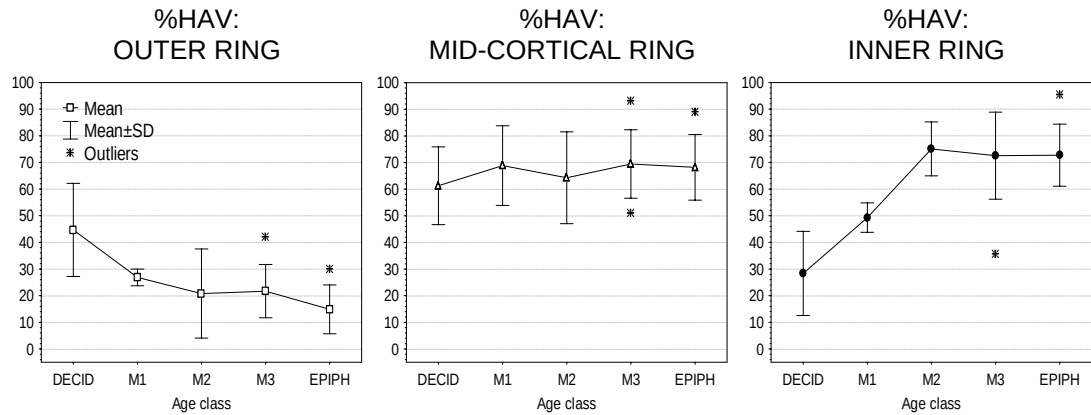


FIGURE 6-15 b. Plots of variation in % HAV across age class, with rings considered separately: *H. lar* midshaft humerus.

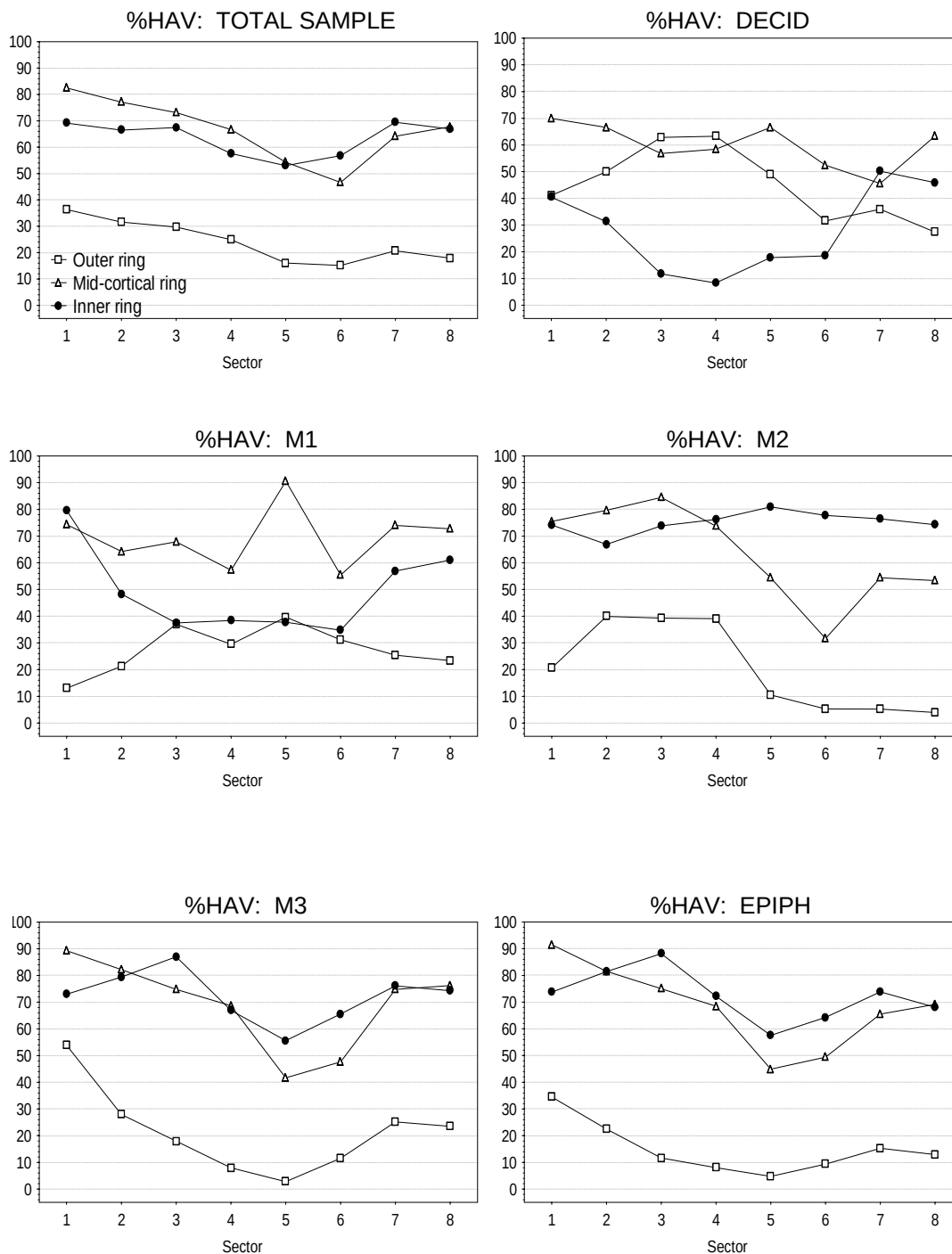


FIGURE 6-16 a. Plots of mean % HAV across radial sectors by ring, with age classes combined and considered separately: *H. lar* midshaft humerus.

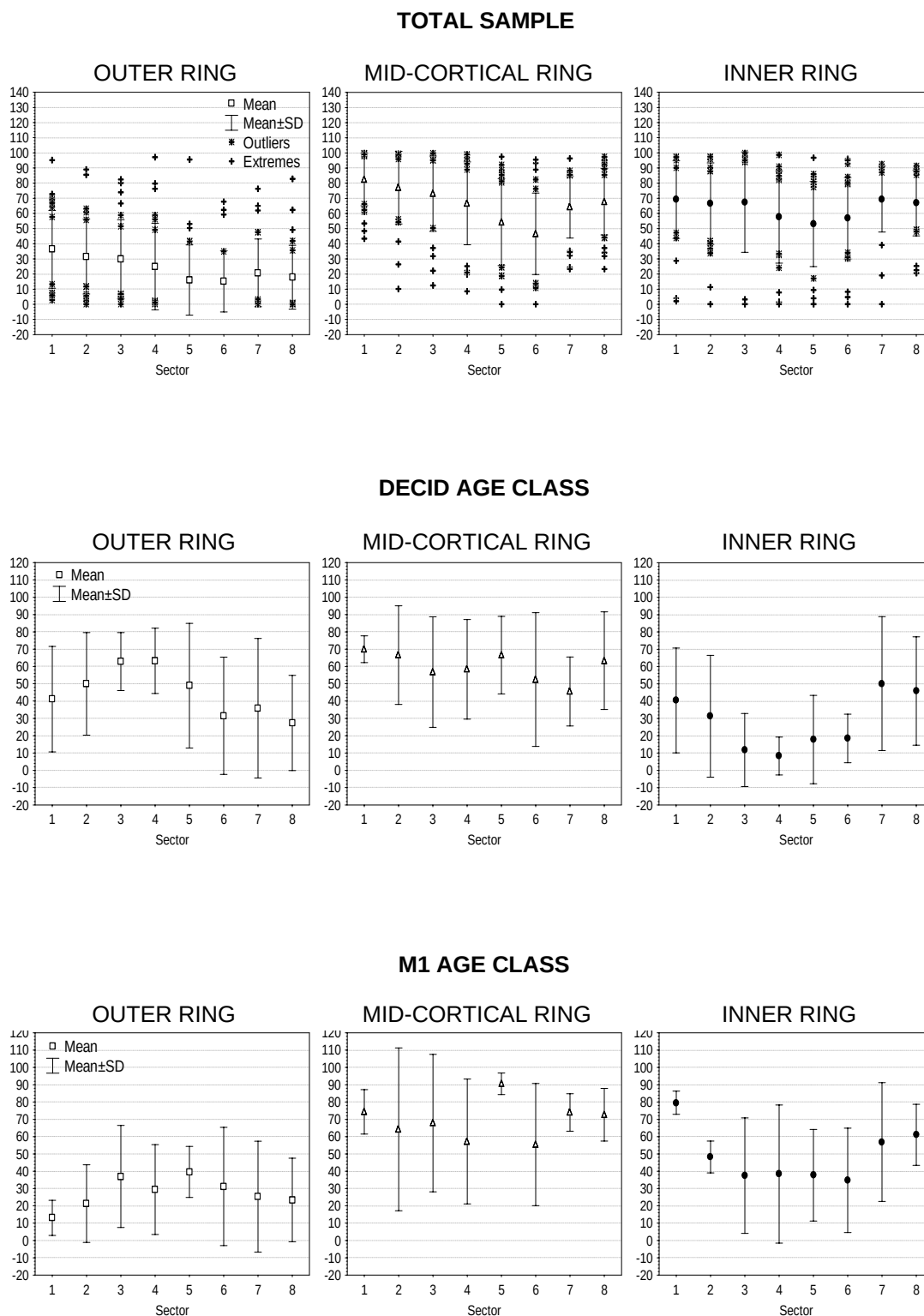


FIGURE 6-16 b. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *H. lar* midshaft humerus.

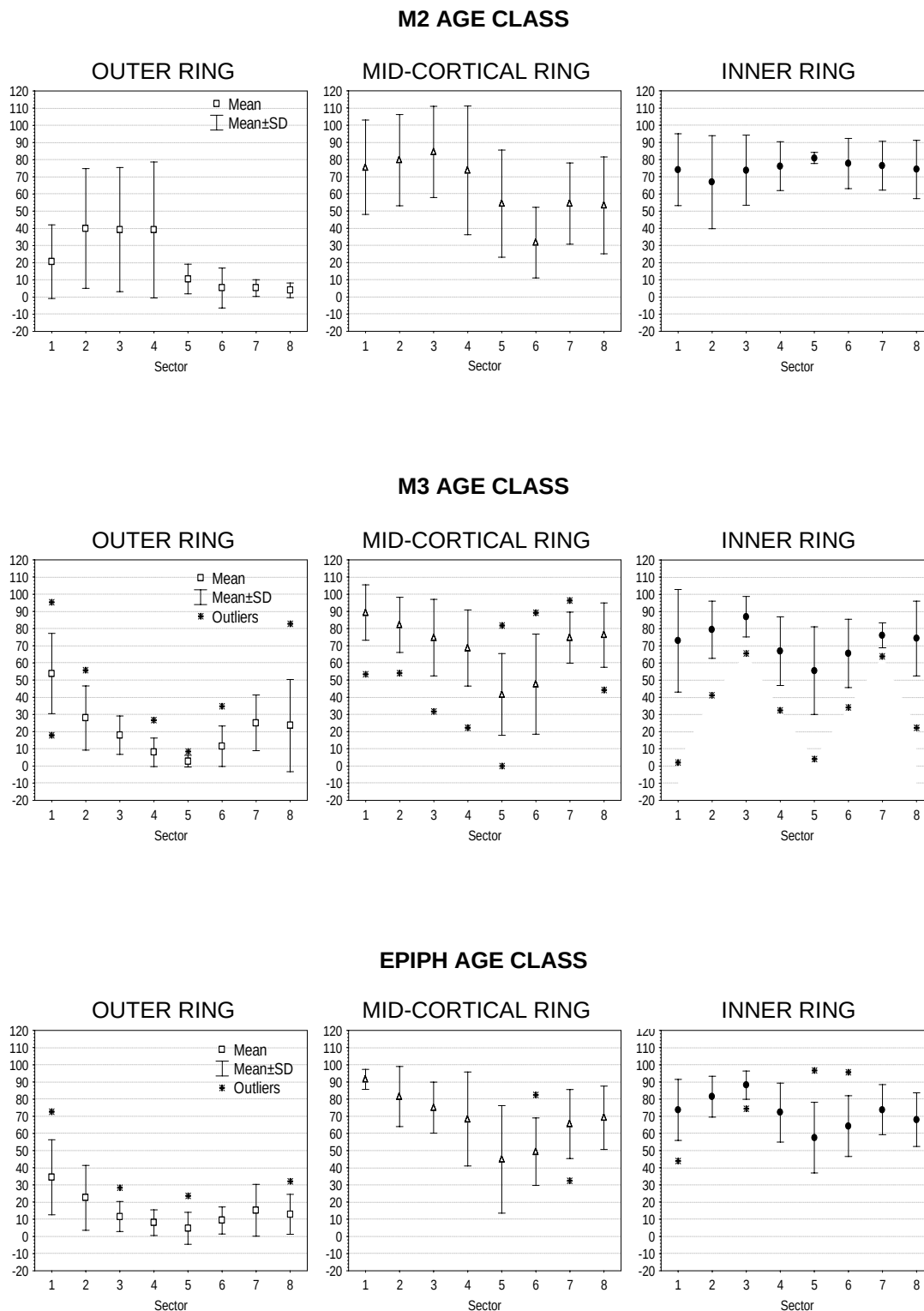
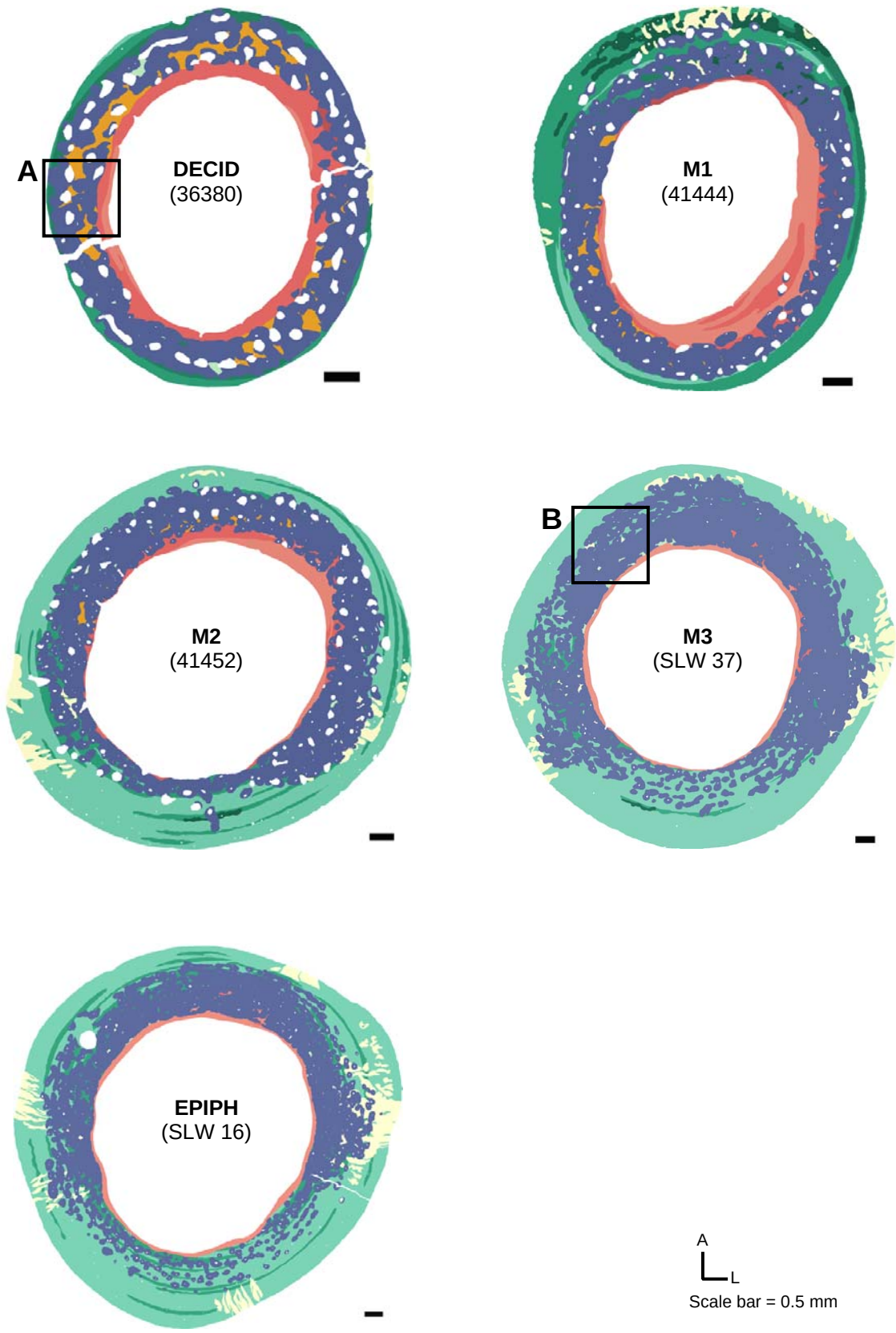


FIGURE 6-16 b cont.. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *H. lar* midshaft humerus.

FIGURE 6-17 a. Intracortical remodeling at the midshaft humerus of *H. lar*:
Example tissue maps by age class. Bone regions occupied by secondary osteonal bone are mapped in purple, as depicted in Figure 6-1. Other bone tissue types represented here were recognized on the basis of microstructural features described in Chapter 5 of this thesis. Insets show locations of FIGURES 6-17 a and b.



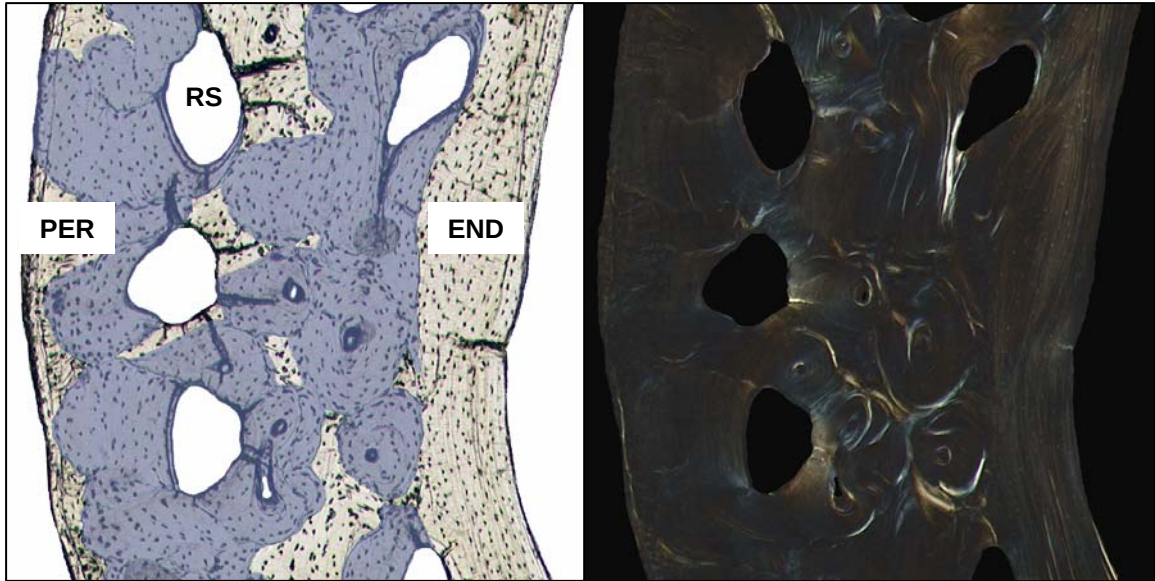


FIGURE 6-17 b. Intracortical remodeling in the midshaft humerus of *H. lar* (MCZ 36380, DECID age class): Secondary osteons in endosteal regions of the medial cortex. Left: LM image. Right: CPL image. Secondary osteons (mapped in purple) are concentrated at the boundary between endosteal (END) and periosteal (PER) cortex, and within endosteal compacted coarse cancellous bone. Large resorption spaces (RS) are common. Secondary osteons are often large and irregular in shape, and show evidence of drift. (Primary bone and secondary osteons commonly appear dark in CPL, particularly in younger individuals.)

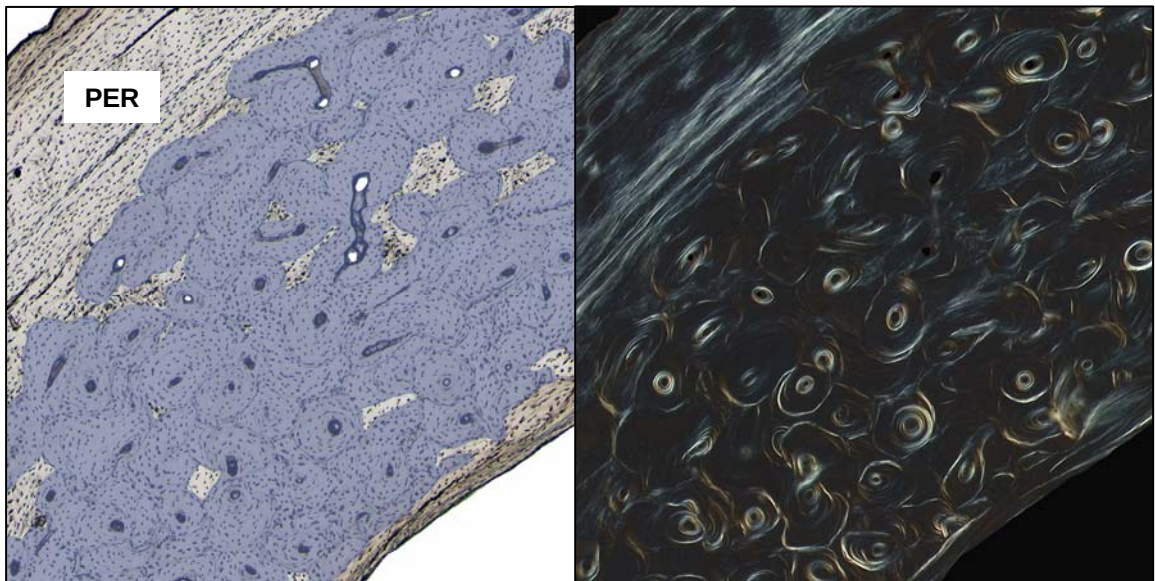


FIGURE 6-17 c. Intracortical remodeling in the midshaft humerus of *H. lar* (SLW 37, M3 age class): Secondary osteons in the anteromedial cortex. Left: LM image. Right: CPL image. Secondary osteons (mapped in purple) are concentrated at the boundary between endosteal and periosteal cortex, and are distributed throughout older periosteal (PER) cortex.

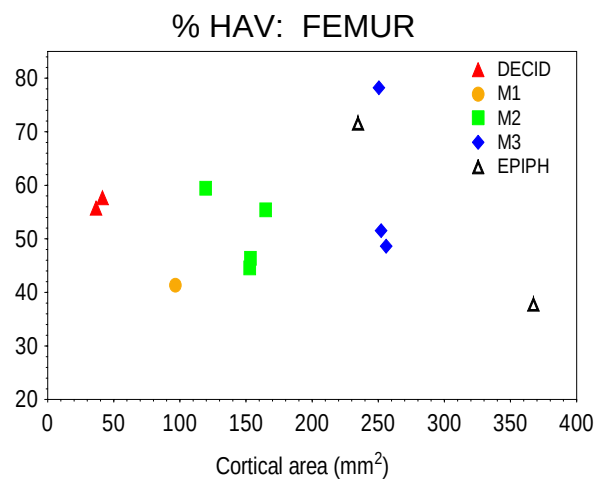


FIGURE 6-18. Scatterplot of whole-section % HAV by cortical area and categorized by age class: *P. troglodytes* midshaft femur.

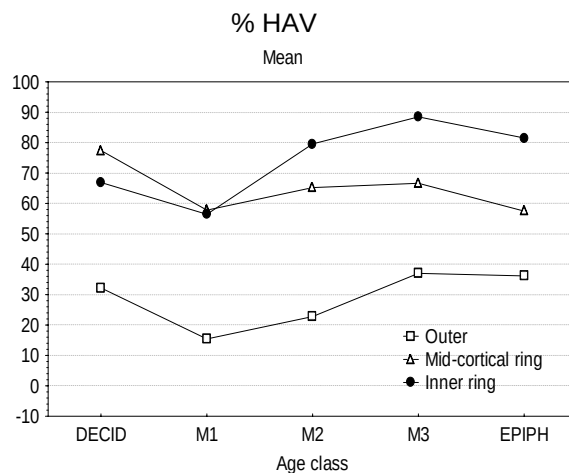


FIGURE 6-19 a. Plot of age class mean % HAV, with rings considered separately: *P. troglodytes* midshaft femur.

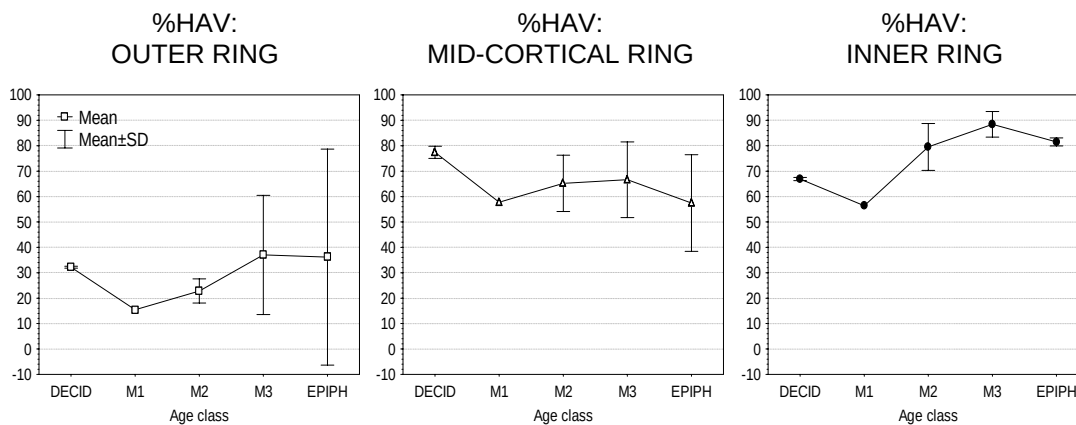


FIGURE 6-19 b. Plots of variation in % HAV across age class, with rings considered separately: *P. troglodytes* midshaft femur.

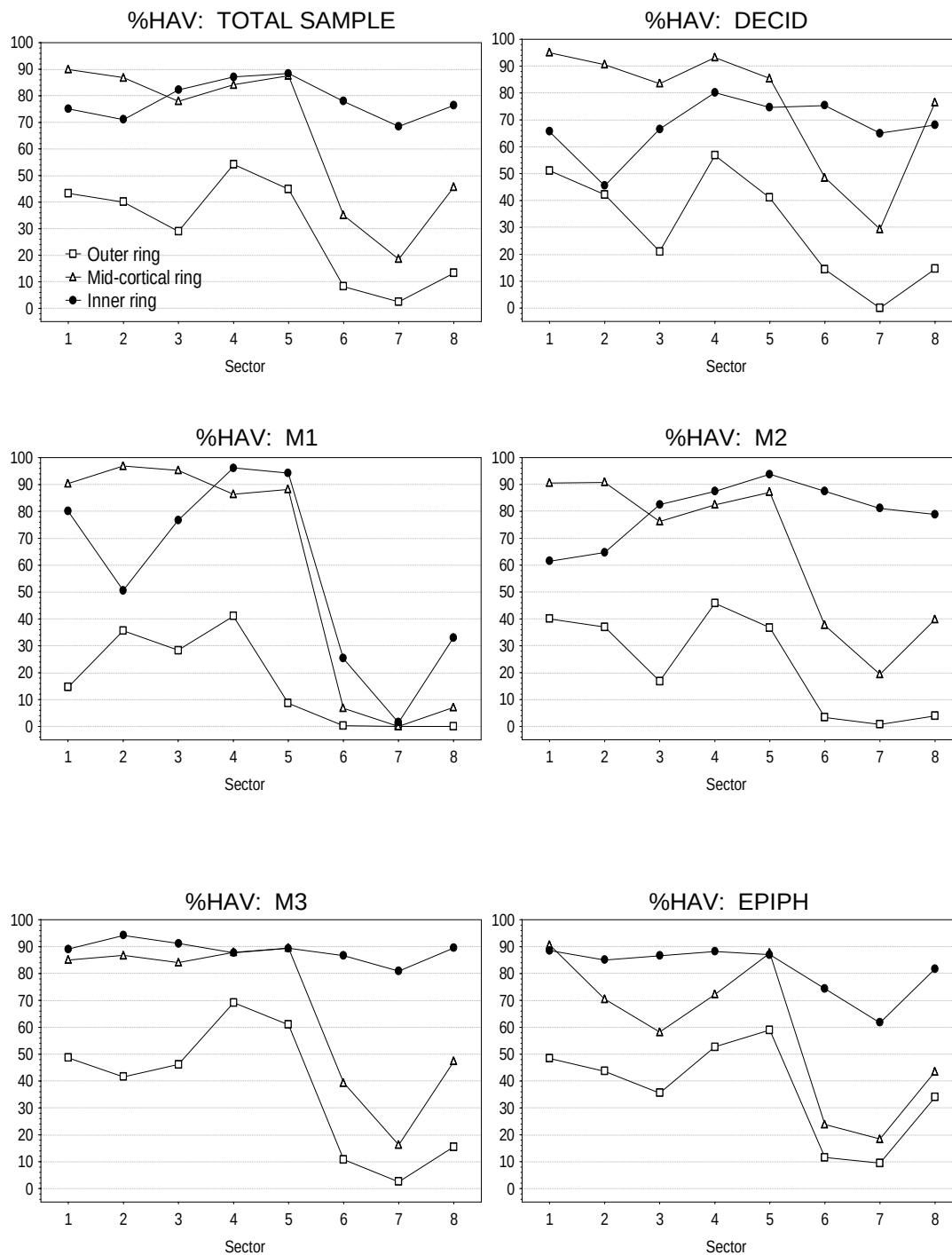


FIGURE 6-20 a. Plots of mean % HAV across radial sectors by ring, with age classes combined and considered separately: *P. troglodytes* midshaft femur.

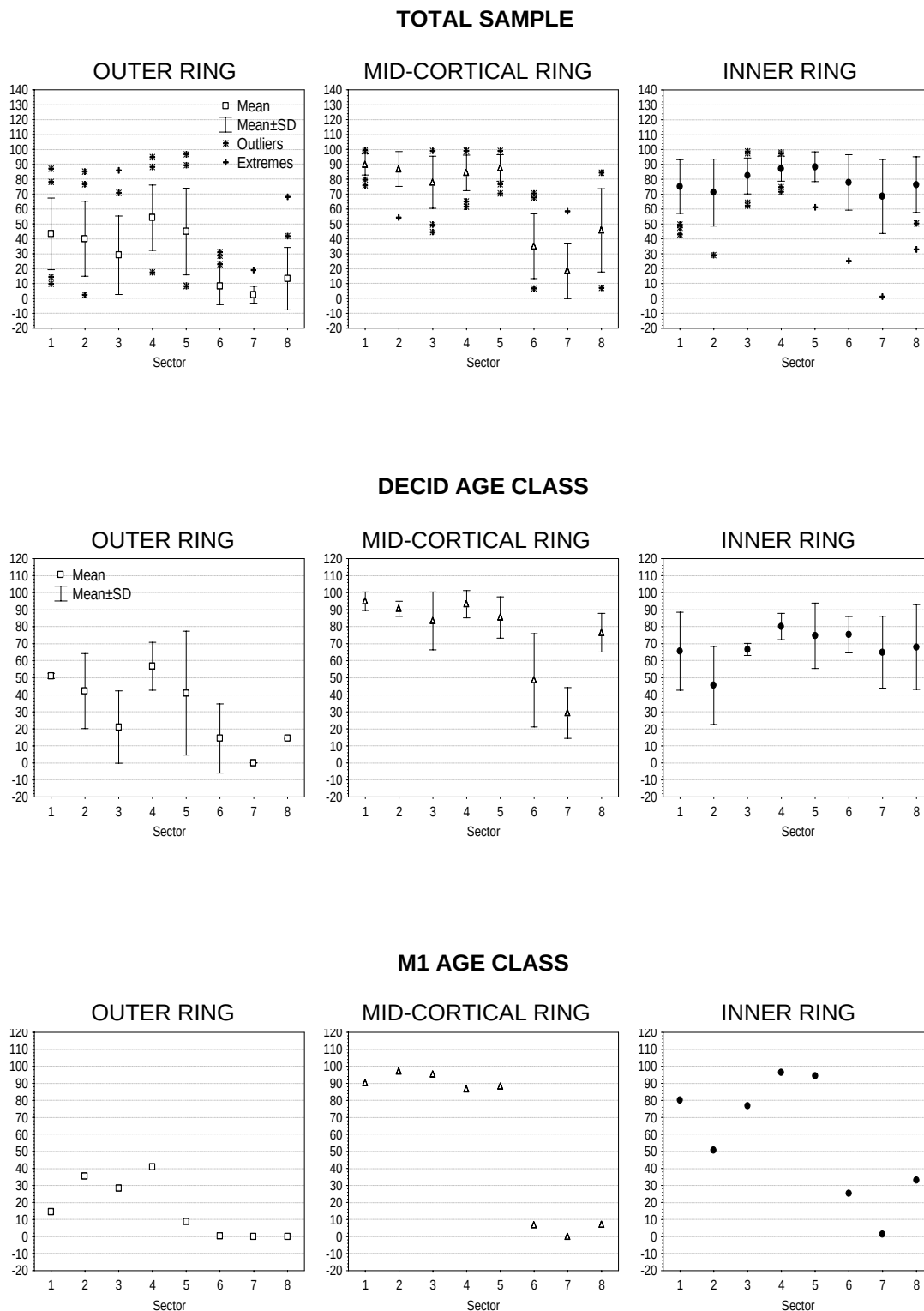


FIGURE 6-20 b. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *P. troglodytes* midshaft femur.

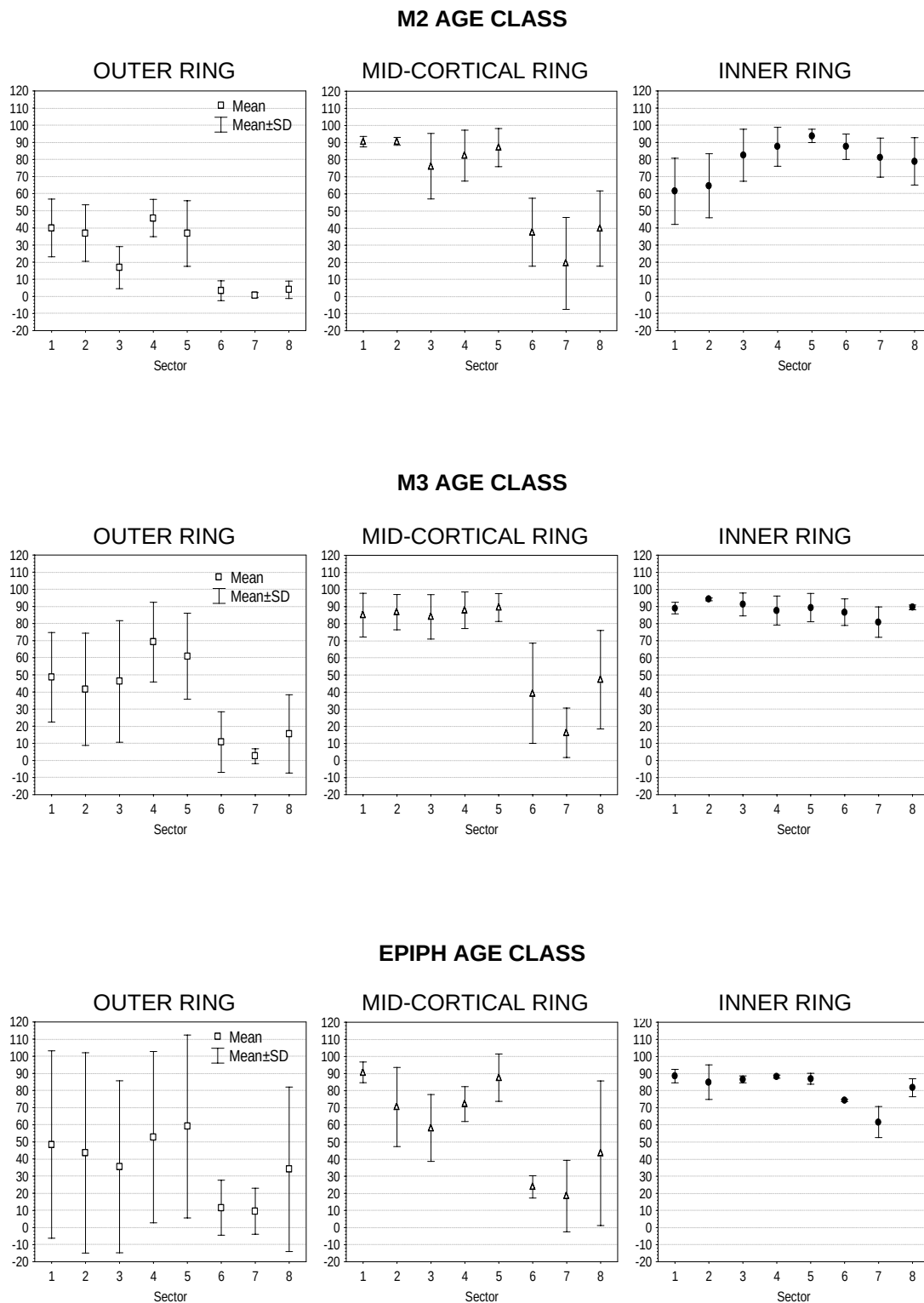
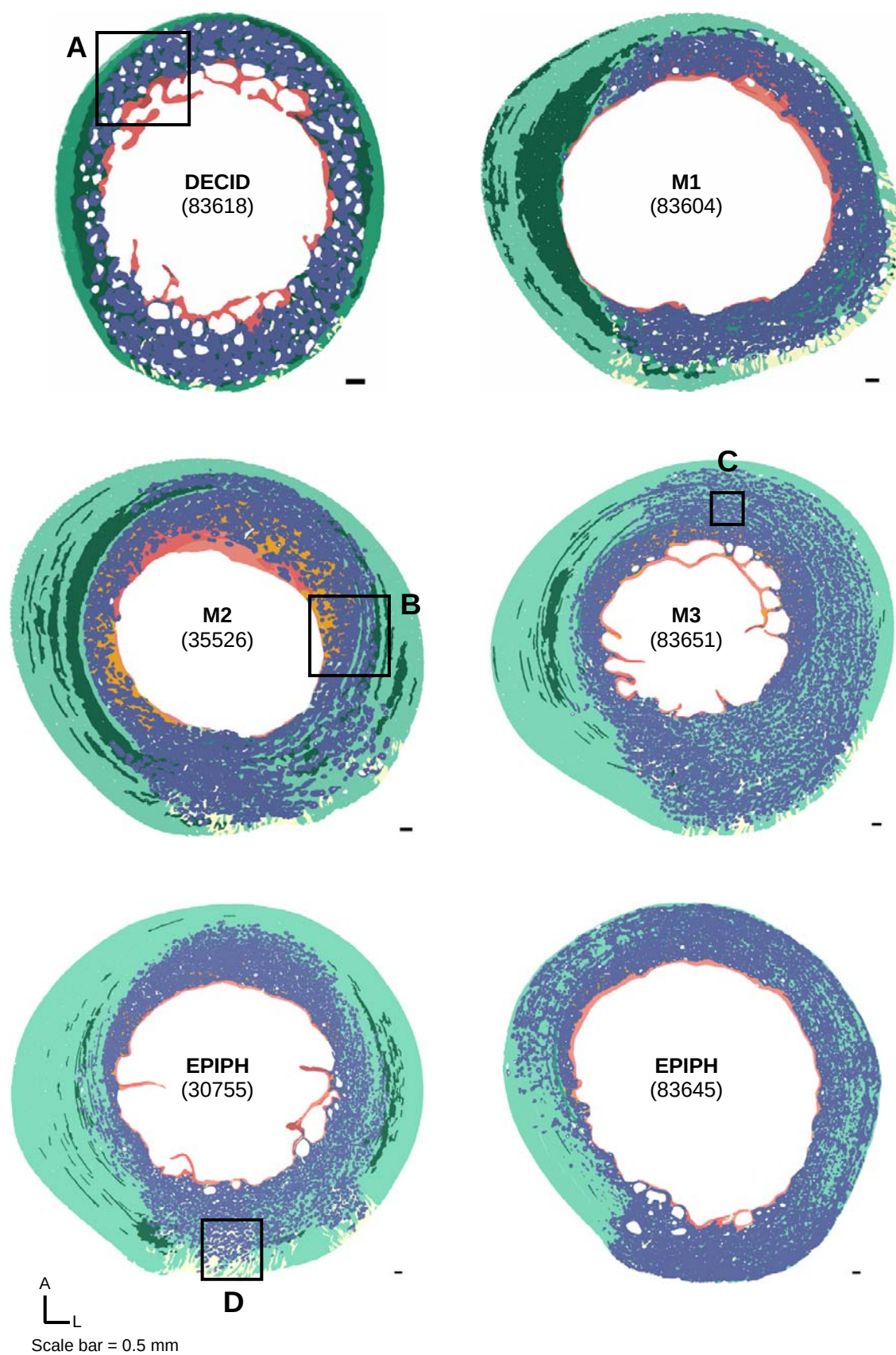


FIGURE 6-20 b cont.. Plots of variation in % HAV across radial sectors by ring, with age classes combined and considered separately: *P. troglodytes* midshaft femur.

FIGURE 6-21 a. Intracortical remodeling at the midshaft femur of *P. troglodytes*: Example tissue maps by age class. Two EIPPH individuals are shown here, to represent the range of variation observed among individuals in this age class. Similar variation in the extent of remodeling is observed in the M3 age class, not depicted here. Bone regions occupied by secondary osteonal bone are mapped in purple, as depicted in Figure 6-1. Other bone tissue types represented here were recognized on the basis of microstructural features described in Chapter 5 of this thesis. Insets show locations of FIGURES 6-21 a - d.



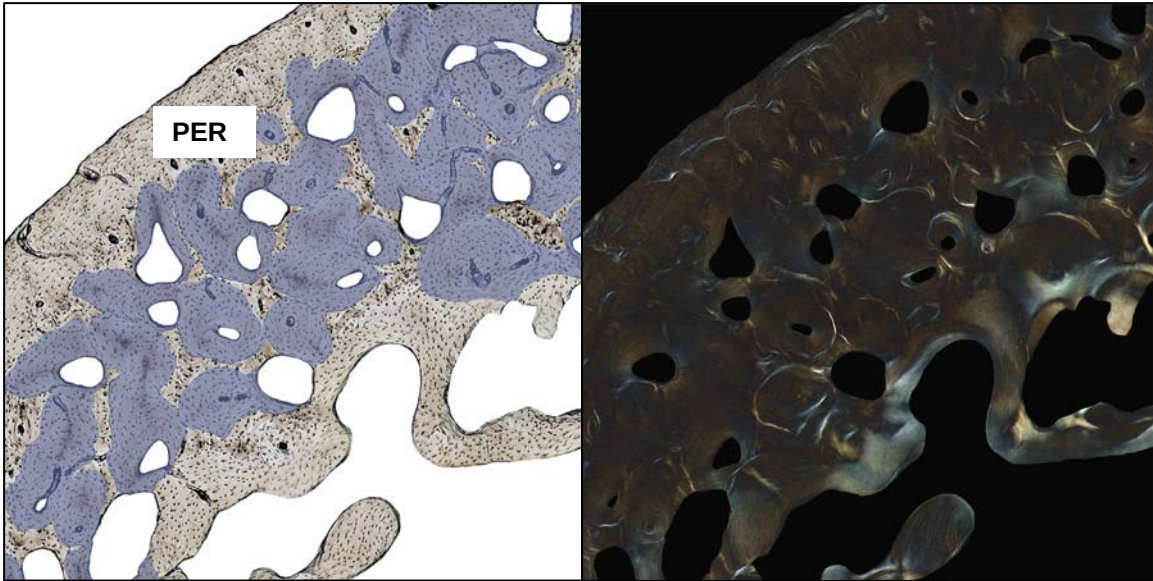


FIGURE 6-21 b. Intracortical remodeling in the midshaft femur of *P. troglodytes* (MNH 83618, DECID age class): Secondary osteons in inner periosteal regions of the anteromedial cortex. *Left:* LM image. *Right:* CPL image. Secondary osteons (mapped in purple) are densely distributed inner periosteal (PER) cortex. Primary tissue to the lower right of this image is endosteal coarse cancellous bone.

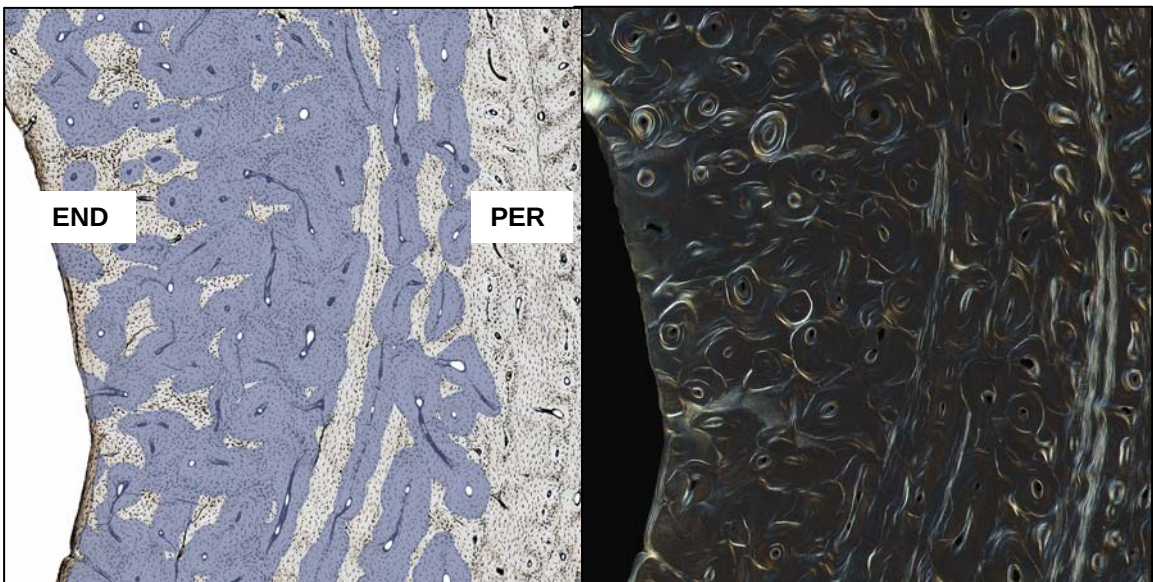


FIGURE 6-21 c. Intracortical remodeling in the midshaft femur of *P. troglodytes* (MNH 35526, M2 age class): Secondary osteons at the endosteal – periosteal boundary in the lateral cortex. *Left:* LM image. *Right:* CPL image. Secondary osteons (mapped in purple) are concentrated at the boundary between endosteal (END) and periosteal (PER) cortex. They are densely-distributed in older regions of the endosteal cortex, where they are commonly associated with interstitial tissues having a compacted coarse cancellous organization. Secondary osteons are also distributed in older and vascular tissues of periosteal origin.

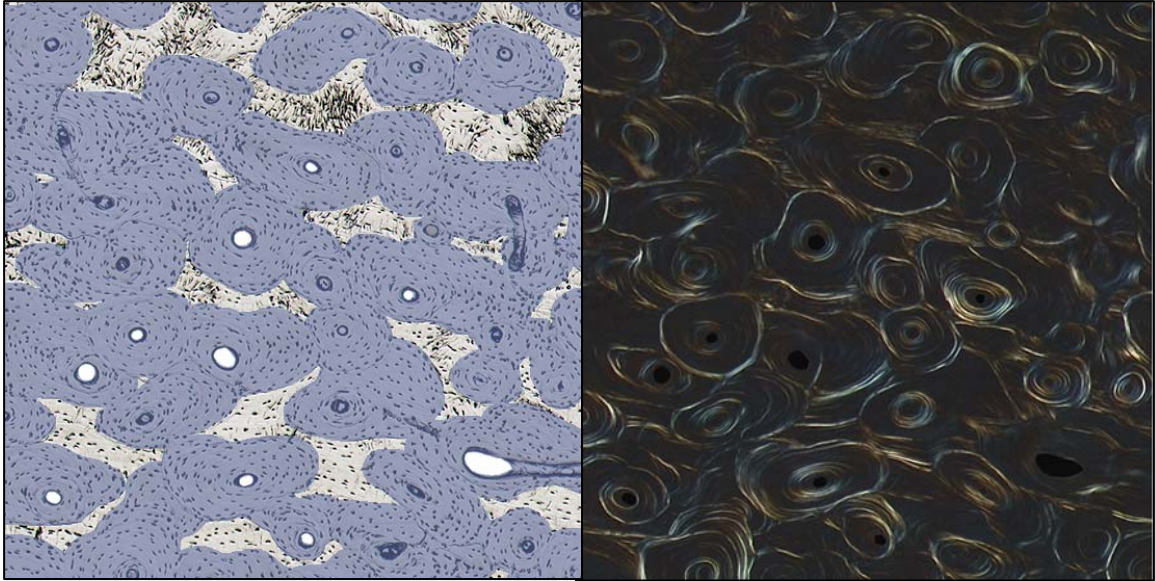


FIGURE 6-21 d. Intracortical remodeling in the midshaft femur of *P. troglodytes* (MNH 83651, M3 age class): Secondary osteons in periosteal regions of the anterior cortex. *Left*: LM image. *Right*: CPL image. Secondary osteons (mapped in purple) are densely distributed throughout the periosteal cortex here, where they are associated with less vascular interstitial bone.

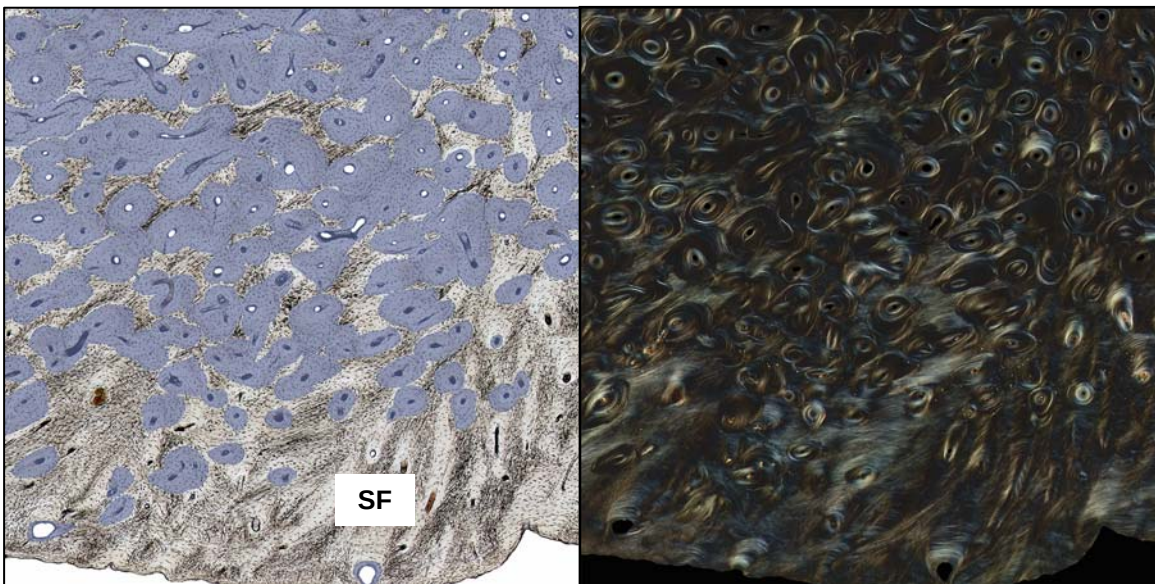


FIGURE 6-21 e. Intracortical remodeling in the midshaft femur of *P. troglodytes* (MNH 30755, EIPPH age class): Secondary osteons and Sharpey's fibers in the posterior cortex. *Left*: LM image. *Right*: CPL image. Secondary osteons (mapped in purple) invade more recently-deposited periosteal tissues here, where they are associated with Sharpey's fibers (SF).

CHAPTER 7.

CONCLUSIONS

This study was motivated by an interest in the significance of bone microstructure for life history investigations. It was designed to provide a baseline understanding of ontogenetic and regional variation in bone microstructural organization in a range of catarrhine taxa, and to consider the manner in which this variation reflects 1) aspects of life history, namely taxon-specific growth patterns, and 2) local mechanically-influenced dynamics of bone growth and remodeling. In ontogenetic series' comprised exclusively of wild-collected individuals, two microstructural features (primary bone tissue types and secondary intracortical remodeling) and one macrostructural feature (cross-sectional geometry) were examined at the midshaft femur and humerus of *Chlorocebus aethiops* and *Hylobates lar*, and at the midshaft femur of *Pan troglodytes*. Important results and their significance are summarized below, followed by a discussion of the broader implications of this research for studies of primate life history, and future directions.

Cross-sectional geometry

The analysis of cross-sectional geometry provided a useful means of quantifying variability in attributes of bone size and shape, which occurs as a consequence of growth and remodeling of the midshaft femur and humerus during ontogeny. First, nonparametric LOWESS regression was used to characterize patterns of growth-related variation in geometric properties and other body dimensions (i.e., bone length and body mass, where possible). Secondly, ontogenetic variation was examined in several cross-

sectional geometric properties describing the distribution of bone material around single cross-sections (i.e., cross-sectional shape), and which are hypothesized to have mechanical relevance: Z_y/Z_x , theta, and $I_{\max}/I_{\min}$. This provided an important context for interpretation of ontogenetic and regional (i.e., within cross-section) variability in primary bone microstructure and secondary intracortical remodeling. Further, as previous studies have focused largely on adults, this research also provides insight into the manner in which adult patterns of diaphyseal geometry are acquired during postnatal ontogeny.

Results of this study were consistent with earlier reports indicating that growth in diaphyseal cross-sectional geometric properties most closely parallels growth in body mass and lags behind growth in bone length. Further, cross-sectional geometric dimensions in the current sample exhibited broadly similar characteristics of postnatal growth as reported previously for other body dimensions (particularly body mass). Observations for *C. aethiops* were consistent with a pattern of bimaturism reported by previous investigators, and males showed evidence of more rapid growth compared to females following M2 emergence. In *H. lar*, males and females showed largely overlapping distributions across age classes for most variables examined. In *P. troglodytes*, small samples precluded assessment of growth in geometric properties in males and females separately. However, ontogenetic variation in geometric properties was consistent with a period of relatively rapid growth between M2 and M3 emergence (and possibly thereafter). Variation in primary bone microstructure examined in Chapter 5 of this thesis was also expected to reflect these growth trends.

Results of analyses of geometric shape variables were consistent with previous

studies based on adults. However, this study also provided new information concerning ontogenetic variability in diaphyseal cross-sectional geometry. Several trends were identified. An age-related increase in relative mediolateral bending rigidity at the midshaft femur and humerus was apparent in most taxa, although this pattern was not always statistically significant. The midshaft femur of *C. aethiops* showed the opposite pattern. Further, in many cases, the orientation of $I_{\max}$ did not correspond to anatomically-defined axes. In *C. aethiops*, age-related trends in diaphyseal proportions were accompanied by little change in the orientation of $I_{\max}$, while considerable variability in $I_{\max}$ orientation was observed within and among age classes of *H. lar* and *P. troglodytes*. With the exception of the midshaft femur of *H. lar*, observations in the latter taxa reflect a mediolateral shift in the location of $I_{\max}$ from a more antero-posterior orientation in the DECID age classes, and a consequent increase in relative mediolateral bending rigidity that appeared most marked during early ontogeny.

Observations of ontogenetic and taxonomic variability in measures of diaphyseal shape provided an important context for understanding regional variability in bone microstructural features examined in following analyses. The significance of these results for skeletal function was also considered. The prevailing pattern of increased mediolateral bending rigidity observed at most skeletal sites examined in the current study is consistent with recent evidence concerning *in vivo* strain patterns and limb kinetics, indicating the importance of mediolateral bending forces generated in the limb skeletons of primates during locomotor behaviors (see Chapter 4). Results also have implications for understanding variability in limb loadings associated with positional behavior ontogeny. Despite the greater variety of positional behaviors practiced by infant

primates compared to older conspecifics, previous behavioral studies suggest that species-typical locomotor preferences emerge very early in development (see Chapter 4). Thus, unidirectional trends in relative mediolateral bending rigidity and $I_{\max}$ orientation may reflect largely quantitative (rather than qualitative) shifts in mechanical loadings as individuals attain locomotor competence and adult body size. However, additional studies characterizing *in vivo* strains associated with locomotor behaviors other than quadrupedalism and involving more variable limb postures are clearly needed to explore this issue further. Studies of both adult and immature primates will also be important.

Primary bone microstructure

Given an empirical relationship between the microscopic organization of primary bone tissues and the rates at which they are deposited, primary bone histo-diversity represents a potentially rich source of information about growth-related aspects of life history. However, primary bone microstructure has remained largely unexplored in recent systematic investigations of primates, with a few exceptions. The current study aimed to 1) describe ontogenetic, regional and taxonomic variability in patterns of primary bone microstructural organization, and 2) consider the potential of this variability (particularly as reflected in proportions of primary periosteal bone tissue types) for discriminating primate growth patterns. Results presented here were meant to provide a baseline understanding of ontogenetic variability in primary bone microstructure in a range of catarrhine taxa, and to establish a foundation from which more informed questions may be addressed in future studies concerning the influence of life history on particular features of the microstructural organization of primary bone.

Quantitative results demonstrated that proportions of primary periosteal bone tissue types vary significantly across age classes within all taxa examined. In accordance with predictions, younger age classes were characterized by higher proportions of tissues associated with faster depositional rates (namely, fibro-lamellar tissue types and/or parallel-fibered bone), while older age classes were characterized by higher proportions of tissues associated with slow depositional rates (i.e., lamellar bone). However, taxa differed in the timing of this transition, and in the particular tissue types observed in individuals of similar biological age (defined on the basis of dental emergence and epiphyseal union). Intraspecific patterns in whole cross-section proportions of primary bone tissue types are summarized below.

In *C. aethiops*, the first postnatal year was characterized by a transition from fibro-lamellar bone to deposition of less-vascularized lamellar bone, the latter characterized by predominantly longitudinal simple vascular canals and small primary osteons. This transition began very early in postnatal ontogeny, before the deciduous (DECID) dentition was fully erupted. Evidence of recent periosteal deposition at the midshaft femur was almost exclusively lamellar at the midshaft femur in individuals having emerged first permanent molars (M1s). There was relatively little change in periosteal tissue type proportions in older age classes. However, while growth in cross-sectional dimensions at the midshaft femur and humerus (Chapter 4) was consistent with bimaturism and slightly elevated growth rates in male vervets as reported previously for body mass and linear dimensions, significant differences among sexes in the proportions of primary bone tissue types were *not* observed. Fibro-lamellar bone was observed in slightly higher proportions at the midshaft humerus during later ontogeny, despite its

greater extent of secondary intracortical remodeling, and this was interpreted to reflect locally faster depositional rates associated with marked posteromedial cortical drift.

In *H. lar*, primary bone tissue type proportions were strongly influenced by metaphyseal growth remodeling in the DECID and M1 age classes. A history of metaphyseal remodeling was indicated by several observations: 1) endosteal bone, most notably compacted coarse cancellous tissue, comprised a considerable proportion of total cortical area; and 2) reversal lines observed in the primary periosteal cortex indicated a prior history of bone resorption at the periosteal surface. These observations aside, the primary periosteal cortex at the midshaft femur and humerus was comprised largely of less-vascularized lamellar-zonal tissues at all stages of ontogeny. Individuals with emergent DECID dentitions and M1s were characterized by a larger proportion of tissues having a parallel-fibered matrix component compared to vervets of similar dental emergence status. A substantial increase in the proportion of lamellar bone was observed in individuals having emergent M2s, and evidence of recent periosteal deposition was predominantly lamellar in all older age classes. Males and females exhibited considerable overlap in tissue type proportions in all age classes.

In *P. troglodytes*, a significant decrease in the proportions of fibro-lamellar and parallel-fibered tissues, and an increase in the proportion of lamellar bone, was observed with age at the midshaft femur. The majority of primary periosteal cortex was comprised of lamellar-zonal tissues in all age classes; these tissues had a parallel-fibered matrix component in the DECID age class, while they were predominantly lamellar in older age classes. However, fibro-lamellar tissues (particularly FLC-LZPO) also comprised a considerable proportion of cortical area, with similar mean proportions observed across

DECID, M1 and M2 age classes. This latter observation can be interpreted to represent a period of relatively rapid bone growth during this time. Qualitative assessment indicated that reticular and/or laminar vascularization of fibro-lamellar bone was common in the M2 age class. Lamellar bone observed in older age classes was vascularized by simple vascular canals and scattered primary osteons.

In sum, when interpreted within the context of local growth and secondary remodeling history (the latter discussed further in Chapter 6), patterns of bone microstructural development (as reflected in the proportions of primary periosteal bone tissue types) observed within and among taxa revealed several broad consistencies with growth patterns reported previously for other body dimensions. In both *C. aethiops* and *H. lar*, the transition to periosteal deposition of predominantly lamellar bone was coincident with body mass growth rates of 1 kg / year or less, reported for captive animals by previous investigators. This transition in bone tissue types occurred much earlier (relative to dental emergence status) in vervets compared to gibbons. Slightly increased proportions of bone tissue types having a parallel-fibered matrix component in the M1 and, to a lesser extent, M2 age classes in gibbons is in general accordance with the observation that body mass growth velocities are above 1 kg/year (and slightly faster than rates of mass growth in vervets) during the first three years of life (Leigh, 1996; Leigh and Shea, 1995). However, lower proportions of fibro-lamellar tissue types in gibbons compared to vervets, particularly in the DECID age class, may be interpreted to reflect a history of metaphyseal growth remodeling at this skeletal site during earlier stages of ontogeny.

Observations of *P. troglodytes* were less easily reconciled with their considerably

faster body mass growth velocities than other taxa examined here (e.g., as reported for captive animals by Leigh, 1996). While the M1 and M2 age classes exhibited higher proportions of fibro-lamellar tissues than observed in individuals of similar dental emergence status in other taxa, the DECID age class of *C. aethiops* exhibited substantially higher proportions of fibro-lamellar tissue types compared to *P. troglodytes* DECID individuals. Several possible explanations were proposed to account for this inconsistency, including the substantially higher proportion of secondary intracortical remodeling observed in *P. troglodytes*, and the possibility that growth rate variability may be reflected in other features of primary bone tissues which were not quantitatively examined in the current study. These features will be discussed in further detail below.

Secondary intracortical remodeling.

As intracortical remodeling is a partially destructive process, characterization of ontogenetic and regional variability in proportions of secondary osteonal bone provided an important context for interpretations of primary bone microstructure. This study had two specific aims: 1) to determine whether proportions of secondary osteonal bone (% HAV) at the midshaft femur and humerus vary within taxa with respect to age during postnatal ontogeny, and 2) to determine whether secondary osteonal bone shows a patterned regional distribution within cross-sections. Tissue age was expected to be an important factor influencing the distribution of intracortical remodeling across circumferential rings within cross-sections, by virtue of its consequences for increased loading cycles and accumulation of bone fatigue. As bone mechanical properties vary considerably in tension and compression, long bones subjected to bending were also

expected to exhibit significant variability in proportions of secondary osteonal bone across radial sectors. As few studies have examined both ontogenetic and regional variation in intracortical remodeling in nonhuman primates, the current study also provides important insight into the manner in which its distribution may reflect local dynamics of bone growth and skeletal function.

First, results indicated that while the absolute area of secondary osteonal bone was positively correlated with age class in all taxa, % HAV showed a weak positive correlation with age only in *C. aethiops*. In light of regional patterns of intracortical remodeling (see below), the lack of a significant age trend in *H. lar* and *P. troglodytes* may reflect differences in the amount of bone resorbed at the endosteal surface during bone growth. This may be particularly true if the rate of accumulation of new secondary osteons slows by the time skeletal maturity is reached, as has been shown previously for other nonhuman primates (see Chapter 6). The proportion of secondary osteonal bone does not provide information about the rate at which new remodeling events are initiated, and the extent to which these differences reflect variation in other parameters of intracortical remodeling warrants further study.

Secondly, results also demonstrated significant variation in % HAV across three circumferential rings and eight radial sectors within cross-sections. Mean proportions were usually higher in the inner and mid-cortical rings compared to the outer ring (the latter usually representing cortical regions of more recent origin), with some exceptions. However, no consistent pattern of intracortical remodeling characterized inner versus mid-cortical regions across all skeletal sites and taxa examined. The midshaft femur of *C. aethiops*, which exhibited the least intracortical remodeling of all taxa and elements

examined, showed consistently higher % HAV in the inner cortical ring. Other taxa and skeletal sites showed a more complicated pattern, with considerable overlap or age-related variability in % HAV among inner and mid-cortical rings. Variation among radial sectors was considered relative to cross-sectional geometric properties (as determined in Chapter 4). In *C. aethiops* and *P. troglodytes*, proportions of secondary osteonal bone were increased in radial sectors generally coinciding with the plane of greatest structural rigidity to bending (i.e., $I_{\max}$). However, the distribution of intracortical remodeling across sectors did not show clear correspondence to structural proportions observed at the midshaft femur and humerus of *H. lar*.

Results suggest that variability in intracortical remodeling within bone cross-sections may be influenced by both developmental and mechanical factors, and at times, these may be difficult to tease apart. Qualitative observations suggest that the distribution of intracortical remodeling across both circumferential rings and radial sectors was strongly influenced by local dynamics of bone remodeling at periosteal and endosteal surfaces during growth. In addition to its preferential distribution in inner and mid-cortical rings noted above, those radial sectors characterized by the highest proportions of secondary osteonal bone were often comprised of periosteal bone tissues interpreted to be of older depositional history. This was most evident in skeletal sites undergoing marked cortical drift, including the *C. aethiops* midshaft humerus, the anterior and adjacent sectors of the *C. aethiops* midshaft femur during early ontogeny, and the lateral cortex of the *P. troglodytes* midshaft femur in the M1-M2 age classes.

Remodeling at periosteal and endosteal surfaces is thought to be a primary mechanism by which mechanical properties of bone are altered in response to variable

loading conditions during growth (see Chapter 2). The above observations suggest that future studies should consider the influence of bone growth remodeling (and its consequences for tissue age) on observed distributions of intracortical remodeling whenever possible. This point has been made by previous researchers as well (e.g., Bouvier and Hylander, 1996; Bromage, 1992; Bromage and Boyde, n.d.; McMahon et al., 1995; Skedros et al., 2001).

In the absence of available *in vivo* strain data, the relationship between aspects of the *in vivo* strain stimulus itself (e.g., strain magnitude or strain mode) and observed distributions of intracortical remodeling could not be directly tested. The observation that % HAV was higher in the posterior cortex at the midshaft femur in all taxa suggests that forces associated with muscle pull may have a significant influence on the distribution of intracortical remodeling (but see below), in addition to other strains generated during locomotor behaviors. Future studies would benefit from examination of the distribution of preferred collagen fiber orientation, as a source of information about the prevailing strain mode conditions under which intracortical remodeling was initiated (e.g., Ascenzi and Bonucci, 1967; Ascenzi and Bonucci, 1968, 1972; Ascenzi et al., 1973; Simkin and Robin, 1974).

Qualitative observations also indicated that local circumstances of bone growth remodeling may also influence secondary osteon distributions in ways that are not related in an obvious manner to tissue age or mechanical strain history, in accordance with earlier proposals by Enlow (Enlow, 1962a, 1963, 1976; Hoyte and Enlow, 1966). First, developmental origin (e.g., primary tissue type), particularly relating to endosteal growth and compaction of coarse cancellous bone, appeared to have an important influence on

the distribution of secondary osteons in growing bone. Secondary osteons were distributed not only in older periosteal regions, but they were frequently concentrated at the boundary between cortical regions of endosteal and periosteal origin. Intracortical remodeling was often prevalent in endosteal regions, where it was commonly observed in regions of compacted coarse cancellous bone. Secondly, secondary osteons were commonly observed in association with Sharpey's fibers at muscle attachment sites. While this pattern may have mechanical significance, as mentioned above, intracortical remodeling may also provide a mechanism for maintaining muscle attachments during growth, particularly on resorptive bone surfaces. Future studies of intracortical remodeling and its significance for skeletal function should consider these factors whenever possible. Such factors may contribute to intra-skeletal and taxonomic variability in secondary intracortical remodeling, independently of differences in the mechanical loading environment.

Broader implications for life history research and future directions

A major focus of evolutionary approaches to the study of primate life history has been on anatomical features that can be observed in mineralized tissues, and which can be used to infer schedules of growth and maturation in once-living animals in paleontological contexts. Mineralized tissues provide an important perspective as they are known to record a *history* of their growth, rather than the developmental status of an individual at the time of death. To date, such efforts in anthropology have focused largely on aspects of dental development. Research on other vertebrate groups has demonstrated the potential of primary bone microstructure as another important source of

life history information in both modern and paleontological contexts. Despite the promising results of this work, however, the significance of primary bone microstructure for interpreting aspects of growth and life history in primates has been surprisingly unexplored in systematic studies, with a few exceptions (Castanet et al., 2004; Enlow, 1962b; Herrmann and Danielmeyer, 1994; Newell-Morris and Sirianni, 1982). The current study was designed to address this issue. Results presented here suggest that investigations of primary bone microstructure hold great promise for life history investigations of primates. Further, this study identified a number of avenues to be explored in future research.

Results of this study demonstrated considerable variability in primary bone microstructure in several catarrhine taxa characterized by differences in growth-related aspects of life history. All taxa examined here were characterized by age-related transition from rapidly-forming and loosely-organized tissue types (e.g., fibro-lamellar bone) observed during early growth stages, to slowly-forming and more highly ordered tissue types (e.g., lamellar bone) observed during later growth stages. Interestingly, however, taxa differed in the timing of this transition, and in the particular primary bone tissue types deposited at different stages of ontogeny. As described above, this intra- and interspecific variability revealed several broad consistencies with expectations based on growth patterns reported previously for other body dimensions (namely, body mass). In other cases, however, correspondence of quantitative variability in primary bone tissue type proportions with expectations was not observed.

In some cases, mixed support for predictions of the current study reflected complications associated with histological investigations of primary bone tissues more

generally. As also shown in this study, intra-skeletal and taxonomic variability in local patterns of bone growth (e.g., metaphyseal remodeling and cortical drift) and secondary remodeling may have an important influence on observed proportions of primary bone tissue types. It is also interesting to note, however, that even in those taxa exhibiting more extensive secondary intracortical remodeling, this remodeling showed a highly-patterned distribution within cross-sections. Broad expanses of primary periosteal bone (often encompassing the entire width of the periosteal cortex) were left *in tact*, and thus were amenable to study. Growth and secondary remodeling processes are recognizable on the basis of histological features described by previous investigators (see Chapter 2), and thus, their influence on interpretations of growth – related aspects of life history from bone can and should be considered whenever possible (also see Ricqlès, 1993). Future studies would benefit from detailed examinations across a broader range of skeletal sites, to improve our understanding of the manner in which local dynamics of bone growth and remodeling vary both intra-skeletally and taxonomically. Such studies would also benefit by incorporating serial sectioning protocols, to better understand how whole bone remodeling changes during the growth process determine bone microstructural organization at a local level (e.g., Enlow, 1962b; Enlow, 1963).

Qualitative observations suggested that variations in bone growth rates may be discriminated from features of primary bone microstructure not explicitly measured, and which cannot be appreciated from the classification of primary bone tissues into more broadly-defined tissue ‘types’ as was done in the current study. Variability in several features of primary bone could be visually appreciated, and may contribute to our understanding of the relationship between primary bone microstructure and life history.

The current study identified several variables which warrant attention in future studies:

Vascular patterns. While bimaturism and slightly elevated male growth rates in *C. aethiops* (as revealed in diaphyseal cross-sectional dimensions, and reported by other researchers for body mass growth) were not revealed in the quantitative analysis of primary bone tissue types, sex differences in the overall vascularity of primary periosteal tissues were visually apparent. Age-related variation in features of the primary vascular network was also observed in *P. troglodytes*. In this latter taxon, zones of highly-vascularized tissue were observed to alternate with less vascular bone layers (and, in some cases, growth marks) in some individuals, resulting in a distinctively repetitive microstructural pattern locally. As suggested by recent vital-labeling studies of other vertebrate groups (e.g., Castanet et al., 2000; Margerie et al., 2002), such changes in characteristics of the primary vascular network (particularly as reflected in the size, density, and possibly orientation, of vascular canals) likely have significance for discriminating subtle or temporary variations in bone depositional rates during periods of relatively slow or fast overall growth.

Growth marks: annuli and resting lines. Annuli and resting lines (the latter commonly referred to as “lines of arrested growth”) were observed in individuals of all taxa examined here. These structures varied in their number and strength among individuals, and they may also vary in relation to age (i.e., they often appeared more numerous in older compared to younger individuals). In some individuals, similarities in the number and distribution of resting lines across elements (i.e., at the midshaft femur and humerus) could also be appreciated. The significance of growth marks for discriminating life history parameters (e.g., growth rates, longevity) and age at death has

been most widely recognized by investigators of other vertebrate groups. A recent study of *Microcebus murinus* (Castanet et al., 2004) suggests that similar studies of other primates hold great promise.

Collagen fiber orientation of primary bone. In *H. lar*, in which the primary periosteal cortex was largely comprised of slow-forming lamellar-zonal tissue types, temporary or short-term variations in bone depositional rates were suggested by apparently repetitive changes in the polarization contrast of lamellar bone. A similar microstructural pattern was reported in a recent study of alligator femora (Lee, 2004). This observation is interesting, given that the preferred orientation of collagen fibers in bone is primarily understood in relation to its significance for mechanical properties of bone (see Chapter 5). However, it is in keeping with suggestions of other researchers that collagen orientation in primary bone tissues (as opposed to secondarily-remodeled bone) may be determined in part by developmental constraints associated with circumstances of bone growth remodeling, including the particular tissue type within which collagen orientation is established (e.g., Bromage and Boyde, n.d.).

Future quantitative examinations of these features may provide insight into the manner in which specific characteristics of primate ontogenies (e.g., sex differences in growth rates or duration, occurrence of a subadult growth spurt), not revealed by investigation of primary tissue ‘types’ alone, may be interpreted from bone histology. Such studies would benefit tremendously by incorporation of conspecifics from other geographic areas as well as *known* individuals, thus making it possible to address questions concerning the manner in which environment or specific events in life history (e.g., weaning or stress) may influence bone growth, and thus be recorded in

microstructural features. Comparisons with other mineralized tissues (e.g., enamel; Dirks et al., 2002; Macho et al., 1996) would also be particularly interesting in this latter respect, and may yield insight into the relative sensitivities of different mineralized tissues in recording aspects of individual life histories.

Finally, future studies would benefit considerably by expanding the taxonomic range of this sample. Primates as a group exhibit considerable diversity in their pre- and postnatal ontogenies, and this diversity is suggested to underlie important differences in life history (e.g., Leigh, 1996; Leigh, 2001; Leigh and Blomquist, 2007). Thus, primates provide a wealth of opportunities to address many of the questions raised above. Incorporation of more controlled contrasts among closely-related species (*e.g.*, including gorillas, siamangs, and patas monkeys) differing in body size and life history would allow for more focused studies of the relationship between specific characteristics of postnatal ontogenies (e.g., growth rates, growth durations) and bone microstructural organization, independently of local mechanical influences on bone growth and development (*i.e.*, associated with species differences in positional behavior). A more broadly comparative approach would also make it possible to begin addressing questions concerning the influence of phylogeny on patterns of bone microstructure. The current study provides an important foundation for such work, and results presented here demonstrate the potential of bone microstructural analyses as an independent line of inquiry into primate life history in both modern and paleontological contexts.

APPENDIX A.

MECHANICAL PROPERTIES OF BONE

The abilities of bone to resist functional loading can be characterized using basic engineering principles derived from the study of mechanics, which are reviewed here. Structures, such as whole bones, can be subjected to three modes of loading - compression, tension and shear – although most loading regimes involve a combination of these. (Figure A-1). The amount of deformation produced by loading can be graphically represented in the form of a load-deformation curve, which is divided into two regions. (1) In the region of *elastic deformation*, the relationship between load and deformation is linear and the structure returns to its original shape after loading. The slope of this region of the curve is referred to as the *stiffness* or *rigidity* of the structure. (2) The relationship between load and deformation is non-linear in the region of *plastic deformation*, and there is irreversible deformation in the structure after the applied load is removed. The point at which plastic deformation begins is referred to as the *yield point*. If the load continues to increase, fracture will eventually occur at the *failure point* (Martin et al., 1998; Turner and Burr, 1993). (Figure A-2).

The concepts of stress and strain are central to considerations of the mechanical properties of bone as a material. *Stress* refers to the magnitude of the load applied, and is defined as the force per unit area. *Strain* refers to deformation in the tissue under an applied load, and is usually defined as a proportional change in length or some other dimension. The relationship between stress and strain can be depicted as a stress-strain curve, which is similar to the load-deformation curve. The *elastic* or *Young's modulus* is

the slope of the stress-strain curve in the region of elastic deformation, and it represents a measure of material *stiffness*. The reciprocal of stiffness is *compliance*. The *yield stress* is the stress at the yield point, while the *failure stress* is the stress at failure. Materials are said to be *ductile* if they undergo a large amount of post-yield deformation before fracture. The opposite is true for *brittle* materials. The area under the stress-strain curve represents the energy required to fracture the structure, and is referred to as the *toughness* (Currey, 1990; Martin et al., 1998; Turner and Burr, 1993).

When bone is loaded cyclically (i.e., repetitively), the result is a process referred to as *fatigue*. Fatigue involves a gradual decline in elastic modulus and yield strength over time, associated with the gradual accumulation of microscopic damage which is most evident at the light microscope level in the form of linear microcracks. The pattern of material property degradation varies according to loading mode (i.e., tension and compression). However, if fatigue damage is allowed to accumulate, bone may eventually progress to fatigue failure at stresses lower than the failure stress under monotonic loading. Thus, the ability of bone to prevent or repair the accumulation of fatigue microdamage has an important influence on bone strength (Martin et al., 1998; Turner and Burr, 1993).

Bone *strength* is often more generally defined as its ability to withstand loading without failure. However, in analyses of mechanical properties, strength is usually defined more specifically as the stress (or load) at the yield or failure points (Martin et al., 1998; Turner and Burr, 1993). Currey (Currey, 1999, 2002, 2003) has discussed the importance of *stiffness* as a determinant of bone strength, and the trade-off between *stiffness* and *toughness* in optimizing resistance to failure. The measures of bone strength

discussed above are determined by: (1) *structural* properties, particularly the amount and distribution of bone (e.g., cross-sectional geometry); and (2) *material* properties, which are dependent on the microscopic organization and composition of bone (e.g., porosity, mineralization, collagen fiber orientation, fatigue damage) (Martin, 1993; Martin et al., 1998). Changes in the structural and material properties of bone are accomplished by growth remodeling and secondary intracortical remodeling, respectively.

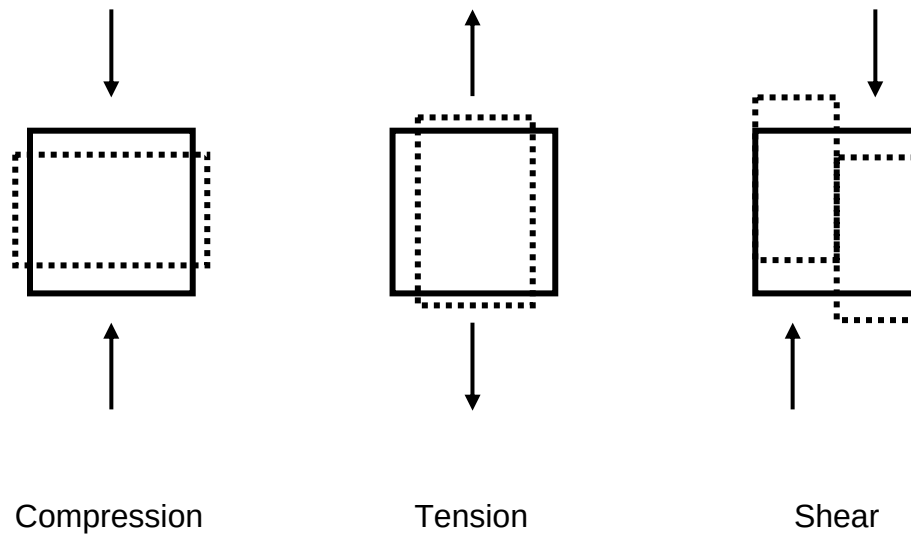


FIGURE A-1. Modes of loading.

Compressive loading results in the shortening of a structure along the axis of loading, although it is often stretched in the direction perpendicular to this axis. Tensile loading results in the opposite pattern. Shear loading results in the sliding of one region of a structure relative to another region.

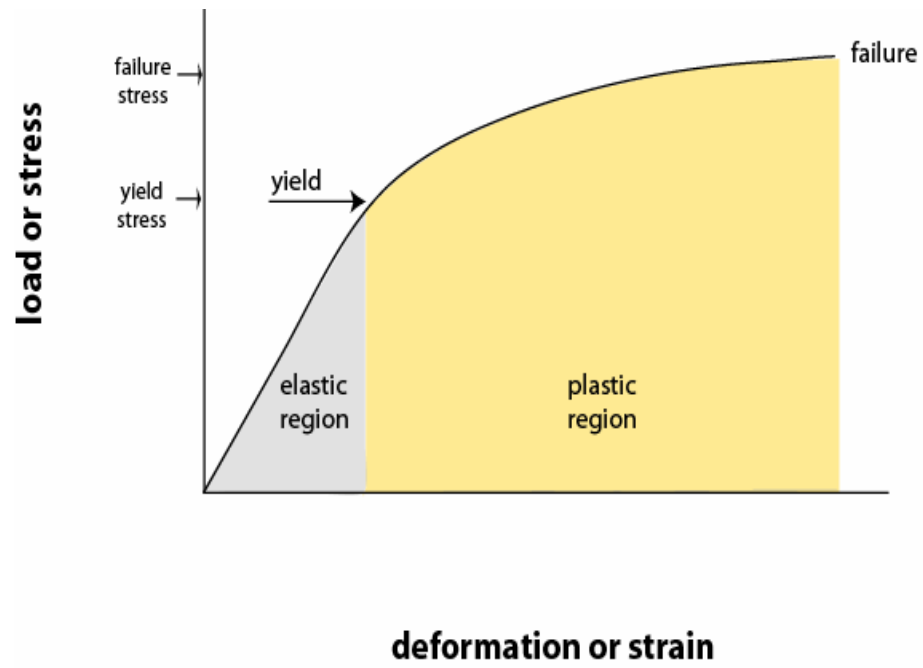


FIGURE A-2. Stress – strain curve.

APPENDIX B.
SAMPLE DATA

TABLE B-1. Summary sample data.

ID#	LOCALITY (Collection notes)	DATE	COLLECTOR	SEX	ELEMENTS	AGE CLASS	BODY MASS (g)	FEM LGTH (mm)	HUM LGTH (mm)
<i>Chlorocebus aethiops</i> (SLW collection)									
62	northwest Uganda	1947	Washburn	M	RFEM, LHUM	DECID	453	47.8	41.0
92	northwest Uganda	1947	Washburn	M	RFEM, RHUM	DECID	340	49.0	43.2
76	northwest Uganda	1947	Washburn	F	RFEM, RHUM	DECID	907	76.3	65.2
27	northwest Uganda	1947	Washburn	M	RFEM, RHUM	DECID	907	68.6	57.5
33	northwest Uganda	1947	Washburn	M	RFEM, RHUM	DECID	1077	69.9	60.2
21	northwest Uganda	1947	Washburn	F	RFEM, RHUM	M1	1474	87.0	72.0
11	northwest Uganda	1947	Washburn	F	RFEM, LHUM	M1	1814	89.2	73.3
37	northwest Uganda	1947	Washburn	F	RFEM, RHUM	M1	1930	91.1	78.5
47	northwest Uganda	1947	Washburn	F	RFEM, LHUM	M1	1701	91.6	77.0
96	northwest Uganda	1947	Washburn	F	RFEM, RHUM	M1	1701	88.4	74.5
73	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M1	1814	91.7	79.3
34	northwest Uganda	1947	Washburn	F	RFEM, LHUM	M2	2041	99.5	85.2
35	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M2	2268	102.8	83.3
72	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M2	2268	112.3	94.2
74	northwest Uganda	1947	Washburn	F	RFEM	M2	2268	106.8	n.d.
78	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M2	3629	120.3	96.8
80	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M2	2722	110.4	87.3
94	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M2	4082	127.1	104.7
14	northwest Uganda	1947	Washburn	F	RFEM, RHUM	M3	3402	120.7	101.5
15	northwest Uganda	1947	Washburn	F	RFEM, RHUM	M3	2948	113.0	95.3
60	northwest Uganda	1947	Washburn	F	RFEM	M3	3402	113.4	n.d.
70	northwest Uganda	1947	Washburn	F	RFEM, RHUM	M3	3969	120.5	98.0
13	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M3	3856	126.5	107.7

TABLE B-1 continued. Summary sample data.

ID#	LOCALITY (from collection notes)	DATE	COLLECTOR	SEX	ELEMENTS	AGE CLASS	BODY MASS (g)	FEM LGTH (mm)	HUM LGTH (mm)
68	northwest Uganda	1947	Washburn	M	RFEM, LHUM	M3	5443	135.3	114.7
91	northwest Uganda	1947	Washburn	M	RFEM, RHUM	M3	4490	139.8	108.5
101	northwest Uganda	1947	Washburn	M	RFEM, RHUM	n.d. ¹	n.d.	129.7	107.5
16	northwest Uganda	1947	Washburn	F	RFEM, RHUM	EIPPH	3515	111.0	93.3
25	northwest Uganda	1947	Washburn	F	RFEM, RHUM	EIPPH	2608	114.3	92.2
43	northwest Uganda	1947	Washburn	F	RFEM, RHUM	EIPPH	3629	119.7	97.3
18	northwest Uganda	1947	Washburn	M	RFEM, RHUM	EIPPH	5443	137.5	113.5
64	northwest Uganda	1947	Washburn	M	RFEM, RHUM	EIPPH	7258	149.0	119.0
75	northwest Uganda	1947	Washburn	M	RFEM, RHUM	EIPPH	5670	136.2	113.2
<i>Hylobates lar</i> (MCZ collection)									
41462	Chiengmai region, Thailand	1937	A. P. E.	F	RFEM, RHUM	DECID	680	91.2	104.7
41466	Chiengmai region, Thailand	1937	A. P. E.	F	RFEM, RHUM	DECID	680	78.8	93.8
35456	Chiengmai region, Thailand	1937	A. P. E.	M	RFEM, RHUM	DECID	680	95.0	111.3
36380	Chiengmai region, Thailand	1937	A. P. E.	M	RFEM, RHUM	DECID	680	100.8	118.2
41482	Chiengmai region, Thailand	1937	A. P. E.	F	RFEM, RHUM	M1	1361	111.5	126.0
41502	Chiengmai region, Thailand	1937	A. P. E.	F	RFEM, RHUM	M1	1814	132.0	152.2
41444	Chiengmai region, Thailand	1937	A. P. E.	M	LFEM, RHUM	M1	1361	127.7	148.5
41452	Chiengmai region, Thailand	1937	A. P. E.	F	RFEM, RHUM	M2	2722	153.2	179.5
41498	Chiengmai region, Thailand	1937	A. P. E.	M	RFEM, RHUM	M2	2041	135.7	160.8
41473	Chiengmai region, Thailand	1937	A. P. E.	F	RFEM, RHUM	M2	2948	155.8	169.2
41461	Chiengmai region, Thailand	1937	A. P. E.	M	RFEM, RHUM	M2	4082	172.8	200.2
41491	Chiengmai region, Thailand	1937	A. P. E.	M	RFEM, RHUM	M2	3175	160.0	189.5
41429	Chiengmai region, Thailand	1937	A. P. E.	F	LFEM, LHUM	M3	4309	182.8	210.8
41475	Chiengmai region, Thailand	1937	A. P. E.	F	LFEM, LHUM	M3	4082	167.7	192.2

TABLE B-1 continued. Summary sample data.

ID#	LOCALITY (from collection notes)	DATE	COLLECTOR	SEX	ELEMENTS	AGE CLASS	BODY MASS (g)	FEM LGTH (mm)	HUM LGTH (mm)
<i>Hylobates lar</i> (SLW collection)									
6	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	M2	n.d.	184.3	218.0
32	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	M3	n.d.	204.0	238.0
37	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	M3	n.d.	178.3	206.8
38	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	M3	n.d.	170.0	198.2
39	Chiengmai region, Thailand	1937	Washburn	M	RFEM, LHUM	M3	n.d.	189.0	205.3
5	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	M3	n.d.	195.5	230.5
10	Chiengmai region, Thailand	1937	Washburn	F	RFEM, RHUM	n.d. ¹	n.d.	181.5	208.8
35	Chiengmai region, Thailand	1937	Washburn	F	RFEM, RHUM	n.d. ¹	n.d.	179.8	208.8
11	Chiengmai region, Thailand	1937	Washburn	F	RFEM, RHUM	EPIPH	n.d.	185.3	214.5
30	Chiengmai region, Thailand	1937	Washburn	F	RFEM, RHUM	EPIPH	n.d.	175.5	206.5
4	Chiengmai region, Thailand	1937	Washburn	F	RFEM, RHUM	EPIPH	n.d.	178.3	206.5
16	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	EPIPH	n.d.	187.0	204.0
26	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	EPIPH	n.d.	177.8	206.2
31	Chiengmai region, Thailand	1937	Washburn	M	RFEM, RHUM	EPIPH	n.d.	186.5	224.2
<i>Pan troglodytes</i> (MNH collection)									
83614	Kamerun: Vogebeinga?; Lokundje		Jacob	F	RFEM	DECID	n.d.	99.3	n.d.
83618	Kamerun: Longji	1907	Paschen	n.d.	RFEM, RHUM	DECID	n.d.	95.3	97.3
83604	Kamerun: Bipindi	1906	Zenker	n.d.	RFEM, RHUM	M1	n.d.	146.8	153.3
32052	Kongo, Gabun usw (außer Kamerun) + Fundort die z. zt. Nicht auffb.: in Matadi an Bord genommen	1921	Hagenbeck-G.	F	RFEM, RHUM	M2	n.d.	178.5	183.0
83673	Kamerun: 8 Std. östl. V. Lolodorf, Ukalla Nsama	1907	Jacob	M	RFEM, RHUM	M2	n.d.	176.7	188.7
83643	Kamerun: Molundu	1910	v.d. Marwitz	n.d.	RFEM	M2	n.d.	178.8	n.d.
35526	Kamerun: zw. Kribi u. Yaunde		Dr. Lacroix V.	n.d.	RFEM, RHUM	M2 ²	n.d.	209.3	213.2
83685	Kongo, Gabun usw (außer Kamerun) + Fundort die z. zt. Nicht auffb.: Mayumba	1903	Hesselbarth	F	RFEM, RHUM	M3	n.d.	237.7	249.7

TABLE B-1 continued. Summary sample data.

ID#	LOCALITY (from collection notes)	DATE	COLLECTOR	SEX	ELEMENTS	AGE CLASS	BODY MASS (g)	FEM LGTH (mm)	HUM LGTH (mm)
17011	Kongo, Gabun usw (außer Kamerun) + Fundort die z. zt. Nicht auffb.: westl. Singa	1914	v. Ramsay	F	RFEM, RHUM	M3	n.d.	246.7	251.5
83651	Kamerun: Bipindi	1900	Zenker	F	RFEM	M3	n.d.	223.5	n.d.
83619	Kamerun: Süd wald zw. Japoma und Edea	1911	Schäfer	n.d.	RFEM, RHUM	EIPPH	n.d.	228.2	244.5
83645	Kamerun: südl. V. Bipindi	1907	Zenker	n.d.	RFEM, LHUM	EIPPH	n.d.	273.5	298.0
30755	Kamerun: Ajos-Höhe, Akonolinga	1920	Reichenow	M	LFEM, LHUM	EIPPH	n.d.	271.1	269.1

n.d. = no data available

¹ These individuals were assigned to the M3 age class for analysis, based on epiphyseal union and diaphyseal length.

² This individual was questionably associated with a skull having only permanent M1s erupted. It was reassigned to the M2 age class based on bone length.

APPENDIX C.

RECONSTRUCTION PROTOCOL

Impression and Epoxy Materials

Exaflex Injection Type (Low-viscosity) hydrophilic vinyl polysiloxane impression material– base and catalyst

Exaflex Putty Type O (Very high viscosity) hydrophilic vinyl polysiloxane impression material – base and catalyst

RS Epoxy Resin Two part quick set

Other materials

Putty knife

Blunt-tip scissors or scalpel

Cotton balls

Plastic-tip tweezers

Compressed air

Osteometric board with a ¼” groove cut down the center

center

Reconstruction Protocol

Molding the area to be removed

1. The midshaft region, encompassing the area to be removed, is coated with low-viscosity, high-resolution Exaflex injection type silicone impression material and allowed to cure.
2. To ensure that the orientation and dimensions of the bone are maintained in the reconstruction, a low-resolution mold spanning the inferior aspect of the bone is produced. The bone is gently pressed into a bed of Exaflex high viscosity silicone putty. This bed does not completely enclose the bone, but rather rises up approximately halfway around it, to facilitate easy removal of the bone after curing. The lower aspect of the high viscosity Exaflex mold is pressed into a ¼” groove cut down the center of an osteometric board, to create a longitudinal “lip” that can later be used for realignment of the mold.
3. After the high-resolution mold has cured (step 1), and is now bonded below to the low-resolution mold (step 2), it is slit open with blunt-tip scissors. Using plastic-tipped tweezers, the mold is gently eased away from the bone, and the bone is removed from the mold for sectioning.

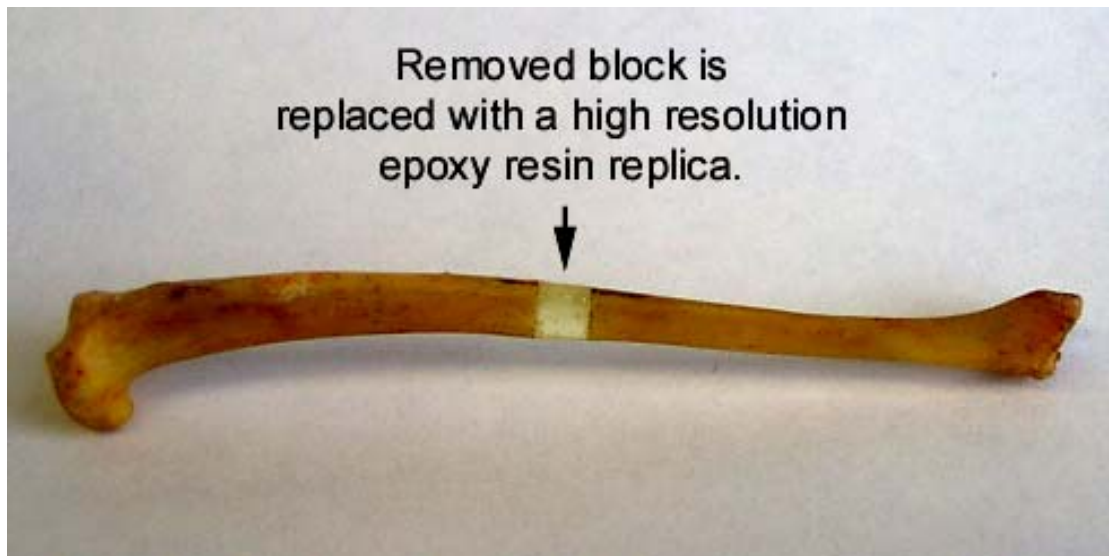
Reconstruction, following sectioning

4. The mold is re-aligned on the osteometric board, by re-fitting its undersurface into the ¼ inch groove.
5. The two ends of the bone are re-fit back into the mold.
6. High-resolution epoxy resin is injected into the mold in the region spanning the removed block. An initial thin layer of epoxy is blown (using compressed air) into the mold, to ensure accurate replication of microscopic surface topography.

The epoxy replica is allowed to cure over the course of one hour, after which time the epoxy replica has adhered to the cut ends of the bone.

7. Following cure, the bone is carefully removed from the mold. It is now reconstructed to its original disposition and dimensions.

FIGURE C-1. Image of a sample bone reconstruction.



APPENDIX D.

IMAGING AND IMAGE EDITING PROCEDURES

Non-Quantitative Imaging in CPL

1. The microscope was configured with 5X objective and a CPL filter arrangement.
2. Illumination intensity was adjusted.
 - a. In Syncroscopy Montage Explorer:
 - i. White balance was performed. No shade or color correction.
 - ii. Illumination was adjusted until the green color band approached a 5-250 range of values, assuming the presence of longitudinal and transverse collagen fibrils, respectively.
 - iii. From this point, it was necessary to increase the illumination of the specimen in order to illuminate dark areas of the section.

Imaging in LM

1. The quarter wave and linear polarizing filters were swung out of position.
2. The aperture was set to position “2”.
3. In Syncroscopy Montage Explorer:
 - a. The lighting and Contour was adjusted (between 30-60) so that important microstructural features (e.g., cement lines) were clearly discernable.
 - b. White balance, shade and color correction were performed.

Image Editing and Creation of Binary Image Masks in Adobe Photoshop

1. On the LM image, editing out of cracks in the medium and other background artifacts was done using the ‘Magic Eraser’ function (threshold value of 40) and manual editing of small features at 100%.
2. Resorption bays, large Haversian canals, and other large non-bone spaces were filled in with the hard round ‘Eraser’ tool (size 19 pixels) at 100%.
3. The image was converted to binary using the ‘Threshold’ function, such that the bone area appeared black and the background appeared white.
4. A ‘Dust & Scratches’ filter was applied (radius 2 pixels).
5. The resulting image “mask” was saved as a .TIFF file, and used for calculation of cross-sectional geometric properties.
6. This mask was overlain on the original LM image to produce an edited version of the LM image.
 - a. Select – Color range (white pixels)
 - b. Copy
 - c. Paste onto the original LM image, using the Layers function.
 - d. Save as .TIFF file.
7. This mask was Inverted in Photoshop, so that non-bone areas were black. This inverted mask was then overlaid on the original CPL image using the procedures outlined in step 6 above.

Image Orientation

As described in Chapter 4, the anterior orientation was recorded on the bone block prior to sample removal and chemical preparation. Bone blocks were mounted with their proximal surface up and with their anterior cortex (and the anterior orientation mark) positioned at the top of the slide. A second anterior orientation mark was also made on the undersurface of the slide itself, so that section orientation after imaging could be verified using adjacent histological details.

Because there was occasional rotation of the block prior to curing of the dental adhesives as it was affixed to the slide, section orientation was verified in acquired images using histological details.

1. All digital images were reduced (scaled to fit paper size) and printed.
2. As per our original method of establishing orientation prior to removal of bone samples, the anterior-posterior plane was reconstructed on the digital (printed) image:
 - a. A graph overlay (printed on overhead material) was positioned over the image so that 1) the Y axis passed through the anterior orientation mark, and 2) the farthest medial and lateral extents of the periosteal margin of the cross-section was equidistant from the Y axis.
 - b. In this way, the anteroposterior axis was positioned such that it passed through the anterior orientation point and the midpoint of the medio-lateral axis of the cross-section.
3. The digital image was then re-aligned in Adobe Photoshop (Rotate function and Guides), using adjacent histological details.
4. Left side bones were horizontally-flipped, to preserve a common orientation for later image quantification.

Manual mapping of primary and secondary bone tissue types in Adobe Photoshop

LM and CPL images were imported into Adobe Photoshop 6.0, where they were superimposed for visualization purposes and for the creation of bone tissue type maps.

1. As described above, a binary overlay was created from the original LM image, on which most non-bone areas (e.g., medullary canals, large resorption spaces, large cracks and other preparation artifacts, but not small vascular canals and Haversian spaces) were deleted for later quantitative analysis.
2. Areas representing distinct primary and secondary bone tissue types were manually traced and color-coded on separate layers, using the 'Layers' function.
3. Qualitative observations (e.g., concerning growth remodeling patterns, growth arrest lines, vascular patterns) were annotated on a separate layer.
4. The binary image overlay was inverted, so that non-bone areas were assigned to black.

Creation of flattened tissue type maps for Optimas Image Analysis software.

The following procedures were carried out on tissue type maps created in Adobe Photoshop 6.0. Only those color-coded layers representing different bone tissue types were selected for the creation of composite tissue maps from which quantitative data were collected.

1. Anterior orientation was recorded on the image by a small white point, for later orientation of radial segments.
2. The tissue map with layers was reduced to 25% (*C. aethiops* and *H. lar*) or 12.5% (*P. troglodytes*) of original pixel dimensions.
3. To eliminate the anti-aliasing (i.e., fuzzy borders) effect along color boundaries, each color layer was re-filled with the appropriate color using the Fill or Paint Bucket tool, with the following options:
 - a. Anti-alias unchecked
 - b. Contiguous unchecked
 - c. The threshold value was adjusted such that color boundaries were flat.
4. Layers were flattened.
5. The canvas was enlarged to allow sufficient space between the periosteal bone margin and the image edge for radial segmentation.
6. The image was converted to grayscale, and saved for later import into Optimas Image Analysis system.

Editing out cracks and trabeculae, where necessary.

Cracks which open to the endosteal or periosteal surface and large trabeculae were edited out of the processed image, as they created problems in the identification of the endosteal outline in Optimas software. Cracks were closed at the surface and trabeculae were cut off using a black line 2 pixels in width.

APPENDIX E.

CROSS-SECTIONAL GEOMETRY: RAW DATA AND DESCRIPTIVE STATISTICS (Chapter 4)

TABLE E-1 a. Cross-sectional geometry raw data: *Chlorocebus aethiops*. NIH Image (MomentMacro) output.

ID #	TA	CA	Xbar	Ybar	Ix	Iy	Imax	Imin	Theta	Zx	Zy	Max x rad	Max y rad
<i>Chlorocebus aethiops</i>: FEMUR													
11	29.12	16.50	3.23	3.24	53.87	56.12	59.96	50.03	-38.47	16.88	17.83	3.15	3.19
13	53.20	33.38	4.12	4.58	214.77	173.67	216.35	172.09	-79.09	45.24	43.37	4.00	4.75
14	51.78	25.38	4.33	4.38	154.91	160.71	167.14	148.48	-35.95	37.21	38.40	4.19	4.16
15	53.79	30.13	4.54	4.39	162.43	209.85	210.96	161.33	-8.60	38.25	47.05	4.46	4.25
16	57.33	29.02	4.43	4.75	214.64	182.86	217.21	180.29	-74.70	42.06	42.42	4.31	5.10
18	67.19	45.99	4.82	4.88	328.43	319.52	346.23	301.72	-50.77	67.43	68.53	4.66	4.87
21	29.36	16.72	3.18	3.31	57.46	53.78	60.12	51.12	-57.11	17.83	17.58	3.06	3.22
25	47.79	26.50	4.02	4.30	152.51	137.75	154.67	135.59	-70.34	35.92	35.33	3.90	4.25
27	23.67	13.09	3.05	2.86	32.80	38.86	39.35	32.31	-15.29	12.14	13.26	2.93	2.70
33	23.73	14.22	2.99	2.88	34.92	40.89	42.41	33.40	-24.25	12.66	14.23	2.87	2.76
34	37.20	21.34	3.85	3.48	86.54	94.70	97.25	83.99	-25.99	23.77	25.41	3.73	3.64
35	51.18	23.35	4.29	4.40	156.29	137.78	158.89	135.17	-70.64	36.16	33.66	4.09	4.32
37	35.70	16.24	3.89	3.73	67.10	76.62	80.89	62.82	-29.10	19.46	21.45	3.57	3.45
43	52.37	32.29	4.12	4.44	192.98	179.55	202.17	170.37	-57.49	44.44	44.26	4.06	4.34
47	25.96	17.62	3.01	2.96	46.45	50.67	53.74	43.38	-32.99	15.78	17.05	2.97	2.94
60	49.98	25.90	4.17	4.26	146.47	159.31	165.61	140.17	-29.83	35.59	39.34	4.05	4.12
62	11.80	6.81	2.01	2.12	8.86	9.33	9.54	8.65	-28.99	4.42	4.69	1.99	2.00
64	100.96	56.40	6.08	5.95	678.14	626.13	682.29	621.97	-74.79	112.91	104.98	5.96	6.01
68	77.48	42.67	5.08	5.40	414.12	356.44	421.62	348.94	-71.26	70.93	72.00	4.95	5.84
70	64.63	32.24	5.02	4.74	253.47	245.41	264.69	234.19	-52.67	52.69	52.45	4.68	4.81
73	36.72	17.72	3.84	3.74	78.68	79.64	88.38	69.95	-43.51	21.65	22.16	3.59	3.64
74	43.88	24.76	4.08	4.11	127.63	121.55	136.77	112.40	-52.22	31.37	31.85	3.82	4.07
75	66.48	47.86	4.79	5.03	345.30	306.45	354.09	297.66	-66.75	70.24	65.65	4.67	4.92
76	27.09	14.00	3.21	3.12	42.38	48.68	51.89	39.17	-30.14	14.25	15.50	3.14	2.97
78	58.81	29.00	4.68	4.55	202.03	206.17	210.05	198.15	-34.81	43.62	45.84	4.50	4.63
80	42.61	25.25	3.80	3.92	124.71	117.74	128.14	114.31	-60.13	30.27	31.46	3.74	4.12

TABLE E-1 a continued. Cross-sectional geometry raw data: *Chlorocebus aethiops*.

ID #	TA	CA	Xbar	Ybar	Ix	Iy	Imax	Imin	Theta	Zx	Zy	Max x rad	Max y rad
91	74.40	44.43	4.99	5.01	368.55	367.87	390.97	345.44	-45.42	72.82	75.27	4.89	5.06
92	12.65	6.50	2.12	2.29	10.23	9.21	10.67	8.77	-61.32	4.72	4.56	2.02	2.17
94	69.24	38.08	4.86	4.98	321.85	291.31	335.43	277.73	-60.98	62.44	60.95	4.78	5.15
96	33.50	15.88	3.44	3.58	69.24	61.50	73.78	56.96	-58.70	18.91	18.52	3.32	3.66
101	59.91	31.53	4.39	4.67	227.25	211.47	228.67	210.04	-73.96	49.44	47.73	4.43	4.60
<i>Chlorocebus aethiops</i>: HUMERUS													
11	25.43	14.93	3.10	2.83	38.77	47.88	51.30	35.35	27.57	14.01	15.57	3.07	2.77
13	50.28	28.82	4.22	4.45	173.91	168.42	221.22	121.12	46.57	40.01	39.72	4.24	4.35
14	56.14	26.28	4.61	4.77	177.96	202.50	245.38	135.08	38.57	38.63	41.49	4.88	4.61
15	52.40	28.55	4.69	4.40	147.89	214.35	225.27	136.97	20.60	35.05	45.46	4.72	4.22
16	48.47	25.11	4.34	4.09	135.03	165.69	189.26	111.46	33.39	33.67	36.16	4.58	4.01
18	60.19	43.42	4.74	4.87	269.42	307.04	381.67	194.79	39.19	57.44	60.56	5.07	4.69
21	30.63	15.43					68.35	44.87					
25	46.75	24.87	4.07	4.26	136.87	145.11	173.05	108.94	41.31	33.70	34.00	4.27	4.06
27	24.23	13.39	2.93	2.98	36.05	39.60	45.27	30.37	38.11	12.50	12.85	3.08	2.88
33	22.25	13.24	2.94	2.75	30.65	36.70	41.12	26.23	33.01	11.55	12.27	2.99	2.65
34	33.44	19.25	3.55	3.30	65.10	83.43	89.72	58.81	26.81	20.34	22.78	3.66	3.20
35	37.90	19.58	3.96	3.82	81.34	98.86	109.63	70.57	31.67	21.78	25.64	3.86	3.73
37	29.81	15.40	3.84	3.01	47.28	72.81	85.15	34.95	29.72	16.01	19.27	3.78	2.95
43	52.52	31.54	4.44	4.49	170.79	213.98	233.59	151.17	29.20	39.05	45.69	4.68	4.37
47	27.32	15.93	3.19	3.09	46.73	54.54	62.46	38.81	35.37	15.42	16.53	3.30	3.03
62	11.46	7.06	1.83	2.04	9.77	8.33	10.81	7.29	57.10	4.87	4.32	1.93	2.01
64	94.13	54.86	6.39	5.37	473.52	749.89	793.60	429.81	20.28	91.21	115.90	6.47	5.19
68	72.03	38.35	5.35	4.77	274.34	391.49	411.67	254.17	20.97	58.75	72.23	5.42	4.67
70	51.00	27.22	4.77	3.89	127.88	207.22	210.39	124.70	11.09	34.46	44.78	4.63	3.71
73	35.52	19.13	3.69	3.43	76.60	87.81	105.16	59.24	37.93	22.36	23.56	3.73	3.43
74	32.12	15.91	3.37	3.28	57.91	66.97	75.39	49.50	34.76	18.12	17.89	3.74	3.20
75	68.87	46.44	5.13	5.06	327.56	374.60	446.62	255.53	37.87	66.29	74.11	5.05	4.94
76	26.43	13.57	3.17	2.99	37.21	49.67	53.33	33.55	25.47	12.78	14.18	3.50	2.91
78	52.93	26.70	4.76	4.07	144.72	203.29	218.67	129.34	24.52	36.06	43.57	4.67	4.01
80	38.98	21.58	3.80	3.74	94.63	104.19	122.88	75.94	39.12	26.11	27.55	3.78	3.62

TABLE E-1 a continued. Cross-sectional geometry raw data: *Chlorocebus aethiops* humerus.

ID #	TA	CA	Xbar	Ybar	Ix	Iy	Imax	Imin	Theta	Zx	Zy	Max x rad	Max y rad
91	66.32	40.54	5.60	4.64	242.99	377.85	396.27	224.58	19.12	53.54	68.99	5.48	4.54
92	12.14	6.47	2.07	1.99	8.60	9.70	9.99	8.32	24.37	4.41	4.63	2.09	1.95
94	57.19	34.69	4.34	4.92	240.68	209.46	278.30	171.84	53.53	49.77	47.82	4.38	4.84
96	30.54	16.03	3.47	3.24	54.86	64.74	79.95	39.65	37.91	17.35	18.18	3.56	3.16
101	63.69	37.41	4.80	5.09	282.12	257.16	315.45	223.83	52.91	56.12	55.18	4.66	5.03

TABLE E-1 b. Cross-sectional geometry raw data: *Hylobates lar*. NIH Image (MomentMacro) output.

ID #	TA	CA	Xbar	Ybar	Ix	Iy	Imax	Imin	Theta	Zx	Zy	Max x rad	Max y rad
<i>Hylobates lar</i> : FEMUR													
5	74.52	42.03	5.19	4.94	348.16	363.10	366.24	345.02	22.60	68.68	72.38	5.02	5.07
6	86.63	48.58	5.69	5.41	473.98	492.99	504.16	462.81	31.31	88.74	89.74	5.49	5.34
10	73.70	47.92	5.30	4.92	358.48	403.74	410.72	351.50	20.08	75.38	78.98	5.11	4.76
11	72.01	51.84	4.84	4.92	383.15	380.00	393.97	369.18	48.66	74.18	75.98	5.00	5.17
16	87.34	66.71	5.72	5.27	523.69	631.16	646.38	508.47	19.40	101.78	112.14	5.63	5.15
26	88.00	55.45	5.89	5.20	487.50	581.64	582.48	486.66	-5.36	93.02	102.48	5.68	5.24
30	76.38	47.07	5.26	4.81	368.15	425.36	425.36	368.15	-0.18	71.39	81.33	5.23	5.16
31	79.71	47.08	5.17	5.28	421.50	423.25	452.64	392.11	44.17	80.59	80.93	5.23	5.23
32	76.00	53.30	5.20	4.83	398.48	441.98	443.72	396.75	11.07	77.57	85.90	5.15	5.14
35	64.52	47.73	4.76	4.62	307.10	310.72	311.45	306.37	22.37	66.42	67.66	4.59	4.62
37	73.23	53.95	5.14	4.74	385.28	412.12	412.26	385.14	4.12	73.80	82.11	5.02	5.22
38	68.51	46.41	4.93	4.76	338.19	333.12	338.21	333.10	86.43	72.38	69.90	4.77	4.67
39	74.15	44.38	4.91	5.09	371.57	360.21	372.81	358.97	72.60	74.77	74.49	4.84	4.97
4	83.28	54.89	5.31	5.50	495.81	486.58	543.56	438.82	47.53	87.57	90.90	5.35	5.66
35456	15.50	8.09	2.26	2.30	14.34	14.90	15.09	14.15	26.64	6.38	6.38	2.33	2.25
36380	19.01	11.36	2.44	2.73	25.51	21.61	25.59	21.53	82.07	9.69	9.14	2.36	2.63
41429	66.62	43.30	4.74	4.71	307.78	312.19	329.80	290.17	41.81	63.14	65.74	4.75	4.87
41444	28.06	17.29	3.14	3.03	51.17	55.12	55.34	50.95	-12.91	17.32	18.01	3.06	2.95
41452	48.91	31.50	4.10	4.03	166.24	164.72	171.16	159.80	-48.82	39.28	40.72	4.05	4.23
41461	62.40	40.67	4.62	4.58	277.84	265.49	280.49	262.83	67.17	62.25	58.64	4.53	4.46
41462	16.94	9.80	2.25	2.47	19.88	17.26	20.10	17.03	74.24	8.23	7.53	2.29	2.41
41466	14.66	7.68	2.13	2.23	13.36	12.86	13.60	12.62	60.16	5.94	5.91	2.18	2.25
41473	46.83	29.81	4.07	4.00	153.58	148.65	154.42	147.81	-69.09	39.82	38.37	3.87	3.86
41475	58.22	32.02	4.43	4.27	213.46	216.33	226.04	203.75	-41.29	45.52	49.57	4.36	4.69
41482	24.55	14.64	2.88	2.85	38.28	41.57	41.58	38.27	2.74	13.78	14.36	2.90	2.78
41491	48.70	29.72	4.12	3.91	158.94	160.72	165.13	154.53	40.17	38.43	39.95	4.02	4.14
41498	31.35	17.41	3.31	3.19	61.95	63.47	64.18	61.24	29.40	19.07	19.23	3.30	3.25
41502	28.89	20.99	3.08	3.24	64.82	57.32	64.95	57.19	-82.46	19.78	19.10	3.00	3.28

TABLE E-1 b continued. Cross-sectional geometry raw data: *Hylobates lar*.

ID #	TA	CA	Xbar	Ybar	Ix	Iy	Imax	Imin	Theta	Zx	Zy	Max x rad	Max y rad
<i>Hylobates lar</i>: HUMERUS													
4	84.36	49.30	5.82	5.00	412.05	531.00	535.40	407.65	10.69	82.19	92.73	5.73	5.01
5	70.38	37.06	5.06	4.73	295.08	310.45	316.40	289.14	27.84	60.75	63.98	4.85	4.86
6	84.19	38.80	5.82	5.27	396.58	405.52	421.30	380.80	38.62	71.28	71.87	5.64	5.56
10	77.29	45.24	5.34	4.98	372.55	417.53	421.24	368.84	15.43	74.03	78.53	5.32	5.03
11	67.44	43.86	4.82	4.51	308.74	325.70	325.72	308.72	-2.05	64.68	66.69	4.88	4.77
16	83.78	53.81	5.38	5.13	476.29	501.70	522.17	455.82	33.74	86.85	92.64	5.42	5.48
26	93.79	53.33	5.63	5.72	542.95	602.94	610.97	534.92	18.96	96.92	107.97	5.58	5.60
30	79.66	40.84	5.38	5.02	369.73	400.12	403.28	366.56	17.06	72.08	76.15	5.25	5.13
31	77.93	42.70	5.19	5.09	381.88	387.79	403.41	366.25	40.42	73.69	73.51	5.28	5.18
32	75.69	51.29	5.09	4.97	409.72	408.12	422.69	395.16	46.67	81.26	80.18	5.09	5.04
35	70.75	46.54	5.16	4.76	324.61	381.20	386.70	319.11	16.57	69.90	76.23	5.00	4.64
37	73.35	50.03	5.16	4.96	380.83	390.56	407.83	363.57	38.65	76.77	77.77	5.02	4.96
38	71.26	43.28	4.94	4.83	325.01	360.52	363.53	321.99	-15.64	66.75	70.69	5.10	4.87
39	79.96	43.53	5.56	5.06	372.66	428.04	428.53	372.16	5.38	73.54	79.34	5.40	5.07
35456	18.01	8.30	2.28	2.61	21.47	15.42	21.48	15.42	88.97	8.08	6.70	2.30	2.66
36380	18.87	9.97	2.38	2.67	24.39	18.28	24.39	18.28	-89.24	9.03	7.87	2.32	2.70
41429	70.29	39.84	4.84	4.95	314.12	318.85	330.07	302.91	39.98	65.49	64.16	4.97	4.80
41444	28.79	17.87	2.87	3.22	64.17	48.02	64.25	47.95	86.09	19.23	17.10	2.81	3.34
41452	49.51	30.15	4.25	4.06	154.82	172.64	178.10	149.37	25.83	38.89	41.83	4.13	3.98
41461	64.58	40.42	4.81	4.67	269.30	296.04	298.37	266.96	-15.82	59.46	63.14	4.69	4.53
41462	22.08	11.59	2.43	2.90	36.61	23.87	36.84	23.64	82.42	12.10	10.07	2.37	3.03
41466	17.30	8.25	2.19	2.64	19.90	14.96	20.66	14.20	69.97	7.72	6.82	2.19	2.58
41475	61.05	33.75	4.73	4.10	209.05	266.72	266.96	208.81	3.66	46.41	53.58	4.98	4.50
41482	28.39	15.21	3.30	2.90	49.14	50.59	50.81	48.93	19.66	15.42	15.74	3.21	3.19
41491	50.99	29.95	4.02	4.28	176.97	162.19	178.25	160.91	-74.21	42.32	40.95	3.96	4.18
41498	30.89	17.19	3.25	3.04	61.71	60.74	66.61	55.84	47.60	18.77	19.28	3.15	3.29
41502	27.25	19.75	3.11	3.03	53.97	53.06	55.58	51.44	51.31	17.98	17.66	3.01	3.00

TABLE E-1 c. Cross-sectional geometry raw data: *Pan troglodytes* femur. NIH Image (MomentMacro) output.

ID #	TA	CA	Xbar	Ybar	Ix	Iy	Imax	Imin	Theta	Zx	Zy	Max x rad	Max y rad
<i>Pan troglodytes</i>: FEMUR													
17011	351.02	256.06	11.13	10.58	8467.74	9533.05	9564.98	8435.80	9.68	812.45	865.96	11.01	10.42
30755	506.66	367.19	13.59	12.41	17111.05	20701.21	20701.29	17110.98	0.26	1397.18	1542.04	13.42	12.25
32052	176.90	119.48	7.96	7.72	2136.68	2217.89	2276.28	2078.28	-32.89	279.07	283.58	7.82	7.66
35526	201.72	153.09	8.64	8.04	2816.25	3323.64	3457.10	2682.80	-24.53	364.98	393.75	8.44	7.72
83604	164.31	96.36	7.78	7.12	1609.01	1945.55	1990.20	1564.36	-18.89	230.15	245.00	7.94	6.99
83614	86.42	41.46	5.31	5.45	441.67	410.43	444.90	407.20	72.98	81.11	79.05	5.19	5.45
83618	67.81	36.64	4.44	5.12	323.33	242.18	323.35	242.16	89.08	64.64	55.98	4.33	5.00
83643	229.32	164.72	8.89	9.30	4019.80	3663.93	4256.89	3426.85	-57.69	447.50	429.64	8.53	8.98
83645	417.95	234.73	11.76	11.89	11434.75	11051.58	12244.98	10241.35	-50.51	963.38	943.05	11.72	11.87
83651	315.63	252.28	11.09	9.99	7002.70	8215.96	8258.16	6960.50	10.39	712.69	757.44	10.85	9.83
83673	198.05	153.42	8.43	7.85	2740.02	3160.72	3186.65	2714.09	-13.55	341.04	373.96	8.45	8.03
83685	362.90	250.34	11.31	11.07	9179.97	9677.84	9696.67	9161.13	10.81	847.80	877.68	11.03	10.83

TABLE E-2 a. Cross-sectional geometry descriptive statistics by age class and sex: *Chlorocebus aethiops*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>DECID females</u>							<u>DECID females</u>					
Body mass	1	907.000					1	907.000				
Bone length	1	76.250					1	65.200				
TA	1	27.090					1	26.430				
CA	1	14.000					1	13.570				
MA	1	13.090					1	12.860				
lmax	1	51.890					1	53.330				
lmin	1	39.170					1	33.550				
lmax/lmin	1	1.325					1	1.590				
Theta	1	-30.140					1	25.470				
J	1	91.060					1	86.880				
Z _x	1	14.250					1	12.780				
Z _y	1	15.500					1	14.180				
Z _y /Z _x	1	1.088					1	1.110				
Z _p	1	29.807					1	27.108				
<u>DECID males</u>							<u>DECID males</u>					
Body mass	4	694.250	680.000	340.00	1077.00	353.768	4	694.250	680.000	340.00	1077.00	353.768
Bone length	4	58.825	58.790	47.80	69.92	12.060	4	50.475	50.350	41.00	60.20	9.775
TA	4	17.963	18.160	11.80	23.73	6.634	4	17.520	17.195	11.46	24.23	6.660
CA	4	10.155	9.950	6.50	14.22	4.070	4	10.040	10.150	6.47	13.39	3.790
MA	4	7.808	7.830	4.99	10.58	2.663	4	7.480	7.340	4.40	10.84	2.966
lmax	4	25.493	25.010	9.54	42.41	17.818	4	26.798	25.965	9.99	45.27	19.013
lmin	4	20.783	20.540	8.65	33.40	13.947	4	18.053	17.275	7.29	30.37	11.960
lmax/lmin	4	1.202	1.217	1.10	1.27	0.070	4	1.435	1.487	1.20	1.57	0.161
Theta	4	-32.463	-26.620	-61.32	-15.29	20.060	4	38.148	35.560	24.37	57.10	13.849
J	4	46.275	45.550	18.19	75.81	31.757	4	44.850	42.830	18.10	75.65	30.959
Z _x	4	8.485	8.430	4.42	12.66	4.527	4	8.333	8.210	4.41	12.50	4.285
Z _y	4	9.185	8.975	4.56	14.23	5.281	4	8.518	8.450	4.32	12.85	4.676
Z _y /Z _x	4	1.061	1.077	0.97	1.12	0.068	4	1.007	1.039	0.89	1.06	0.081
Z _p	4	17.696	17.368	9.12	26.93	9.831	4	16.879	16.535	9.06	25.38	8.974

TABLE E-2 a continued. Cross-sectional geometry descriptive statistics by age class and sex: *Chlorocebus aethiops*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>M1 females</u>							<u>M1 females</u>					
Body mass	5	1724.000	1701.000	1474.00	1930.00	168.889	5	1724.000	1701.000	1474.00	1930.00	168.889
Bone length	5	89.450	89.170	87.00	91.58	1.893	5	75.060	74.500	72.00	78.50	2.663
TA	5	30.728	29.360	25.96	35.70	3.860	5	28.746	29.810	25.43	30.63	2.287
CA	5	16.592	16.500	15.88	17.62	0.654	5	15.544	15.430	14.93	16.03	0.446
MA	5	14.136	12.640	8.34	19.46	4.433	5	13.202	14.410	10.50	15.20	2.106
lmax	5	65.698	60.120	53.74	80.89	11.215	5	69.442	68.350	51.30	85.15	13.567
lmin	5	52.862	51.120	43.38	62.82	7.364	5	38.726	38.810	34.95	44.87	4.008
lmax/lmin	5	1.239	1.239	1.18	1.30	0.053	4	1.878	1.813	1.45	2.44	0.442
Theta	5	-43.274	-38.470	-58.70	-29.10	13.776	4	32.643	32.545	27.57	37.91	4.812
J	5	118.562	111.240	97.12	143.72	18.494	5	94.828	101.270	46.52	120.10	30.390
Z _x	5	17.772	17.830	15.78	19.46	1.493	4	15.698	15.715	14.01	17.35	1.385
Z _y	5	18.486	17.830	17.05	21.45	1.739	4	17.388	17.355	15.57	19.27	1.654
Z _y /Z _x	5	1.041	1.056	0.98	1.10	0.056	4	1.109	1.092	1.05	1.20	0.068
Z _p	5	36.279	35.427	32.87	40.94	3.083	4	33.239	33.796	29.67	35.69	2.933
<u>M1 males</u>							<u>M1 males</u>					
Body mass	1	1814.000					1	1814.000				
Bone length	1	91.670					1	79.300				
TA	1	36.720					1	32.120				
CA	1	17.720					1	15.910				
MA	1	19.000					1	16.210				
lmax	1	88.380					1	75.390				
lmin	1	69.950					1	49.500				
lmax/lmin	1	1.263					1	1.523				
Theta	1	-43.510					1	34.760				
J	1	158.320					1	124.880				
Z _x	1	21.650					1	18.120				
Z _y	1	22.160					1	17.890				
Z _y /Z _x	1	1.024					1	0.987				
Z _p	1	43.798					1	35.991				
<u>M2 females</u>							<u>M2 females</u>					
Body mass	2	2154.500	2154.500	2041.00	2268.00	160.513	1	2041.000	2041.000	2041.00	2041.00	

TABLE E-2 a continued. Cross-sectional geometry descriptive statistics by age class and sex: *Chlorocebus aethiops*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
Bone length	2	103.165	103.165	99.50	106.83	5.183	1	85.200	85.200	85.20	85.20	
TA	2	40.540	40.540	37.20	43.88	4.723	1	33.440	33.440	33.44	33.44	
CA	2	23.050	23.050	21.34	24.76	2.418	1	19.250	19.250	19.25	19.25	
MA	2	17.490	17.490	15.86	19.12	2.305	1	14.190	14.190	14.19	14.19	
lmax	2	117.010	117.010	97.25	136.77	27.945	1	89.720	89.720	89.72	89.72	
lmin	2	98.195	98.195	83.99	112.40	20.089	1	58.810	58.810	58.81	58.81	
lmax/lmin	2	1.187	1.187	1.16	1.22	0.042	1	1.526	1.526	1.53	1.53	
Theta	2	-39.105	-39.105	-52.22	-25.99	18.547	1	26.810	26.810	26.81	26.81	
J	2	215.210	215.205	181.24	249.18	48.041	1	148.530	148.530	148.53	148.53	
Z _x	2	27.570	27.570	23.77	31.37	5.374	1	20.340	20.340	20.34	20.34	
Z _y	2	28.630	28.630	25.41	31.85	4.554	1	22.780	22.780	22.78	22.78	
Z _y /Z _x	2	1.042	1.042	1.02	1.07	0.038	1	1.120	1.120	1.12	1.12	
Z _p	2	56.172	56.172	49.18	63.16	9.884	1	43.303	43.303	43.30	43.30	
M2 males							M2 males					
Body mass	4	3175.250	3175.500	2268.00	4082.00	827.974	5	2993.800	2722.000	2268.00	4082.00	823.879
Bone length	4	115.125	115.335	102.75	127.08	10.715	5	93.260	94.200	83.30	104.70	8.352
TA	4	55.460	54.995	42.61	69.24	11.322	5	44.504	38.980	35.52	57.19	9.833
CA	4	28.920	27.125	23.35	38.08	6.542	5	24.336	21.580	19.13	34.69	6.521
MA	4	26.540	28.820	17.36	31.16	6.271	5	20.168	18.320	16.39	26.23	4.109
lmax	4	208.128	184.470	128.14	335.43	91.345	5	166.928	122.880	105.16	278.30	77.652
lmin	4	181.340	166.660	114.31	277.73	73.481	5	101.386	75.940	59.24	171.84	47.746
lmax/lmin	4	1.141	1.148	1.06	1.21	0.065	5	1.651	1.620	1.55	1.78	0.085
Theta	4	-56.640	-60.555	-70.64	-34.81	15.314	5	37.354	37.930	24.52	53.53	10.749
J	4	389.470	351.130	242.45	613.16	164.422	5	268.316	198.820	164.41	450.14	125.301
Z _x	4	43.123	39.890	30.27	62.44	13.989	5	31.216	26.110	21.78	49.77	11.845
Z _y	4	42.978	39.750	31.46	60.95	13.548	5	33.628	27.550	23.56	47.82	11.207
Z _y /Z _x	4	0.999	1.008	0.93	1.05	0.056	5	1.091	1.055	0.96	1.21	0.101
Z _p	4	86.135	79.675	61.69	123.50	27.488	5	64.994	53.735	45.92	97.64	22.880
M3 females							M3 females					
Body mass	4	3430.250	3402.000	2948.00	3969.00	418.096	3	3439.667	3402.000	2948.00	3969.00	511.541
Bone length	4	116.905	116.960	113.00	120.70	4.271	3	98.267	98.000	95.30	101.50	3.109
TA	4	55.045	52.785	49.98	64.63	6.577	3	53.180	52.400	51.00	56.14	2.657

TABLE E-2 a continued. Cross-sectional geometry descriptive statistics by age class and sex: *Chlorocebus aethiops*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
CA	4	28.413	28.015	25.38	32.24	3.322	3	27.350	27.220	26.28	28.55	1.141
MA	4	26.633	25.240	23.66	32.39	4.023	3	25.830	23.850	23.78	29.86	3.490
lmax	4	202.100	189.050	165.61	264.69	46.725	3	227.013	225.270	210.39	245.38	17.560
lmin	4	171.043	154.905	140.17	234.19	42.989	3	132.250	135.080	124.70	136.97	6.606
lmax/lmin	4	1.186	1.156	1.13	1.31	0.085	3	1.716	1.687	1.64	1.82	0.090
Theta	4	-31.763	-32.890	-52.67	-8.60	18.211	3	23.420	20.600	11.09	38.57	13.955
J	4	373.140	343.955	305.78	498.88	88.802	3	359.267	362.240	335.10	380.46	22.826
Z _x	4	40.935	37.730	35.59	52.69	7.913	3	36.047	35.050	34.46	38.63	2.257
Z _y	4	44.310	43.195	38.40	52.45	6.668	3	43.910	44.780	41.49	45.46	2.123
Z _y /Z _x	4	1.091	1.069	1.00	1.23	0.104	3	1.224	1.297	1.07	1.30	0.129
Z _p	4	85.269	80.542	74.85	105.14	14.105	3	80.526	80.357	80.18	81.04	0.452
<u>M3 males</u>							<u>M3 males</u>					
Body mass	3	4596.333	4490.000	3856.00	5443.00	798.826	3	4596.333	4490.000	3856.00	5443.00	798.826
Bone length	4	132.813	132.500	126.50	139.75	5.893	4	109.600	108.100	107.50	114.70	3.427
TA	4	66.248	67.155	53.20	77.48	11.591	4	63.080	65.005	50.28	72.03	9.216
CA	4	38.003	38.025	31.53	44.43	6.490	4	36.280	37.880	28.82	40.54	5.143
MA	4	28.245	29.175	19.82	34.81	6.247	4	26.800	26.030	21.46	33.68	5.072
lmax	4	314.403	309.820	216.35	421.62	106.962	4	336.153	355.860	221.22	411.67	87.474
lmin	4	269.128	277.740	172.09	348.94	91.472	4	205.925	224.205	121.12	254.17	58.275
lmax/lmin	4	1.171	1.170	1.09	1.26	0.076	4	1.655	1.692	1.41	1.83	0.185
Theta	4	-67.433	-72.610	-79.09	-45.42	15.030	4	34.893	33.770	19.12	52.91	17.355
J	4	583.535	587.560	388.44	770.56	197.809	4	542.070	580.065	342.33	665.83	143.091
Z _x	4	59.608	60.185	45.24	72.82	14.290	4	52.105	54.830	40.01	58.75	8.339
Z _y	4	59.593	59.865	43.37	75.27	16.367	4	59.030	62.085	39.72	72.23	14.845
Z _y /Z _x	4	0.993	0.990	0.96	1.03	0.037	4	1.124	1.111	0.98	1.29	0.158
Z _p	4	119.201	119.998	88.79	148.02	30.548	4	111.729	117.614	79.71	131.98	22.981
<u>EIPPH females</u>							<u>EIPPH females</u>					
Body mass	3	3250.667	3515.000	2608.00	3629.00	559.477	3	3250.667	3515.000	2608.00	3629.00	559.477
Bone length	3	115.000	114.300	111.00	119.70	4.392	3	94.267	93.300	92.20	97.30	2.684
TA	3	52.497	52.370	47.79	57.33	4.771	3	49.247	48.470	46.75	52.52	2.962
CA	3	29.270	29.020	26.50	32.29	2.903	3	27.173	25.110	24.87	31.54	3.784
MA	3	23.227	21.290	20.08	28.31	4.444	3	22.073	21.880	20.98	23.36	1.202

TABLE E-2 a continued. Cross-sectional geometry descriptive statistics by age class and sex: *Chlorocebus aethiops*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
lmax	3	191.350	202.170	154.67	217.21	32.644	3	198.633	189.260	173.05	233.59	31.340
lmin	3	162.083	170.370	135.59	180.29	23.474	3	123.857	111.460	108.94	151.17	23.688
lmax/lmin	3	1.177	1.187	1.14	1.20	0.033	3	1.611	1.588	1.55	1.70	0.079
Theta	3	-67.510	-70.340	-74.70	-57.49	8.947	3	34.633	33.390	29.20	41.31	6.150
J	3	353.430	372.540	290.26	397.50	56.113	3	322.490	300.720	281.98	384.77	54.744
Z _x	3	40.807	42.060	35.92	44.44	4.396	3	35.473	33.700	33.67	39.05	3.098
Z _y	3	40.670	42.420	35.33	44.26	4.715	3	38.617	36.160	34.00	45.69	6.220
Z _y /Z _x	3	0.996	0.996	0.98	1.01	0.012	3	1.084	1.074	1.01	1.17	0.081
Z _p	3	81.471	84.485	71.23	88.70	9.117	3	74.250	70.016	67.70	85.03	9.407
<u>EIPPH males</u>							<u>EIPPH males</u>					
Body mass	3	6123.667	5670.000	5443.00	7258.00	988.897	3	6123.667	5670.000	5443.00	7258.00	988.897
Bone length	3	140.900	137.500	136.20	149.00	7.045	3	115.233	113.500	113.20	119.00	3.265
TA	3	78.210	67.190	66.48	100.96	19.705	3	74.397	68.870	60.19	94.13	17.632
CA	3	50.083	47.860	45.99	56.40	5.550	3	48.240	46.440	43.42	54.86	5.929
MA	3	28.127	21.200	18.62	44.56	14.290	3	26.157	22.430	16.77	39.27	11.704
lmax	3	460.870	354.090	346.23	682.29	191.796	3	540.630	446.620	381.67	793.60	221.472
lmin	3	407.117	301.720	297.66	621.97	186.080	3	293.377	255.530	194.79	429.81	121.995
lmax/lmin	3	1.145	1.148	1.10	1.19	0.046	3	1.851	1.846	1.75	1.96	0.106
Theta	3	-64.103	-66.750	-74.79	-50.77	12.227	3	32.447	37.870	20.28	39.19	10.557
J	3	867.990	651.750	647.95	1304.27	377.834	3	834.010	702.150	576.46	1223.41	343.037
Z _x	3	83.527	70.240	67.43	112.91	25.485	3	71.647	66.290	57.44	91.21	17.511
Z _y	3	79.720	68.530	65.65	104.98	21.923	3	83.523	74.110	60.56	115.90	28.846
Z _y /Z _x	3	0.960	0.935	0.93	1.02	0.049	3	1.148	1.118	1.05	1.27	0.111
Z _p	3	163.275	135.981	135.92	217.92	47.325	3	156.182	140.571	118.13	209.85	47.811

TABLE E-2 b. Cross-sectional geometry descriptive statistics by age class and sex: *Hylobates lar*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>DECID females</u>							<u>DECID females</u>					
Body mass	2	680.000	680.000	680.00	680.00	0.000	2	680.000	680.000	680.00	680.00	0.000
Bone length	2	85.000	85.000	78.80	91.20	8.768	2	99.250	99.250	93.80	104.70	7.707
TA	2	15.800	15.800	14.66	16.94	1.612	2	19.690	19.690	17.30	22.08	3.380
CA	2	8.740	8.740	7.68	9.80	1.499	2	9.920	9.920	8.25	11.59	2.362
MA	2	7.060	7.060	6.98	7.14	0.113	2	9.770	9.770	9.05	10.49	1.018
lmax	2	16.850	16.850	13.60	20.10	4.596	2	28.750	28.750	20.66	36.84	11.441
lmin	2	14.825	14.825	12.62	17.03	3.118	2	18.920	18.920	14.20	23.64	6.675
lmax/lmin	2	1.129	1.129	1.08	1.18	0.073	2	1.507	1.507	1.45	1.56	0.073
Theta	2	67.200	67.200	60.16	74.24	9.956	2	76.195	76.195	69.97	82.42	8.803
J	2	31.680	31.680	26.22	37.14	7.722	2	47.670	47.670	34.86	60.48	18.116
Z _x	2	7.085	7.085	5.94	8.23	1.619	2	9.910	9.910	7.72	12.10	3.097
Z _y	2	6.720	6.720	5.91	7.53	1.146	2	8.445	8.445	6.82	10.07	2.298
Z _y /Z _x	2	0.955	0.955	0.91	0.99	0.057	2	0.858	0.858	0.83	0.88	0.036
Z _p	2	13.819	13.819	11.84	15.80	2.802	2	18.508	18.508	14.62	22.40	5.504
<u>DECID males</u>							<u>DECID males</u>					
Body mass	2	680.000	680.000	680.00	680.00	0.000	2	680.000	680.000	680.00	680.00	0.000
Bone length	2	97.900	97.900	95.00	100.80	4.101	2	114.750	114.750	111.30	118.20	4.879
TA	2	17.255	17.255	15.50	19.01	2.482	2	18.440	18.440	18.01	18.87	0.608
CA	2	9.725	9.725	8.09	11.36	2.312	2	9.135	9.135	8.30	9.97	1.181
MA	2	7.530	7.530	7.41	7.65	0.170	2	9.305	9.305	8.90	9.71	0.573
lmax	2	20.340	20.340	15.09	25.59	7.425	2	22.935	22.935	21.48	24.39	2.058
lmin	2	17.840	17.840	14.15	21.53	5.218	2	16.850	16.850	15.42	18.28	2.022
lmax/lmin	2	1.128	1.128	1.07	1.19	0.086	2	1.364	1.364	1.33	1.39	0.042
Theta	2	54.355	54.355	26.64	82.07	39.195	2	-0.135	-0.135	-89.24	88.97	126.013
J	2	38.180	38.180	29.24	47.12	12.643	2	39.780	39.780	36.89	42.67	4.087
Z _x	2	8.035	8.035	6.38	9.69	2.341	2	8.555	8.555	8.08	9.03	0.672
Z _y	2	7.760	7.760	6.38	9.14	1.952	2	7.285	7.285	6.70	7.87	0.827
Z _y /Z _x	2	0.972	0.972	0.94	1.00	0.040	2	0.850	0.850	0.83	0.87	0.030
Z _p	2	15.827	15.827	12.77	18.89	4.326	2	15.940	15.940	14.88	17.00	1.500

TABLE E-2 b continued. Cross-sectional geometry descriptive statistics by age class and sex: *Hylobates lar*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>M1 females</u>							<u>M1 females</u>					
Body mass	2	1587.500	1587.500	1361.00	1814.00	320.319	2	1587.500	1587.500	1361.00	1814.00	320.319
Bone length	2	121.750	121.750	111.50	132.00	14.496	2	139.100	139.100	126.00	152.20	18.526
TA	2	26.720	26.720	24.55	28.89	3.069	2	27.820	27.820	27.25	28.39	0.806
CA	2	17.815	17.815	14.64	20.99	4.490	2	17.480	17.480	15.21	19.75	3.210
MA	2	8.905	8.905	7.90	9.91	1.421	2	10.340	10.340	7.50	13.18	4.016
Imax	2	53.265	53.265	41.58	64.95	16.525	2	53.195	53.195	50.81	55.58	3.373
Imin	2	47.730	47.730	38.27	57.19	13.378	2	50.185	50.185	48.93	51.44	1.775
Imax/Imin	2	1.111	1.111	1.09	1.14	0.035	2	1.059	1.059	1.04	1.08	0.030
Theta	2	-39.860	-39.860	-82.46	2.74	60.245	2	35.485	35.485	19.66	51.31	22.380
J	2	100.995	100.995	79.85	122.14	29.904	2	103.380	103.380	99.73	107.03	5.162
Z _x	2	16.780	16.780	13.78	19.78	4.243	2	16.700	16.700	15.42	17.98	1.810
Z _y	2	16.730	16.730	14.36	19.10	3.352	2	16.700	16.700	15.74	17.66	1.358
Z _y /Z _x	2	1.004	1.004	0.97	1.04	0.054	2	1.001	1.001	0.98	1.02	0.027
Z _p	2	33.507	33.507	28.12	38.90	7.624	2	33.391	33.391	31.17	35.61	3.143
<u>M1 males</u>							<u>M1 males</u>					
Body mass	1	1361.000					1	1361.000				
Bone length	1	127.700					1	148.500				
TA	1	28.060					1	28.790				
CA	1	17.290					1	17.870				
MA	1	10.770					1	10.920				
Imax	1	55.340					1	64.250				
Imin	1	50.950					1	47.950				
Imax/Imin	1	1.086					1	1.340				
Theta	1	-12.910					1	86.090				
J	1	106.290					1	112.190				
Z _x	1	17.320					1	19.230				
Z _y	1	18.010					1	17.100				
Z _y /Z _x	1	1.040					1	0.889				
Z _p	1	35.371					1	36.488				

TABLE E-2 b continued. Cross-sectional geometry descriptive statistics by age class and sex: *Hylobates lar*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>M2 females</u>							<u>M2 females</u>					
Body mass	2	2835.000	2835.000	2722.00	2948.00	159.806	1	2722.000				
Bone length	2	154.500	154.500	153.20	155.80	1.838	1	179.500				
TA	2	47.870	47.870	46.83	48.91	1.471	1	49.510				
CA	2	30.655	30.655	29.81	31.50	1.195	1	30.150				
MA	2	17.215	17.215	17.02	17.41	0.276	1	19.360				
lmax	2	162.790	162.790	154.42	171.16	11.837	1	178.100				
lmin	2	153.805	153.805	147.81	159.80	8.478	1	149.370				
lmax/lmin	2	1.058	1.058	1.04	1.07	0.019	1	1.192				
Theta	2	-58.955	-58.955	-69.09	-48.82	14.333	1	25.830				
J	2	316.595	316.595	302.23	330.96	20.315	1	327.460				
Z _x	2	39.550	39.550	39.28	39.82	0.382	1	38.890				
Z _y	2	39.545	39.545	38.37	40.72	1.662	1	41.830				
Z _y /Z _x	2	1.000	1.000	0.96	1.04	0.052	1	1.076				
Z _p	2	79.069	79.069	78.20	79.94	1.234	1	80.757				
<u>M2 males</u>							<u>M2 males</u>					
Body mass	3	3099.333	3175.000	2041.00	4082.00	1022.602	3	3099.333	3175.000	2041.00	4082.00	1022.602
Bone length	4	163.200	166.400	135.70	184.30	20.848	4	192.125	194.850	160.80	218.00	23.964
TA	4	57.270	55.550	31.35	86.63	23.335	4	57.663	57.785	30.89	84.19	22.456
CA	4	34.095	35.195	17.41	48.58	13.547	4	31.590	34.375	17.19	40.42	10.646
MA	4	23.175	20.355	13.94	38.05	10.428	4	26.073	22.600	13.70	45.39	13.604
lmax	4	253.490	222.810	64.18	504.16	189.042	4	241.133	238.310	66.61	421.30	152.915
lmin	4	235.353	208.680	61.24	462.81	172.568	4	216.128	213.935	55.84	380.80	139.573
lmax/lmin	4	1.068	1.068	1.05	1.09	0.017	4	1.131	1.113	1.11	1.19	0.041
Theta	4	42.013	35.740	29.40	67.17	17.416	4	-0.952	11.400	-74.21	47.60	56.306
J	4	488.845	431.490	125.42	966.97	361.604	4	457.263	452.245	122.45	802.10	292.484
Z _x	4	52.123	50.340	19.07	88.74	30.129	4	47.958	50.890	18.77	71.28	22.803
Z _y	4	51.890	49.295	19.23	89.74	29.930	4	48.810	52.045	19.28	71.87	23.600
Z _y /Z _x	4	1.000	1.010	0.94	1.04	0.041	4	1.016	1.018	0.97	1.06	0.039
Z _p	4	104.022	99.610	38.30	178.57	60.058	4	96.806	102.981	38.03	143.23	46.400

TABLE E-2 b continued. Cross-sectional geometry descriptive statistics by age class and sex: *Hylobates lar*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>M3 females</u>							<u>M3 females</u>					
Body mass	2	4195.500	4195.500	4082.00	4309.00	160.513	2	4195.500	4195.500	4082.00	4309.00	160.513
Bone length	4	177.950	180.650	167.70	182.80	6.943	4	205.150	208.800	192.20	210.80	8.685
TA	4	65.765	65.570	58.22	73.70	6.382	4	69.845	70.520	61.05	77.29	6.678
CA	4	42.743	45.515	32.02	47.92	7.460	4	41.343	42.540	33.75	46.54	5.834
MA	4	23.023	24.550	16.79	26.20	4.345	4	28.503	28.875	24.21	32.05	3.476
lmax	4	319.503	320.625	226.04	410.72	75.778	4	351.243	358.385	266.96	421.24	67.599
lmin	4	287.948	298.270	203.75	351.50	61.840	4	299.918	311.010	208.81	368.84	66.903
lmax/lmin	4	1.108	1.123	1.02	1.17	0.065	4	1.181	1.177	1.09	1.28	0.082
Theta	4	10.743	21.225	-41.29	41.81	36.032	4	18.910	16.000	3.66	39.98	15.211
J	4	607.450	618.895	429.79	762.22	136.359	4	651.158	669.395	475.77	790.08	133.389
Z _x	4	62.615	64.780	45.52	75.38	12.516	4	63.958	67.695	46.41	74.03	12.207
Z _y	4	65.488	66.700	49.57	78.98	12.113	4	68.125	70.195	53.58	78.53	11.565
Z _y /Z _x	4	1.049	1.044	1.02	1.09	0.029	4	1.071	1.076	0.98	1.15	0.073
Z _p	4	128.122	131.527	94.98	154.45	24.689	4	132.264	138.005	100.37	152.67	23.392
<u>M3 males</u>							<u>M3 males</u>					
Body mass	0						0					
Bone length	5	187.360	189.000	170.00	204.00	13.503	4	218.375	218.650	198.20	238.00	18.914
TA	5	73.282	74.150	68.51	76.00	2.848	4	72.670	72.305	70.38	75.69	2.367
CA	5	48.014	46.410	42.03	53.95	5.356	4	45.415	46.655	37.06	51.29	6.587
MA	5	25.268	22.700	19.28	32.49	5.588	4	27.255	26.190	23.32	33.32	4.507
lmax	5	386.648	372.810	338.21	443.72	41.435	4	377.613	385.680	316.40	422.69	47.925
lmin	5	363.796	358.970	333.10	396.75	26.738	4	342.465	342.780	289.14	395.16	46.494
lmax/lmin	5	1.061	1.062	1.02	1.12	0.039	4	1.104	1.108	1.07	1.13	0.027
Theta	5	39.364	22.600	4.12	86.43	37.562	4	24.380	33.245	-15.64	46.67	27.773
J	5	750.442	731.780	671.31	840.46	67.922	4	720.073	728.460	605.53	817.84	93.995
Z _x	5	73.440	73.800	68.68	77.57	3.269	4	71.383	71.760	60.75	81.26	9.329
Z _y	5	76.956	74.490	69.90	85.90	6.771	4	73.155	74.230	63.98	80.18	7.324
Z _y /Z _x	5	1.047	1.054	0.97	1.11	0.066	4	1.028	1.033	0.99	1.06	0.034
Z _p	5	150.300	149.191	140.98	163.36	9.398	4	144.575	146.053	124.73	161.47	16.629

TABLE E-2 b continued. Cross-sectional geometry descriptive statistics by age class and sex: *Hylobates lar*.

	FEMUR						HUMERUS					
	N	Mean	Median	Min	Max	S.D.	N	Mean	Median	Min	Max	S.D.
<u>EIPPH females</u>							<u>EIPPH females</u>					
Body mass	0						0					
Bone length	3	179.700	178.300	175.50	185.30	5.048	3	209.167	206.500	206.50	214.50	4.619
TA	3	77.223	76.380	72.01	83.28	5.682	3	77.153	79.660	67.44	84.36	8.734
CA	3	51.267	51.840	47.07	54.89	3.941	3	44.667	43.860	40.84	49.30	4.287
MA	3	25.957	28.390	20.17	29.31	5.032	3	32.487	35.060	23.58	38.82	7.939
lmax	3	454.297	425.360	393.97	543.56	78.881	3	421.467	403.280	325.72	535.40	106.016
lmin	3	392.050	369.180	368.15	438.82	40.507	3	360.977	366.560	308.72	407.65	49.701
lmax/lmin	3	1.154	1.155	1.07	1.24	0.086	3	1.156	1.100	1.06	1.31	0.138
Theta	3	32.003	47.530	-0.18	48.66	27.877	3	8.567	10.690	-2.05	17.06	9.730
J	3	846.350	793.510	763.15	982.39	118.788	3	782.447	769.840	634.44	943.05	154.690
Z _x	3	77.713	74.180	71.39	87.57	8.649	3	72.983	72.080	64.68	82.19	8.790
Z _y	3	82.737	81.330	75.98	90.90	7.559	3	78.523	76.150	66.69	92.73	13.181
Z _y /Z _x	3	1.067	1.038	1.02	1.14	0.063	3	1.072	1.056	1.03	1.13	0.050
Z _p	3	160.425	152.745	150.08	178.45	15.669	3	151.812	148.331	131.49	175.61	22.267
<u>EIPPH males</u>							<u>EIPPH males</u>					
Body mass	0						0					
Bone length	3	183.767	186.500	177.80	187.00	5.173	3	211.467	206.200	204.00	224.20	11.082
TA	3	85.017	87.340	79.71	88.00	4.608	3	85.167	83.780	77.93	93.79	8.020
CA	3	56.413	55.450	47.08	66.71	9.850	3	49.947	53.330	42.70	53.81	6.280
MA	3	28.603	32.550	20.63	32.63	6.905	3	35.220	35.230	29.97	40.46	5.245
lmax	3	560.500	582.480	452.64	646.38	98.723	3	512.183	522.170	403.41	610.97	104.140
lmin	3	462.413	486.660	392.11	508.47	61.853	3	452.330	455.820	366.25	534.92	84.389
lmax/lmin	3	1.207	1.197	1.15	1.27	0.059	3	1.130	1.142	1.10	1.15	0.025
Theta	3	19.403	19.400	-5.36	44.17	24.765	3	31.040	33.740	18.96	40.42	10.982
J	3	1022.913	1069.140	844.75	1154.85	160.135	3	964.517	977.990	769.67	1145.89	188.472
Z _x	3	91.797	93.020	80.59	101.78	10.648	3	85.820	86.850	73.69	96.92	11.649
Z _y	3	98.517	102.480	80.93	112.14	15.978	3	91.373	92.640	73.51	107.97	17.265
Z _y /Z _x	3	1.069	1.102	1.00	1.10	0.056	3	1.059	1.067	1.00	1.11	0.059
Z _p	3	190.530	195.813	161.52	214.26	26.763	3	177.200	179.448	147.16	204.99	28.979

TABLE E-2 c. Cross-sectional geometry descriptive statistics by age class: *Pan troglodytes*.

FEMUR						
	N	Mean	Median	Min	Max	S.D.
<u>DECID</u>						
Bone length	2	97.300	97.300	95.30	99.30	2.828
TA	2	77.115	77.115	67.81	86.42	13.159
CA	2	39.050	39.050	36.64	41.46	3.408
MA	2	38.065	38.065	31.17	44.96	9.751
Imax	2	384.125	384.125	323.35	444.90	85.949
Imin	2	324.680	324.680	242.16	407.20	116.701
Imax/Imin	2	1.214	1.214	1.09	1.34	0.172
Theta	2	81.030	81.030	72.98	89.08	11.384
J	2	708.805	708.805	565.51	852.10	202.650
Z _x	2	72.875	72.875	64.64	81.11	11.646
Z _y	2	67.515	67.515	55.98	79.05	16.313
Z _y /Z _x	2	0.920	0.920	0.87	0.97	0.077
Z _p	2	140.697	140.697	121.22	160.17	27.538
<u>M1</u>						
Bone length	1	146.800				
TA	1	164.310				
CA	1	96.360				
MA	1	67.950				
Imax	1	1990.200				
Imin	1	1564.360				
Imax/Imin	1	1.272				
Theta	1	3554.560				
J	1	3554.560				
Z _x	1	230.150				
Z _y	1	245.000				
Z _y /Z _x	1	1.065				
Z _p	1	476.163				
<u>M2</u>						
Bone length	4	185.825	178.650	176.70	209.30	15.677
TA	4	201.498	199.885	176.90	229.32	21.533

TABLE E-2 c continued. Cross-sectional geometry descriptive statistics by age class: *Pan troglodytes*.

FEMUR						
	N	Mean	Median	Min	Max	S.D.
CA	4	147.678	153.255	119.48	164.72	19.560
MA	4	53.820	53.025	44.63	64.60	8.955
Imax	4	3294.230	3321.875	2276.28	4256.89	816.708
Imin	4	2725.505	2698.445	2078.28	3426.85	551.585
Imax/Imin	4	1.200	1.208	1.10	1.29	0.084
Theta	4	-32.165	-28.710	-57.69	-13.55	18.769
J	4	6019.733	6020.320	4354.57	7683.73	1362.626
Z _x	4	358.148	353.010	279.07	447.50	69.705
Z _y	4	370.233	383.855	283.58	429.64	62.196
Z _y /Z _x	4	1.038	1.047	0.96	1.10	0.062
Z _p	4	729.061	737.999	562.60	877.64	130.258
<u>M3</u>						
Bone length	3	235.967	237.700	223.50	246.70	11.697
TA	3	343.183	351.020	315.63	362.90	24.590
CA	3	252.893	252.280	250.34	256.06	2.909
MA	3	90.290	94.960	63.35	112.56	24.935
Imax	3	9173.270	9564.980	8258.16	9696.67	795.239
Imin	3	8185.810	8435.800	6960.50	9161.13	1121.412
Imax/Imin	3	1.126	1.134	1.06	1.19	0.064
Theta	3	10.293	10.390	9.68	10.81	0.571
J	3	17359.087	18000.780	15218.66	18857.81	1902.548
Z _x	3	790.980	812.450	712.69	847.80	70.067
Z _y	3	833.693	865.960	757.44	877.68	66.297
Z _y /Z _x	3	1.055	1.063	1.04	1.07	0.017
Z _p	3	1625.703	1679.961	1471.82	1725.32	135.180
<u>EPIPH</u>						
Bone length	2	272.300	272.300	271.10	273.50	1.697
TA	2	462.305	462.305	417.95	506.66	62.727
CA	2	300.960	300.960	234.73	367.19	93.663
MA	2	161.345	161.345	139.47	183.22	30.936
Imax	2	16473.135	16473.135	12244.98	20701.29	5979.514
Imin	2	13676.165	13676.165	10241.35	17110.98	4857.562

TABLE E-2 c continued. Cross-sectional geometry descriptive statistics by age class: *Pan troglodytes*.

FEMUR						
	N	Mean	Median	Min	Max	S.D.
lmax/lmin	2	1.203	1.203	1.20	1.21	0.010
Theta	2	-25.125	-25.125	-50.51	0.26	35.900
J	2	30149.300	30149.300	22486.33	37812.27	10837.076
Z _x	2	1180.280	1180.280	963.38	1397.18	306.743
Z _y	2	1242.545	1242.545	943.05	1542.04	423.550
Z _y /Z _x	2	1.041	1.041	0.98	1.10	0.088
Z _p	2	2426.229	2426.229	1906.43	2946.03	735.108

APPENDIX F.

TISSUE TYPES: RAW DATA AND DESCRIPTIVE STATISTICS (Chapters 5 and 6)

TABLE F-1 a. Tissue type raw data collected from whole cross-sections: *Chlorocebus aethiops*. Data reported in number of pixels.

ID#	WOV	FLC	FLC-LZPO	PF	PFLAM	LAM	SF	EPF	EPF-LAM	ELAM	ECCC	HAV	INT	total # pixels
C. aethiops: FEMUR														
11	0	0	5546	2351	39483	497230	55682	0	2447	659	0	38259	0	641657
13	0	0	0	4295	23079	981041	47370	0	14364	18281	45373	167289	0	1301092
14	0	0	3721	0	10729	775986	58197	0	10107	376	52255	73937	0	985308
15	0	0	1846	0	4843	969959	88400	0	2592	29392	14168	47126	0	1158326
16	0	0	0	0	0	1003419	39320	0	7387	1384	6598	63140	0	1121248
18	0	0	0	2222	3175	984309	77801	0	86510	124163	122073	404409	0	1804662
21	0	0	69567	1017	30232	358844	65968	962	21818	17696	0	81756	0	647860
25	0	0	907	1195	40593	793927	50975	3671	20274	10188	14852	83675	0	1020257
27	0	3397	158362	0	59609	227744	35378	0	9314	1944	0	12447	0	508195
33	0	10660	341472	1750	37711	64806	63300	0	2171	3787	3683	22237	0	551577
34	0	0	3321	3679	59611	540876	77460	1235	15705	4093	27860	82244	0	816084
35	0	0	6053	0	0	810073	81340	0	0	0	0	1498	0	898964
37	0	0	14013	0	23882	504259	66292	0	686	728	0	15977	0	625837
43	0	0	0	0	829	934978	56647	0	22674	23839	61476	160112	0	1260555
60	0	0	0	5368	4771	601461	66456	0	73433	58448	93944	103791	0	1007672
62	0	25856	113855	5525	25327	0	13770	0	0	0	0	0	0	184333
64	0	0	0	0	785	1784700	89349	0	0	95049	541	222230	0	2192654
68	0	0	0	1146	3580	1335855	69928	1547	7565	25618	25645	187283	0	1658167
70	0	0	0	735	1571	1091219	79100	0	0	12424	5983	57293	0	1248325
73	0	10106	72867	0	54562	429195	99044	0	9572	6395	0	7360	0	689101
74	0	0	19505	0	29838	592073	94365	840	41980	20418	73000	84232	0	956251
75	0	0	15203	0	14739	1156143	159618	0	94533	71608	165456	196859	0	1874159
76	0	1328	77289	3012	34258	203770	37496	0	12750	811	0	8383	0	379097
78	0	0	2047	0	9206	926288	62925	890	7486	4872	13202	93740	0	1120656
80	0	0	18244	0	49668	761404	47037	0	7507	678	17796	83252	0	985586
91	0	0	12595	0	3137	1174605	68981	0	17076	28165	36780	354720	0	1696059

TABLE F-1 a continued. Tissue type raw data collected from whole cross-sections: *Chlorocebus aethiops*.

ID#	WOV	FLC	FLC-LZPO	PF	PFLAM	LAM	SF	EPF	EPF-LAM	ELAM	ECCE	HAV	INT	total # pixels
92	0	51051	73963	26420	71377	0	26925	654	0	0	0	331	0	250721
94	0	0	6610	0	1406	1189792	96146	6247	19224	12386	77131	74626	0	1483568
96	0	0	14109	15239	57463	384718	71959	4588	12303	937	0	54171	0	615487
101	0	0	3512	0	7020	854922	35902	0	3508	218250	0	101630	0	1224744
C. aethiops: HUMERUS														
11	0	0	12859	0	10239	284715	5176	1626	26239	18699	19039	193932	7418	579942
13	0	0	24341	0	12615	627133	8624	0	22325	66767	41951	310421	129	1114306
14	0	0	62004	0	14135	469705	40284	6365	49955	92747	109356	166156	2817	1013524
15	0	0	23143	0	6160	637203	9176	0	51406	119390	112082	148730	2821	1110111
16	0	0	9269	2814	18214	625315	17040	0	0	28713	15175	239405	2617	958562
18	0	0	20868	0	5850	906439	23787	0	37544	157037	46538	498847	1014	1697924
21*	0	0	215215	0	14876	171260	16272	9996	56581	25288	16683	64395	0	590566
25	0	26355	8734	661	12190	494982	13523	0	41442	71518	39682	256036	0	965123
27	0	28708	185841	7739	38118	113152	17537	11263	25591	5932	3660	72827	0	510368
33	0	3899	293316	2201	48200	70726	29644	4030	10407	0	0	50146	0	512569
34	0	0	77654	0	19191	412263	13396	0	11922	7277	16652	180447	3670	742472
35	0	0	27005	1756	10970	467909	6478	0	8505	108743	30248	97408	4468	763490
37	0	0	41900	1047	69178	239663	14515	9386	24106	19425	15176	154301	8187	596884
43	0	2411	5462	0	4398	658178	19170	2056	16429	91810	51336	370632	4511	1226393
47	0	0	38638	8610	34880	208412	17729	0	44128	7418	31219	220222	7492	618748
62	0	50288	132713	37304	25669	5225	4014	1811	4829	0	799	7145	0	269797
64	0	0	188280	0	9380	1186037	44361	0	0	147053	31459	535177	2105	2143852
68	0	0	41998	0	36239	869369	26328	1519	8248	78477	34504	395419	890	1492991
70	0	0	71436	0	15630	507617	39620	0	20118	51727	76415	271679	0	1054242
72	0	0	14569	3276	11310	329894	20561	0	29924	97981	54872	166420	4938	733745
73	6058	0	59863	5918	21887	250651	17151	0	59918	41353	43109	101862	8182	615952
75	0	0	41414	0	1889	1116291	28113	4304	28664	104275	69375	414488	12546	1821359
76	0	16224	115206	3155	43561	117585	31410	1808	38770	1532	4900	122071	0	496222
78	0	0	47232	0	32711	666755	16263	0	693	55791	31500	185967	0	1036912
80	0	0	21539	0	47027	472551	23715	0	17184	17114	56987	180903	1284	838304
91	0	0	35255	0	32358	825069	6585	0	8317	34729	42751	568040	1893	1554997
92	0	31969	93029	59801	24909	8821	6315	3968	3743	0	1422	13198	0	247175

TABLE F-1 a continued. Tissue type raw data collected from whole cross-sections: *Chlorocebus aethiops*.

ID#	WOV	FLC	FLC-LZPO	PF	PFLAM	LAM	SF	EPF	EPF-LAM	ELAM	ECCE	HAV	INT	total # pixels
94	0	0	84350	2603	12480	742809	20602	0	59739	111750	78313	233727	3099	1349472
96	0	0	77877	577	16037	230333	23001	859	40035	56806	18076	122167	3380	589148
101	0	0	31860	0	3565	858191	3065	0	5436	299397	31389	219415	2591	1454909

TABLE F-1 b. Tissue type raw data collected from whole cross-sections: *Hylobates lar*. Data reported in number of pixels.

ID#	WOV	FLC	FLC-LZPO	PF	PFLAM	LAM	SF	EPF	EPF-LAM	ELAM	ECCE	HAV	INT	total # pixels
<i>H. lar</i>: FEMUR														
4	0	0	966	0	16121	697204	14551	1	10	51359	1144	740493	495	1522344
5	0	0	1700	0	21645	379450	13514	0	1886	76458	0	655533	402	1150588
6	0	0	4563	0	38670	646669	27803	0	0	556	0	614040	0	1332301
10	0	0	4285	0	13165	892311	22785	0	0	32204	805	370413	29	1335997
11	0	0	0	0	24401	677894	19567	0	2226	53824	0	659105	133	1437150
16	0	0	0	0	18347	1082705	45150	0	20133	37758	0	662257	646	1866996
26	0	0	0	470	3167	868259	37670	0	0	9597	0	621563	351	1541077
30	0	0	0	0	13655	883730	8932	0	1650	29381	713	365646	194	1303901
31	0	0	0	0	21080	664370	23685	0	0	37996	0	555809	1111	1304051
32	0	0	0	0	5846	794419	24160	0	957	33198	0	616673	0	1475253
35	0	0	0	0	44279	745238	35824	0	0	32605	0	462737	797	1321480
37	0	0	0	0	31280	684743	72800	0	13899	77784	2657	612910	1329	1497402
38	0	0	5198	0	13813	766848	57547	0	0	37724	388	405345	286	1287149
39	0	0	2910	0	50581	522524	34488	0	1093	62594	0	539646	587	1214423
35456	0	0	4100	10720	29397	31741	4773	1927	11311	47251	994	77773	1467	221454
36380	0	0	1787	31134	84078	36724	11167	0	19593	24003	818	228294	0	437598
41429	0	0	4531	0	12657	813467	16475	0	4327	11301	0	335074	0	1197832
41444	0	0	31600	8321	70493	71222	9643	0	17764	37035	2687	224311	390	473466
41452	0	0	5756	0	50839	408737	39790	0	9536	29800	537	325758	464	871217
41461	0	18319	20668	0	72924	651153	30924	0	9054	3502	6719	309301	430	1122994
41462	0	1160	3043	13060	74994	30462	5069	917	38710	19614	14417	172109	0	373555
41466	0	0	14266	38573	36712	18329	4956	2661	11765	6789	351	76862	696	211960
41473	0	0	9330	0	108423	565774	102656	0	20312	38177	932	315405	597	1161606

TABLE F-1 b continued. Tissue type raw data collected from whole cross-sections: *Hylobates lar*.

ID#	WOV	FLC	FLC-LZPO	PF	PFLAM	LAM	SF	EPF	EPF-LAM	ELAM	ECCE	HAV	INT	total # pixels
41475	0	0	0	0	15208	542856	24565	0	375	30776	0	261189	416	875385
41482	0	0	35211	34500	70366	22696	27758	2305	8480	42937	1418	156239	2003	403913
41491	0	0	8811	0	107515	356574	18012	0	2138	20753	0	300918	0	814721
41498	0	0	4555	0	78489	232250	29199	0	3105	11275	0	122800	103	481776
41502	0	0	20035	2385	114749	152606	31819	0	5488	38802	6120	206399	253	578656
<i>Hylobates lar</i> : HUMERUS														
4	0	0	0	0	30999	396404	38185	0	1	8627	0	878036	1934	1354186
5	0	0	9073	0	69501	335136	17340	0	0	46797	0	948043	1288	1427178
6	0	0	0	17226	58537	379324	22565	0	8892	7573	0	997812	1272	1493201
10	0	0	0	0	21854	549034	17794	0	0	47463	0	613658	1170	1250973
11	0	0	0	0	34468	607627	39229	0	102	94367	7	937103	481	1713384
16	0	0	0	0	144049	862807	148979	0	650	78620	0	870329	834	2106268
26	0	0	5743	0	16816	1047437	65239	0	1900	89345	0	853510	5639	2085629
30	0	0	0	0	34664	686158	74642	0	0	54702	0	741885	837	1592888
31	0	0	1352	0	23096	470433	64354	0	2321	77239	0	529975	0	1168770
32	0	0	4229	0	20361	548129	19582	0	5147	52875	0	765982	1118	1417423
35	0	0	0	0	43497	644754	64776	0	803	36113	0	1027295	3403	1820641
37	0	0	2242	0	14887	551205	60817	0	2491	38551	0	714976	2862	1388031
38	0	0	0	0	8153	556051	53829	0	0	44988	0	529368	1550	1193939
35456	0	5241	3265	9061	22223	9328	2653	5575	44505	83856	3669	127583	0	316959
36380	0	0	0	14574	26172	1380	1400	1140	64258	9120	26821	229677	1706	376248
41429	0	0	0	0	29115	691578	33794	0	2718	21002	1924	761267	2676	1544074
41444	0	0	37163	22099	139489	26888	18897	4829	34277	75558	5152	325313	140	689805
41452	0	0	3444	0	77997	435099	42792	0	26378	39525	3919	533557	965	1163676
41461	0	9069	9895	0	112645	587051	102331	0	10457	19746	1275	702389	905	1555763
41462	0	0	8645	23527	64403	38078	7145	21705	15314	54460	46471	162568	716	443032
41466	0	0	21551	35624	35576	7615	5665	26296	36461	8157	4449	134318	137	315849
41475	0											562088		
41482	0	0	76592	44949	82871	2048	44261	0	13955	67821	12518	239654	270	584939
41491	0	0	5065	2547	235012	125090	22210	0	8208	84170	0	677398	3998	1163698
41498	0	0	15677	21574	182701	153978	32470	0	9848	14364	0	236329	154	667095
41502	0	0	14850	26288	216635	26544	23977	11740	18180	27811	2584	396882	1405	766896

TABLE F-1 c. Tissue type raw data collected from whole cross-sections: *Pan troglodytes*. Data reported in number of pixels.

ID#	WOV	FLC	FLC-LZPO	PF	PFLAM	LAM	SF	EPF	EPF-LAM	ELAM	ECCE	HAV	INT	total # pixels
<i>P. troglodytes</i>: FEMUR														
17011	0	0	128074	0	5344	1007599	85073	0	8212	9745	15090	1191519	728	2451384
30755	0	0	155035	0	386	1904014	92306	413	5005	26613	4998	1328861	5495	3523126
32052	0	0	145641	0	10825	132243	22326	893	3340	2578	0	469316	1876	789038
35526	0	0	184641	0	1051	525158	21916	0	25372	24130	41519	664621	4273	1492681
83604	0	0	178547	0	18062	269924	40612	1638	14778	17521	0	382052	1098	924232
83614	0	23325	113517	128234	99616	83192	39192	59296	50449	0	14910	839023	2984	1453738
83618	0	0	220827	22711	235794	19578	15675	10139	56442	75	0	732764	0	1314005
83643	0	7953	68915	0	3514	435879	90603	0	26265	68464	6635	881550	287	1590065
83645	0	0	1566	0	7569	563065	4835	0	6079	55471	2901	1619447	2367	2263300
83651	0	0	82427	0	5406	1026005	31061	237	2237	11305	8210	1245246	1188	2413322
83673	0	0	202435	0	0	495894	23219	2646	37151	17333	14357	688676	2752	1484463
83685	0	0	4501	0	4704	491131	11938	409	4671	6208	0	1898631	7766	2429959

TABLE F-2 a. Whole-section tissue type proportions: Descriptive statistics for the total *C. aethiops* sample. Data reported as a % of total cortical area.

	N	Mean	Median	Min	Max	Range	Std. Dev.
<i>C. aethiops</i>: FEMUR							
%FLC	30	1.29	0.000	0.00	20.36	20.36	4.424
%FLC-LZPO	30	7.98	0.559	0.00	61.91	61.91	16.857
%FLC + FLC-LZPO	30	9.28	0.559	0.00	75.79	75.79	19.869
%PF	30	0.64	0.000	0.00	10.54	10.54	1.993
%PF-LAM	30	4.27	1.431	0.00	28.47	28.47	6.029
%LAM	30	65.02	71.988	0.00	90.11	90.11	23.751
%SF	30	7.29	6.778	2.93	14.37	11.44	2.966
%EPF	30	0.08	0.000	0.00	0.75	0.75	0.168
%EPF-LAM	30	1.58	1.016	0.00	7.29	7.29	1.813
%ELAM	30	1.97	0.881	0.00	17.82	17.82	3.470
%ECCC	30	2.20	0.923	0.00	9.32	9.32	2.895
%HAV	30	7.67	8.250	0.00	22.41	22.41	5.453
%INT	30	0.00	0.000	0.00	0.00	0.00	0.000
% Total END	30	5.83	3.813	0.00	22.41	22.41	6.148
% Total PER	30	86.50	87.761	59.15	100.00	40.85	10.186
<i>C. aethiops</i>: HUMERUS							
%WOV	30	0.03	0.000	0.00	0.98	0.98	0.180
%FLC	30	1.47	0.000	0.00	18.64	18.64	4.153
%FLC-LZPO	30	11.56	5.336	0.45	57.22	56.78	15.583
%FLC + FLC-LZPO	30	13.04	5.336	0.64	67.83	67.19	18.553
%PF	30	1.48	0.000	0.00	24.19	24.19	4.972
%PF-LAM	30	3.40	1.991	0.10	11.59	11.49	3.410
%LAM	30	45.10	52.173	1.94	65.23	63.30	17.291
%SF	30	2.27	1.791	0.21	6.33	6.12	1.443
%EPF	30	0.35	0.000	0.00	2.21	2.21	0.614
%EPF-LAM	30	3.26	2.040	0.00	9.73	9.73	2.780
%ELAM	30	5.76	5.318	0.00	20.58	20.58	4.811
%ECCC	30	3.76	3.053	0.00	10.79	10.79	2.737
%HAV	30	21.22	22.719	2.65	36.53	33.88	8.425
%INT	30	0.34	0.166	0.00	1.37	1.37	0.438
% Total END	30	13.12	11.584	2.76	25.50	22.74	6.984
% Total PER	30	65.32	62.785	49.82	94.59	44.77	10.763

TABLE F-2 b. Whole-section tissue type proportions: Descriptive statistics for the total *H. lar* sample. Data reported as a % of total cortical area.

	N	Mean	Median	Min	Max	Range	Std. Dev.
<i>H. lar</i>: FEMUR							
%FLC	28	0.07	0.000	0.00	1.63	1.63	0.312
%FLC-LZPO	28	1.28	0.360	0.00	8.72	8.72	2.303
%FLC + FLC-LZPO	28	1.35	0.360	0.00	8.72	8.72	2.336
%PF	28	1.59	0.000	0.00	18.20	18.20	3.959
%PF-LAM	28	7.12	3.127	0.21	20.08	19.87	7.225
%LAM	28	42.68	47.688	5.62	67.91	62.29	19.748
%SF	28	2.95	2.378	0.69	8.84	8.15	1.954
%EPF	28	0.11	0.000	0.00	1.26	1.26	0.299
%EPF-LAM	28	1.42	0.312	0.00	10.36	10.36	2.393
%ELAM	28	4.24	3.245	0.04	21.34	21.29	4.094
%ECCC	28	0.28	0.042	0.00	3.86	3.86	0.745
%INT	28	0.08	0.035	0.00	0.66	0.66	0.155
% Total END	28	6.05	3.504	0.04	27.76	27.72	6.157
% Total PER	28	55.69	55.730	34.21	71.50	37.30	11.464
<i>H. lar</i>: HUMERUS							
%FLC	25	0.09	0.000	0.00	1.65	1.65	0.346
%FLC-LZPO	25	1.42	0.275	0.00	13.09	13.09	2.974
%FLC + FLC-LZPO	25	1.51	0.275	0.00	13.09	13.09	2.979
%PF	25	1.69	0.000	0.00	11.28	11.28	2.888
%PF-LAM	25	7.92	4.870	0.68	28.25	27.57	8.292
%LAM	25	26.73	35.414	0.35	50.22	49.87	17.252
%SF	25	3.23	2.820	0.37	7.57	7.19	1.984
%EPF	25	0.70	0.000	0.00	8.33	8.33	1.905
%EPF-LAM	25	2.51	0.363	0.00	17.08	17.08	4.663
%ELAM	25	5.18	3.626	0.51	26.46	25.95	5.466
%ECCC	25	0.96	0.000	0.00	10.49	10.49	2.472
%HAV	26	49.22	46.867	35.43	66.82	31.40	8.886
%INT	25	0.12	0.085	0.00	0.45	0.45	0.111
% Total END	25	9.34	4.093	0.64	43.41	42.78	10.998
% Total PER	25	41.16	42.325	11.57	60.92	49.35	11.434

TABLE F-2 c. Whole-section tissue type proportions: Descriptive statistics for the total *P. troglodytes* sample. Data reported as a % of total cortical area.

	N	Mean	Median	Min	Max	Range	Std. Dev.
<i>P. troglodytes</i>: FEMUR							
%FLC	12	0.18	0.000	0.00	1.60	1.60	0.472
%FLC-LZPO	12	8.84	6.517	0.07	19.32	19.25	6.985
%FLC + FLC-LZPO	12	9.01	7.319	0.07	19.32	19.25	6.950
%PF	12	0.88	0.000	0.00	8.82	8.82	2.550
%PF-LAM	12	2.45	0.223	0.00	17.94	17.94	5.248
%LAM	12	27.66	28.309	1.49	54.04	52.55	15.189
%SF	12	2.33	2.092	0.21	5.70	5.48	1.622
%EPF	12	0.45	0.014	0.00	4.08	4.08	1.165
%EPF-LAM	12	1.39	1.011	0.09	4.30	4.20	1.420
%ELAM	12	1.14	0.612	0.00	4.31	4.31	1.269
%ECCC	12	0.53	0.241	0.00	2.78	2.78	0.798
%HAV	12	54.02	53.520	37.72	78.13	40.42	11.861
%INT	12	0.14	0.137	0.00	0.32	0.32	0.107
% Total END	12	3.51	3.260	0.46	8.57	8.11	2.679
% Total PER	12	42.33	43.430	21.08	61.07	39.99	11.784

TABLE F-3 a. Whole-section tissue type proportions: Descriptive statistics for *C. aethiops* by age class.
Data reported as a % of total cortical area.

Tissue Type	FEMUR							HUMERUS						
	N	Mean	Median	Min	Max	Range	SD	N	Mean	Median	Min	Max	Range	SD
<u>DECID.</u>								<u>DECID.</u>						
%FLC	5	7.47	1.933	0.35	20.36	20.01	9.176	5	8.25	5.625	0.76	18.64	17.88	7.377
%FLC-LZPO	5	40.94	31.162	20.39	61.91	41.52	19.508	5	40.74	37.637	23.22	57.22	34.01	13.025
%FLC + FLC-LZPO	5	48.41	49.862	20.74	75.79	55.05	22.531	5	48.98	50.571	26.49	67.83	41.34	15.757
%PF	5	2.93	0.795	0.00	10.54	10.54	4.412	5	8.12	1.516	0.43	24.19	23.76	10.603
%PF-LAM	5	13.96	11.730	6.84	28.47	21.63	8.522	5	9.05	9.404	7.47	10.08	2.61	0.996
%LAM	5	22.06	11.749	0.00	53.75	53.75	25.503	5	13.03	13.798	1.94	23.70	21.76	10.130
%SF	5	9.31	9.891	6.96	11.48	4.51	1.998	5	3.92	3.436	1.49	6.33	4.84	2.079
%EPF	5	0.05	0.000	0.00	0.26	0.26	0.117	5	1.13	0.786	0.36	2.21	1.84	0.758
%EPF-LAM	5	1.12	0.394	0.00	3.36	3.36	1.464	5	3.63	2.030	1.51	7.81	6.30	2.731
%ELAM	5	0.26	0.214	0.00	0.69	0.69	0.289	5	0.29	0.000	0.00	1.16	1.16	0.503
%ECCC	5	0.13	0.000	0.00	0.67	0.67	0.299	5	0.52	0.575	0.00	0.99	0.99	0.381
%HAV	5	1.76	2.211	0.00	4.03	4.03	1.702	5	11.33	9.783	2.65	24.60	21.95	8.637
%INT	5	0.00	0.000	0.00	0.00	0.00	0.000	5	0.00	0.000	0.00	0.00	0.00	0.000
%END	5	1.56	1.748	0.00	3.58	3.58	1.471	5	5.57	3.695	2.76	9.47	6.72	3.417
%PER	5	96.67	95.335	94.21	100.00	5.79	2.896	5	83.10	87.400	65.93	94.59	28.67	11.718
<u>M1</u>								<u>M1</u>						
%WOV	5	0.00	0.000	0.00	0.00	0.00	0.000	6	0.16	0.000	0.00	0.98	0.98	0.402
%FLC	5	0.29	0.000	0.00	1.47	1.47	0.656	6	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	5	5.34	2.292	0.86	10.74	9.87	4.885	6	12.48	8.369	2.22	36.44	34.22	12.299
%FLC + FLC-LZPO	5	5.63	2.292	0.86	12.04	11.18	5.304	6	12.48	8.369	2.22	36.44	34.22	12.299
%PF	5	0.60	0.157	0.00	2.48	2.48	1.059	6	0.44	0.137	0.00	1.39	1.39	0.592
%PF-LAM	5	6.38	6.153	3.82	9.34	5.52	2.273	6	4.63	3.138	1.77	11.59	9.82	3.658
%LAM	5	67.65	62.506	55.39	80.57	25.18	10.834	6	38.62	39.624	29.00	49.09	20.09	6.834
%SF	5	11.10	10.593	8.68	14.37	5.70	2.123	6	2.61	2.770	0.89	3.90	3.01	0.977
%EPF	5	0.18	0.000	0.00	0.75	0.75	0.323	6	0.62	0.213	0.00	1.69	1.69	0.796
%EPF-LAM	5	1.45	1.389	0.11	3.37	3.26	1.315	6	6.97	6.964	4.04	9.73	5.69	2.410
%ELAM	5	0.81	0.152	0.10	2.73	2.63	1.131	6	4.72	3.768	1.20	9.64	8.44	3.006
%ECCC	5	0.00	0.000	0.00	0.00	0.00	0.000	6	3.96	3.176	2.54	7.00	4.46	1.730
%HAV	5	6.20	5.963	1.07	12.62	11.55	4.682	6	23.84	23.294	10.90	35.59	24.69	9.640
%INT	5	0.00	0.000	0.00	0.00	0.00	0.000	6	0.96	1.245	0.00	1.37	1.37	0.555
%END	5	2.43	2.317	0.23	6.25	6.02	2.421	6	16.26	15.878	11.31	23.44	12.13	4.978
%PER	5	91.36	93.553	81.13	97.22	16.09	6.722	6	58.93	58.866	49.82	70.72	20.89	7.106

TABLE F-3 a continued. Whole-section tissue type proportions: Descriptive statistics for *C. aethiops* by age class.
Data reported as a % of total cortical area.

Tissue Type	FEMUR							HUMERUS						
	N	Mean	Median	Min	Max	Range	SD	N	Mean	Median	Min	Max	Range	SD
<u>M2</u>								<u>M2</u>						
%FLC	6	0.00	0.000	0.00	0.00	0.00	0.000	6	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	6	0.93	0.559	0.18	2.04	1.86	0.802	6	4.89	4.046	1.99	10.46	8.47	3.119
%FLC + FLC-LZPO	6	0.93	0.559	0.18	2.04	1.86	0.802	6	4.89	4.046	1.99	10.46	8.47	3.119
%PF	6	0.08	0.000	0.00	0.45	0.45	0.184	6	0.14	0.096	0.00	0.45	0.45	0.181
%PF-LAM	6	2.73	1.971	0.00	7.30	7.30	2.982	6	2.54	2.063	0.92	5.61	4.68	1.710
%LAM	6	76.40	78.726	61.92	90.11	28.20	10.532	6	56.25	55.948	44.96	64.30	19.34	6.628
%SF	6	7.55	7.764	4.77	9.87	5.10	2.190	6	1.90	1.686	0.85	2.83	1.98	0.780
%EPF	6	0.12	0.084	0.00	0.42	0.42	0.157	6	0.00	0.000	0.00	0.00	0.00	0.000
%EPF-LAM	6	1.51	1.029	0.00	4.39	4.39	1.553	6	2.22	1.828	0.07	4.43	4.36	1.708
%ELAM	6	0.66	0.468	0.00	2.14	2.14	0.783	6	7.38	6.831	0.98	14.24	13.26	5.604
%ECCC	6	3.21	2.610	0.00	7.63	7.63	2.828	6	4.89	4.883	2.24	7.48	5.24	2.120
%HAV	6	6.82	8.406	0.17	10.08	9.91	3.663	6	19.43	19.757	12.76	24.30	11.55	4.246
%INT	6	0.00	0.000	0.00	0.00	0.00	0.000	6	0.36	0.362	0.00	0.67	0.67	0.267
%END	6	5.50	4.314	0.00	14.25	14.25	5.102	6	14.49	14.700	4.83	24.91	20.08	7.621
%PER	6	87.69	88.068	76.94	99.83	22.89	7.501	6	65.72	67.358	51.74	73.58	21.84	7.583
<u>M3</u>								<u>M3</u>						
%FLC	8	0.00	0.000	0.00	0.00	0.00	0.000	7	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	8	0.20	0.080	0.00	0.74	0.74	0.266	7	3.49	2.267	2.08	6.78	4.69	2.042
%FLC + FLC-LZPO	8	0.20	0.080	0.00	0.74	0.74	0.266	7	3.49	2.267	2.08	6.78	4.69	2.042
%PF	8	0.12	0.029	0.00	0.53	0.53	0.199	7	0.00	0.000	0.00	0.00	0.00	0.000
%PF-LAM	8	0.61	0.446	0.13	1.77	1.65	0.562	7	1.33	1.395	0.25	2.43	2.18	0.776
%LAM	8	75.58	77.079	59.69	87.41	27.73	9.004	7	54.06	56.280	46.34	58.99	12.64	5.053
%SF	8	5.17	5.062	2.93	7.63	4.70	1.668	7	1.68	0.827	0.21	3.97	3.76	1.575
%EPF	8	0.01	0.000	0.00	0.09	0.09	0.033	7	0.10	0.000	0.00	0.63	0.63	0.234
%EPF-LAM	8	1.42	0.732	0.00	7.29	7.29	2.406	7	2.13	1.908	0.37	4.93	4.56	1.927
%ELAM	8	3.98	1.603	0.04	17.82	17.78	5.847	7	8.41	5.992	2.23	20.58	18.35	6.060
%ECCC	8	2.94	1.858	0.00	9.32	9.32	3.089	7	5.59	3.765	2.16	10.79	8.63	3.742
%HAV	8	9.98	9.299	4.07	20.91	16.85	5.380	7	23.07	25.770	13.40	36.53	23.13	8.416
%INT	8	0.00	0.000	0.00	0.00	0.00	0.000	7	0.13	0.122	0.00	0.28	0.28	0.112
%END	8	8.35	5.416	1.47	22.41	20.94	7.590	7	16.24	14.063	5.52	25.50	19.98	8.390
%PER	8	81.67	83.105	67.29	93.94	26.65	9.372	7	60.56	60.371	57.83	65.23	7.40	2.522

TABLE F-3 a continued. Whole-section tissue type proportions: Descriptive statistics for *C. aethiops* by age class.
 Data reported as a % of total cortical area.

Tissue Type	FEMUR							HUMERUS						
	N	Mean	Median	Min	Max	Range	SD	N	Mean	Median	Min	Max	Range	SD
<u>EPIPH</u>								<u>EPIPH</u>						
%FLC	6	0.00	0.000	0.00	0.00	0.00	0.000	6	0.49	0.000	0.00	2.73	2.73	1.102
%FLC-LZPO	6	0.15	0.000	0.00	0.81	0.81	0.326	6	2.43	1.098	0.45	8.78	8.34	3.169
%FLC + FLC-LZPO	6	0.15	0.000	0.00	0.81	0.81	0.326	6	2.92	1.751	0.64	8.78	8.14	3.071
%PF	6	0.04	0.000	0.00	0.12	0.12	0.062	6	0.06	0.000	0.00	0.29	0.29	0.117
%PF-LAM	6	0.84	0.121	0.00	3.98	3.98	1.565	6	0.73	0.398	0.10	1.90	1.80	0.695
%LAM	6	73.18	75.994	54.54	89.49	34.95	12.926	6	56.70	54.495	51.29	65.23	13.95	5.390
%SF	6	4.98	4.402	3.51	8.52	5.01	1.799	6	1.63	1.553	1.40	2.07	0.67	0.257
%EPF	6	0.06	0.000	0.00	0.36	0.36	0.147	6	0.07	0.000	0.00	0.24	0.24	0.107
%EPF-LAM	6	2.38	1.893	0.00	5.04	5.04	2.100	6	1.57	1.457	0.00	4.29	4.29	1.601
%ELAM	6	3.01	2.856	0.12	6.88	6.76	2.491	6	6.62	7.135	3.00	9.25	6.25	2.111
%ECCC	6	3.76	3.166	0.02	8.83	8.80	3.613	6	2.98	3.275	1.47	4.19	2.72	1.242
%HAV	6	11.60	10.320	5.63	22.41	16.78	5.806	6	26.47	25.752	22.76	30.22	7.46	2.858
%INT	6	0.00	0.000	0.00	0.00	0.00	0.000	6	0.25	0.186	0.00	0.69	0.69	0.256
%END	6	9.20	6.684	1.37	18.44	17.07	7.238	6	11.24	12.262	4.58	15.82	11.24	4.155
%PER	6	79.20	82.118	59.15	93.00	33.85	12.229	6	62.04	61.433	56.23	70.17	13.94	6.039

TABLE F-3 b. Whole-section tissue type proportions: Descriptive statistics for *H. lar* by age class.
 Data reported as a % of total cortical area.

Tissue Type	FEMUR							HUMERUS						
	N	Mean	Median	Min	Max	Range	SD	N	Mean	Median	Min	Max	Range	SD
<u>DECID.</u>								<u>DECID.</u>						
%FLC	4	0.08	0.000	0.00	0.31	0.31	0.155	4	0.41	0.000	0.00	1.65	1.65	0.827
%FLC-LZPO	4	2.45	1.333	0.41	6.73	6.32	2.917	4	2.45	1.491	0.00	6.82	6.82	3.022
%FLC + FLC-LZPO	4	2.53	1.488	0.41	6.73	6.32	2.862	4	2.86	2.317	0.00	6.82	6.82	2.872
%PF	4	8.41	5.978	3.50	18.20	14.70	6.693	4	5.83	4.592	2.86	11.28	8.42	3.769
%PF-LAM	4	17.47	18.267	13.27	20.08	6.80	3.025	4	9.94	9.137	6.96	14.54	7.58	3.668
%LAM	4	9.88	8.520	8.15	14.33	6.18	2.974	4	3.58	2.677	0.37	8.59	8.23	3.524
%SF	4	2.10	2.247	1.36	2.55	1.19	0.522	4	1.15	1.225	0.37	1.79	1.42	0.666
%EPF	4	0.59	0.558	0.00	1.26	1.26	0.574	4	3.82	3.329	0.30	8.33	8.02	3.563
%EPF-LAM	4	6.37	5.329	4.48	10.36	5.89	2.695	4	11.53	12.793	3.46	17.08	13.62	5.839
%ELAM	4	8.82	5.368	3.20	21.34	18.13	8.408	4	10.94	7.438	2.42	26.46	24.03	11.328
%ECCC	4	1.17	0.318	0.17	3.86	3.69	1.801	4	5.05	4.269	1.16	10.49	9.33	4.558
%HAV	4	42.41	41.168	35.12	52.17	17.05	8.157	4	45.13	41.389	36.69	61.04	24.35	10.878
%INT	4	0.25	0.164	0.00	0.66	0.66	0.317	4	0.16	0.102	0.00	0.45	0.45	0.204
%END	4	16.95	14.946	10.15	27.76	17.61	8.500	4	31.34	29.036	23.86	43.41	19.55	8.586
%PER	4	40.39	37.068	34.21	53.23	19.03	8.680	4	23.37	24.170	11.57	33.57	22.00	11.067
<u>M1</u>								<u>M1</u>						
%FLC	3	0.00	0.000	0.00	0.00	0.00	0.000	3	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	3	6.28	6.674	3.46	8.72	5.26	2.649	3	6.81	5.387	1.94	13.09	11.16	5.712
%FLC + FLC-LZPO	3	6.28	6.674	3.46	8.72	5.26	2.649	3	6.81	5.387	1.94	13.09	11.16	5.712
%PF	3	3.57	1.757	0.41	8.54	8.13	4.357	3	4.77	3.428	3.20	7.68	4.48	2.525
%PF-LAM	3	17.38	17.421	14.89	19.83	4.94	2.471	3	20.88	20.222	14.17	28.25	14.08	7.063
%LAM	3	15.68	15.043	5.62	26.37	20.75	10.391	3	2.57	3.461	0.35	3.90	3.55	1.935
%SF	3	4.80	5.499	2.04	6.87	4.84	2.492	3	4.48	3.126	2.74	7.57	4.83	2.682
%EPF	3	0.19	0.000	0.00	0.57	0.57	0.329	3	0.74	0.700	0.00	1.53	1.53	0.766
%EPF-LAM	3	2.27	2.099	0.95	3.75	2.80	1.409	3	3.24	2.386	2.37	4.97	2.60	1.496
%ELAM	3	8.39	7.822	6.71	10.63	3.92	2.022	3	8.72	10.954	3.63	11.59	7.97	4.427
%ECCC	3	0.66	0.568	0.35	1.06	0.71	0.362	3	1.07	0.747	0.34	2.14	1.80	0.945
%HAV	3	40.58	38.681	35.67	47.38	11.71	6.079	3	46.63	47.160	40.97	51.75	10.78	5.410
%INT	3	0.21	0.082	0.04	0.50	0.45	0.251	3	0.08	0.046	0.02	0.18	0.16	0.088
%END	3	11.50	12.142	8.71	13.65	4.94	2.531	3	13.78	16.120	7.86	17.37	9.50	5.165
%PER	3	47.72	47.171	40.40	55.58	15.18	7.603	3	39.50	40.200	35.45	42.86	7.41	3.755

TABLE F-3 b continued. Whole-section tissue type proportions: Descriptive statistics for *H. lar* by age class.
Data reported as a % of total cortical area.

Tissue Type	FEMUR							HUMERUS						
	N	Mean	Median	Min	Max	Range	SD	N	Mean	Median	Min	Max	Range	SD
M2								M2						
%FLC	6	0.27	0.000	0.00	1.63	1.63	0.666	5	0.12	0.000	0.00	0.58	0.58	0.261
%FLC-LZPO	6	0.95	0.874	0.34	1.84	1.50	0.507	5	0.74	0.435	0.00	2.35	2.35	0.927
%FLC + FLC-LZPO	6	1.22	0.874	0.34	3.47	3.13	1.133	5	0.86	0.435	0.00	2.35	2.35	0.947
%PF	6	0.00	0.000	0.00	0.00	0.00	0.000	5	0.92	0.219	0.00	3.23	3.23	1.378
%PF-LAM	6	9.01	7.914	2.90	16.29	13.39	4.987	5	13.09	7.240	3.92	27.39	23.47	10.174
%LAM	6	49.02	48.372	43.77	57.98	14.22	4.762	5	26.87	25.403	10.75	37.73	26.98	11.237
%SF	6	4.42	3.660	2.09	8.84	6.75	2.658	5	3.71	3.677	1.51	6.58	5.07	2.100
%EPF	6	0.00	0.000	0.00	0.00	0.00	0.000	5	0.00	0.000	0.00	0.00	0.00	0.000
%EPF-LAM	6	0.76	0.725	0.00	1.75	1.75	0.621	5	1.14	0.705	0.60	2.27	1.67	0.722
%ELAM	6	1.99	2.444	0.04	3.42	3.38	1.468	5	2.91	2.153	0.51	7.23	6.73	2.644
%ECCC	6	0.12	0.031	0.00	0.60	0.60	0.235	5	0.08	0.000	0.00	0.34	0.34	0.146
%HAV	6	33.43	32.239	25.49	46.09	20.60	8.067	5	50.29	45.851	35.43	66.82	31.40	12.280
%INT	6	0.03	0.030	0.00	0.05	0.05	0.024	5	0.12	0.083	0.02	0.34	0.32	0.128
%END	6	2.87	2.897	0.04	5.12	5.07	1.860	5	4.14	3.629	1.10	7.94	6.84	2.822
%PER	6	63.67	63.968	53.87	71.50	17.64	7.308	5	45.45	48.066	31.99	60.92	28.93	12.486
M3								M3						
%FLC	9	0.00	0.000	0.00	0.00	0.00	0.000	7	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	9	0.17	0.148	0.00	0.40	0.40	0.174	7	0.16	0.000	0.00	0.64	0.64	0.241
%FLC + FLC-LZPO	9	0.17	0.148	0.00	0.40	0.40	0.174	7	0.16	0.000	0.00	0.64	0.64	0.241
%PF	9	0.00	0.000	0.00	0.00	0.00	0.000	7	0.00	0.000	0.00	0.00	0.00	0.000
%PF-LAM	9	1.86	1.737	0.40	4.17	3.77	1.213	7	2.01	1.747	0.68	4.87	4.19	1.377
%LAM	9	54.25	56.394	32.98	67.91	34.93	11.664	7	38.93	39.711	23.48	46.57	23.09	7.842
%SF	9	2.62	2.711	1.17	4.86	3.69	1.322	7	2.67	2.189	1.21	4.51	3.29	1.453
%EPF	9	0.00	0.000	0.00	0.00	0.00	0.000	7	0.00	0.000	0.00	0.00	0.00	0.000
%EPF-LAM	9	0.18	0.065	0.00	0.93	0.93	0.302	7	0.11	0.044	0.00	0.36	0.36	0.138
%ELAM	9	3.50	2.931	0.94	6.65	5.70	1.809	7	2.96	3.279	1.36	3.79	2.43	0.965
%ECCC	9	0.03	0.000	0.00	0.18	0.18	0.059	7	0.02	0.000	0.00	0.12	0.12	0.047
%HAV	9	37.35	35.017	27.73	56.97	29.25	9.648	8	51.78	50.406	43.13	66.43	23.29	7.416
%INT	9	0.03	0.035	0.00	0.09	0.09	0.031	7	0.14	0.130	0.08	0.21	0.13	0.052
%END	9	3.71	2.961	1.30	6.81	5.50	1.938	7	3.08	3.279	1.66	4.09	2.43	0.930
%PER	9	58.90	62.456	36.18	70.72	34.54	11.248	7	43.71	44.302	30.20	51.76	21.56	6.515

TABLE F-3 b continued. Whole-section tissue type proportions: Descriptive statistics for *H. lar* by age class.
 Data reported as a % of total cortical area.

Tissue Type	FEMUR							HUMERUS						
	N	Mean	Median	Min	Max	Range	SD	N	Mean	Median	Min	Max	Range	SD
EPIPH								EPIPH						
%FLC	6	0.00	0.000	0.00	0.00	0.00	0.000	6	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	6	0.01	0.000	0.00	0.06	0.06	0.026	6	0.07	0.000	0.00	0.28	0.28	0.113
%FLC + FLC-LZPO	6	0.01	0.000	0.00	0.06	0.06	0.026	6	0.07	0.000	0.00	0.28	0.28	0.113
%PF	6	0.01	0.000	0.00	0.03	0.03	0.012	6	0.00	0.000	0.00	0.00	0.00	0.000
%PF-LAM	6	1.10	1.053	0.21	1.70	1.49	0.537	6	2.68	2.094	0.81	6.84	6.03	2.105
%LAM	6	54.34	53.644	45.80	67.78	21.98	8.170	6	39.87	40.607	29.27	50.22	20.95	7.078
%SF	6	1.61	1.589	0.69	2.44	1.76	0.740	6	4.25	3.907	2.29	7.07	4.78	1.838
%EPF	6	0.00	0.000	0.00	0.00	0.00	0.000	6	0.00	0.000	0.00	0.00	0.00	0.000
%EPF-LAM	6	0.23	0.064	0.00	1.08	1.08	0.423	6	0.05	0.018	0.00	0.20	0.20	0.079
%ELAM	6	2.49	2.584	0.62	3.75	3.12	1.122	6	4.03	4.008	0.64	6.61	5.97	2.042
%ECCC	6	0.02	0.000	0.00	0.08	0.08	0.034	6	0.00	0.000	0.00	0.00	0.00	0.000
%HAV	6	40.16	41.477	28.04	48.64	20.60	7.475	6	48.95	45.960	40.92	64.84	23.92	9.238
%INT	6	0.03	0.028	0.01	0.09	0.08	0.027	6	0.09	0.046	0.00	0.27	0.27	0.101
%END	6	2.74	3.007	0.62	3.90	3.28	1.148	6	4.09	4.069	0.64	6.81	6.17	2.095
%PER	6	57.07	56.700	47.88	69.51	21.63	7.947	6	46.87	48.893	34.38	54.88	20.49	8.223

TABLE F-3 c. Whole-section tissue type proportions: Descriptive statistics for *P. troglodytes* by age class.
Data reported as a % of total cortical area.

Tissue Type	FEMUR						
	N	Mean	Median	Min	Max	Range	SD
<u>DECID</u>							
%FLC	2	0.80	0.802	0.00	1.60	1.60	1.135
%FLC-LZPO	2	12.31	12.307	7.81	16.81	9.00	6.362
%FLC + FLC-LZPO	2	13.11	13.109	9.41	16.81	7.39	5.227
%PF	2	5.27	5.275	1.73	8.82	7.09	5.015
%PF-LAM	2	12.40	12.399	6.85	17.94	11.09	7.843
%LAM	2	3.61	3.606	1.49	5.72	4.23	2.993
%SF	2	1.94	1.944	1.19	2.70	1.50	1.063
%EPF	2	2.43	2.425	0.77	4.08	3.31	2.339
%EPF-LAM	2	3.88	3.883	3.47	4.30	0.83	0.583
%ELAM	2	0.00	0.003	0.00	0.01	0.01	0.004
%ECCC	2	0.51	0.513	0.00	1.03	1.03	0.725
%HAV	2	56.74	56.740	55.77	57.71	1.95	1.378
%INT	2	0.10	0.103	0.00	0.21	0.21	0.145
%END	2	6.82	6.824	5.07	8.57	3.50	2.476
%PER	2	36.33	36.333	33.51	39.16	5.66	4.000
<u>M1</u>							
%FLC	1	0.00					
%FLC-LZPO	1	19.32					
%FLC + FLC-LZPO	1	19.32					
%PF	1	0.00					
%PF-LAM	1	1.95					
%LAM	1	29.21					
%SF	1	4.39					
%EPF	1	0.18					
%EPF-LAM	1	1.60					
%ELAM	1	1.90					
%ECCC	1	0.00					
%HAV	1	41.34					
%INT	1	0.12					
%END	1	3.67					
%PER	1	54.87					

TABLE F-3 c continued. Whole-section tissue type proportions: Descriptive statistics for *P. troglodytes* by age class.
Data reported as a % of total cortical area.

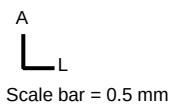
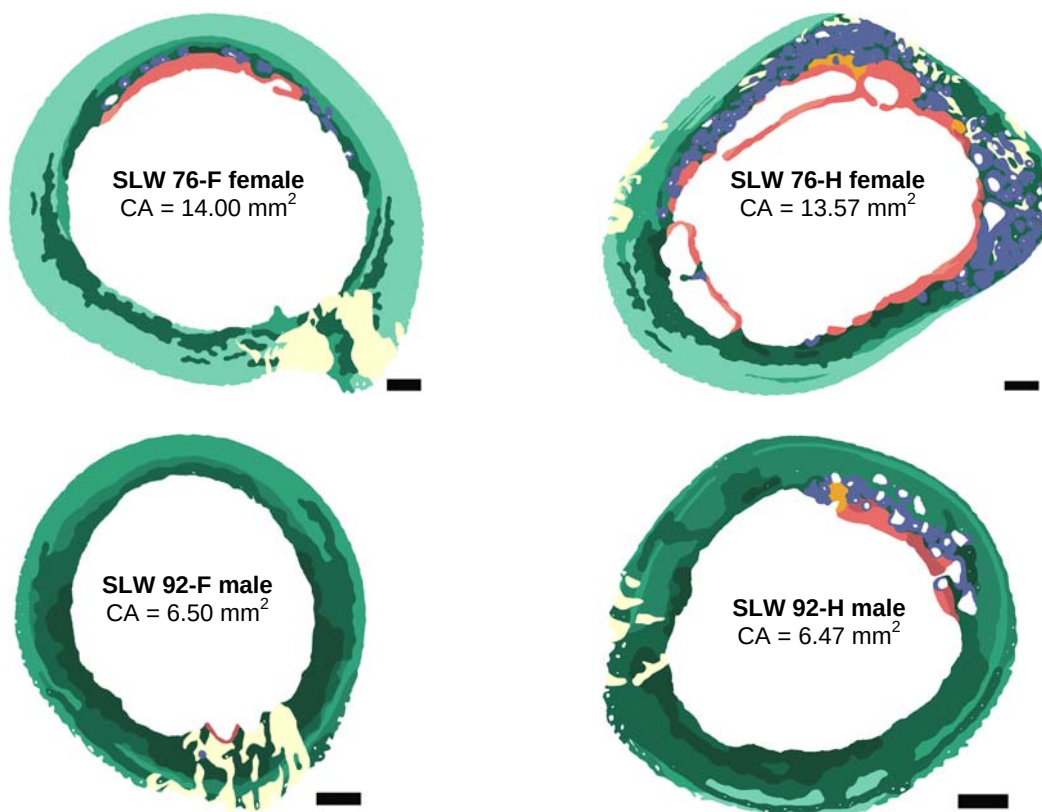
Tissue Type	FEMUR						
	N	Mean	Median	Min	Max	Range	SD
M2							
%FLC	4	0.13	0.000	0.00	0.50	0.50	0.250
%FLC-LZPO	4	12.20	13.003	4.33	18.46	14.12	5.863
%FLC + FLC-LZPO	4	12.32	13.003	4.83	18.46	13.62	5.641
%PF	4	0.00	0.000	0.00	0.00	0.00	0.000
%PF-LAM	4	0.42	0.146	0.00	1.37	1.37	0.644
%LAM	4	28.19	30.409	16.76	35.18	18.42	8.313
%SF	4	2.89	2.197	1.47	5.70	4.23	1.972
%EPF	4	0.07	0.057	0.00	0.18	0.18	0.088
%EPF-LAM	4	1.57	1.676	0.42	2.50	2.08	0.858
%ELAM	4	1.85	1.392	0.33	4.31	3.98	1.720
%ECCC	4	1.04	0.692	0.00	2.78	2.78	1.226
%HAV	4	51.46	50.917	44.53	59.48	14.95	7.163
%INT	4	0.18	0.212	0.02	0.29	0.27	0.117
%END	4	4.54	5.457	0.86	6.37	5.51	2.542
%PER	4	43.82	44.013	38.17	49.09	10.92	5.832
M3							
%FLC	3	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	3	2.94	3.415	0.19	5.22	5.04	2.553
%FLC + FLC-LZPO	3	2.94	3.415	0.19	5.22	5.04	2.553
%PF	3	0.00	0.000	0.00	0.00	0.00	0.000
%PF-LAM	3	0.21	0.218	0.19	0.22	0.03	0.016
%LAM	3	34.61	41.103	20.21	42.51	22.30	12.489
%SF	3	1.75	1.287	0.49	3.47	2.98	1.542
%EPF	3	0.01	0.010	0.00	0.02	0.02	0.008
%EPF-LAM	3	0.21	0.192	0.09	0.33	0.24	0.122
%ELAM	3	0.37	0.398	0.26	0.47	0.21	0.108
%ECCC	3	0.32	0.340	0.00	0.62	0.62	0.308
%HAV	3	59.45	51.599	48.61	78.13	29.53	16.253
%INT	3	0.13	0.049	0.03	0.32	0.29	0.162
%END	3	0.91	0.911	0.46	1.35	0.88	0.442
%PER	3	39.51	47.441	21.08	50.02	28.93	16.014

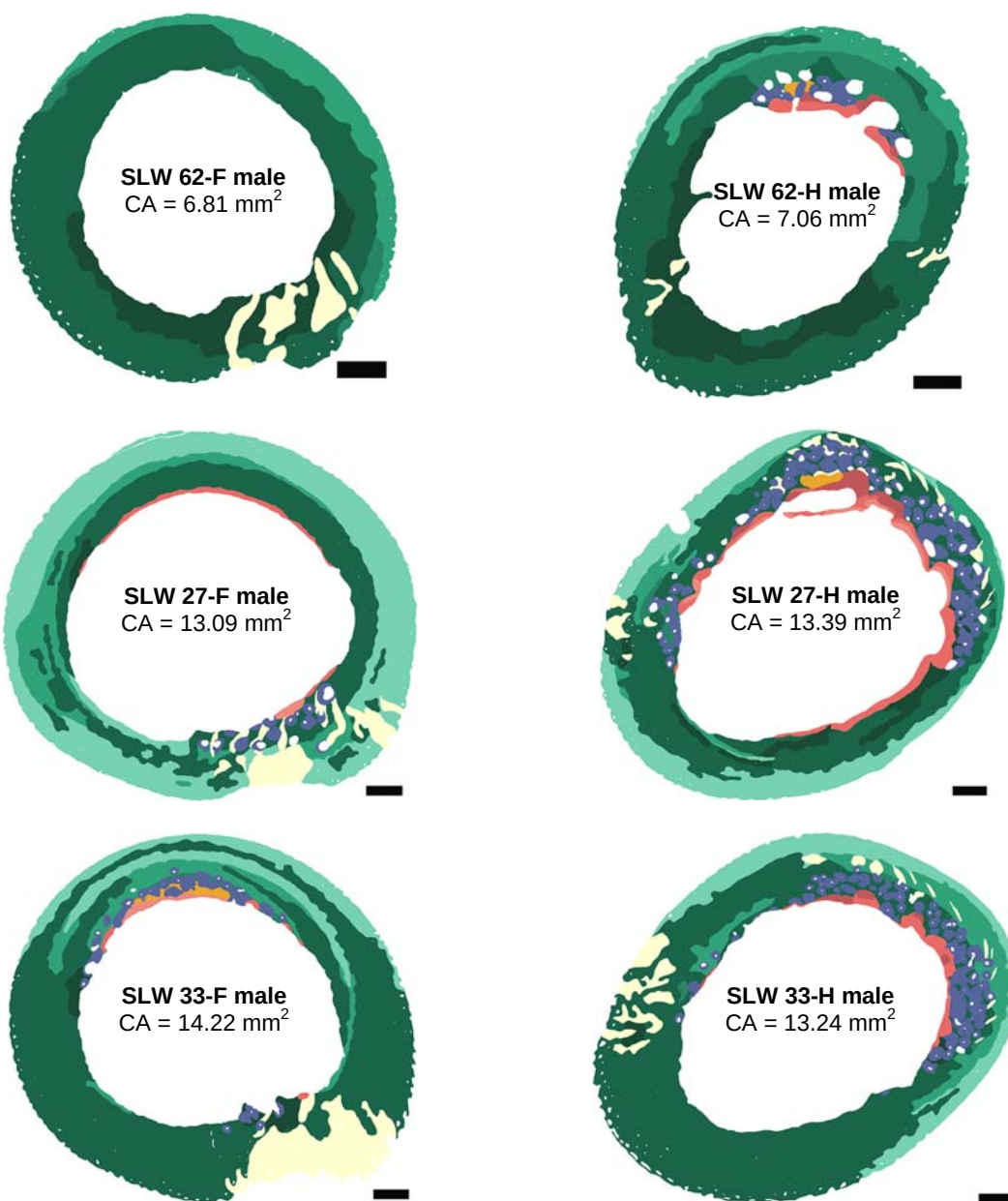
TABLE F-3 c continued. Whole-section tissue type proportions: Descriptive statistics for *P. troglodytes* by age class.
Data reported as a % of total cortical area.

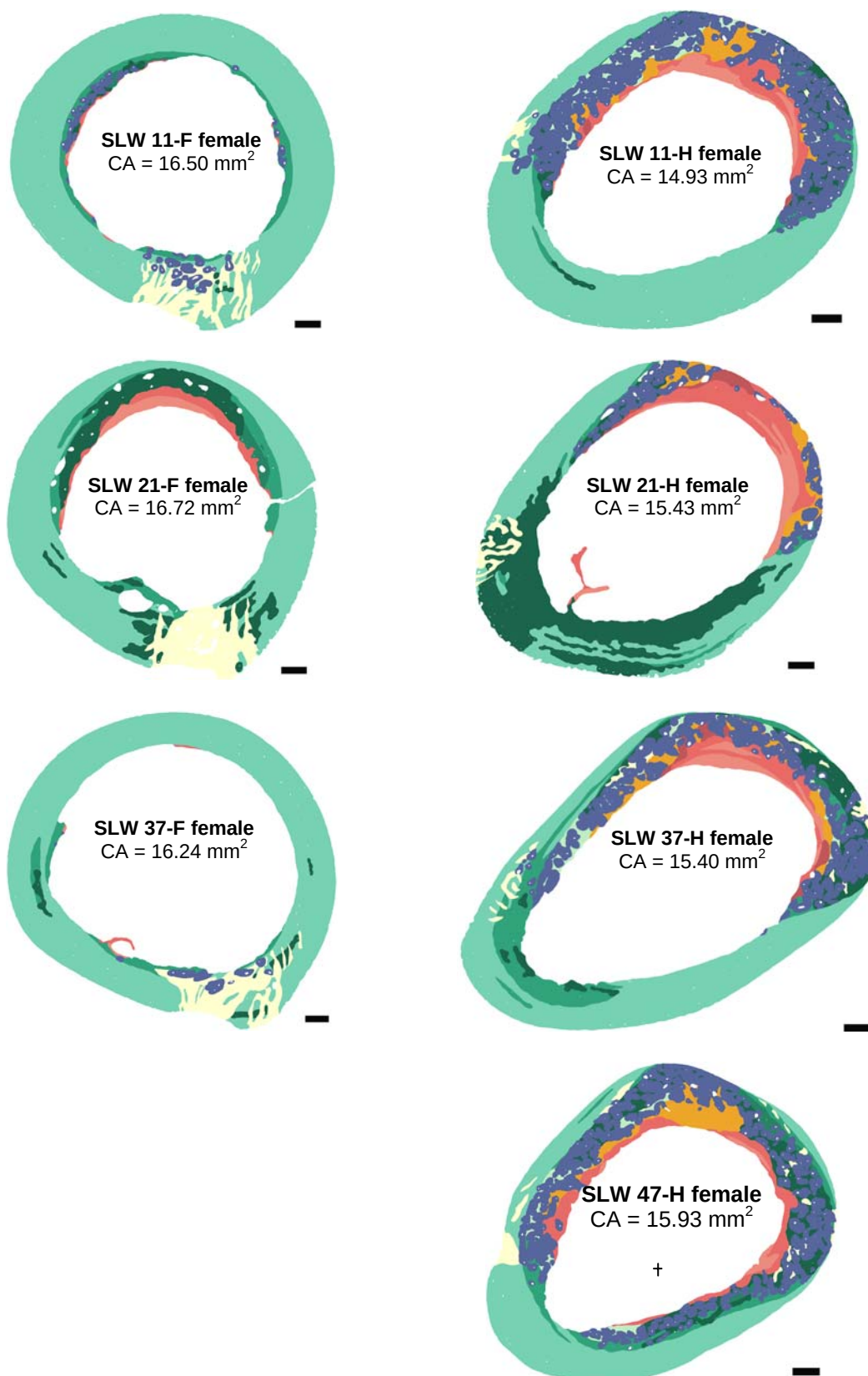
Tissue Type	FEMUR						
	N	Mean	Median	Min	Max	Range	SD
EPIPH							
%FLC	2	0.00	0.000	0.00	0.00	0.00	0.000
%FLC-LZPO	2	2.23	2.235	0.07	4.40	4.33	3.063
%FLC + FLC-LZPO	2	2.23	2.235	0.07	4.40	4.33	3.063
%PF	2	0.00	0.000	0.00	0.00	0.00	0.000
%PF-LAM	2	0.17	0.173	0.01	0.33	0.32	0.229
%LAM	2	39.46	39.461	24.88	54.04	29.17	20.623
%SF	2	1.42	1.417	0.21	2.62	2.41	1.702
%EPF	2	0.01	0.006	0.00	0.01	0.01	0.008
%EPF-LAM	2	0.21	0.205	0.14	0.27	0.13	0.089
%ELAM	2	1.60	1.603	0.76	2.45	1.70	1.199
%ECCC	2	0.14	0.135	0.13	0.14	0.01	0.010
%HAV	2	54.64	54.635	37.72	71.55	33.83	23.924
%INT	2	0.13	0.130	0.10	0.16	0.05	0.036
%END	2	1.95	1.949	1.05	2.85	1.80	1.270
%PER	2	43.29	43.285	25.50	61.07	35.58	25.158

APPENDIX G.**TISSUE TYPE MAPS**

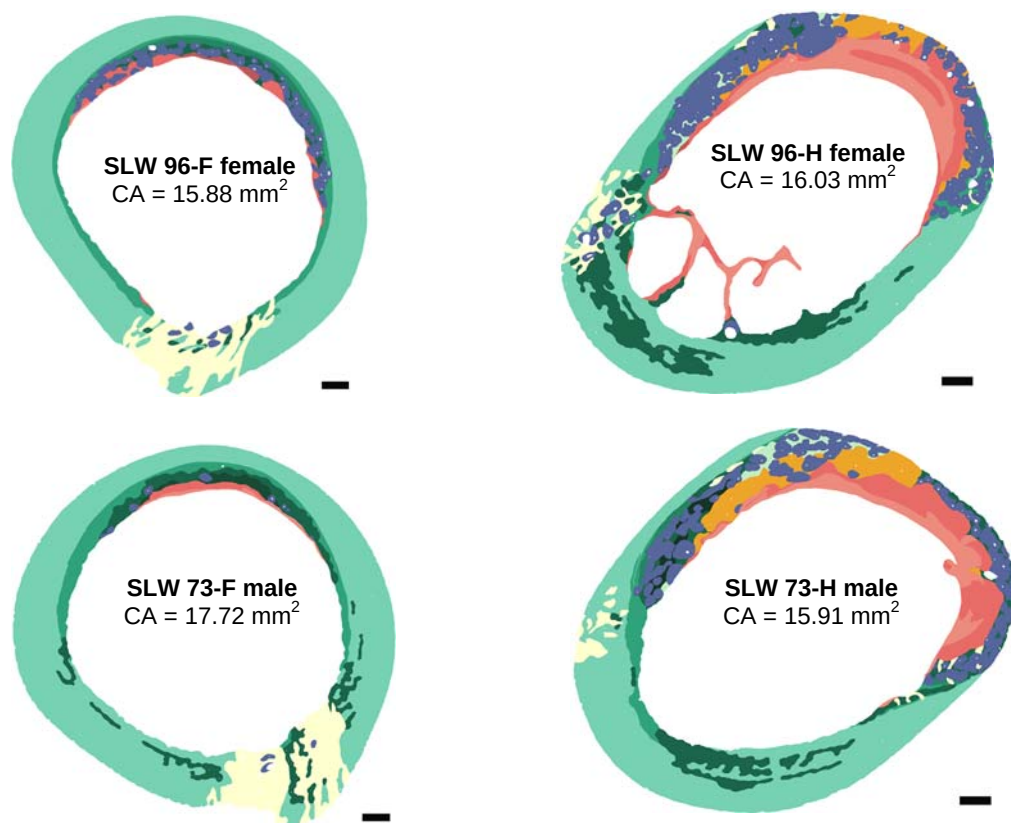
Tissue maps derived from the midshaft femur (F) and humerus (H) of all individuals examined in the current study. Colors denote bone regions represented by different tissue types, as shown in Figure 5-2. Cortical area (CA) dimensions are also provided.

**APPENDIX G-1 a. Tissue maps from *C. aethiops*: DECID age class.**

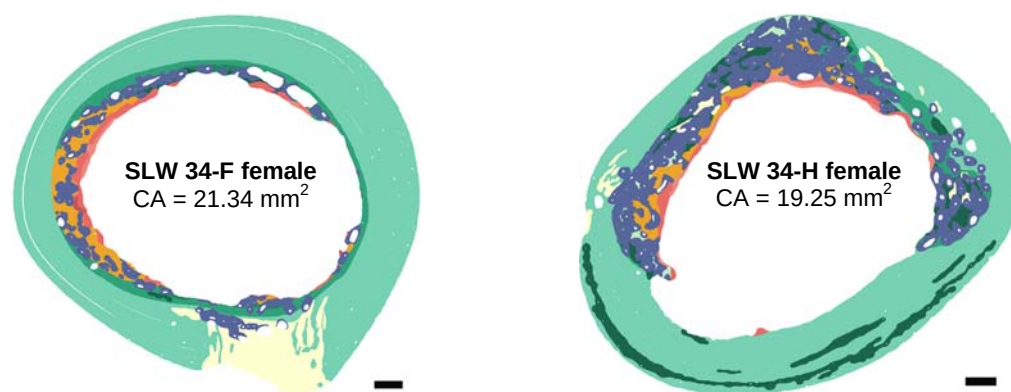
APPENDIX G-1 a continued. Tissue maps from *C. aethiops*: DECID age class.

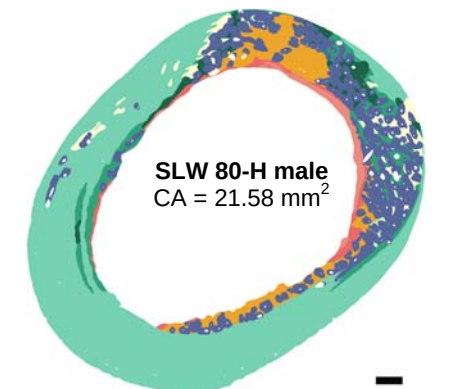
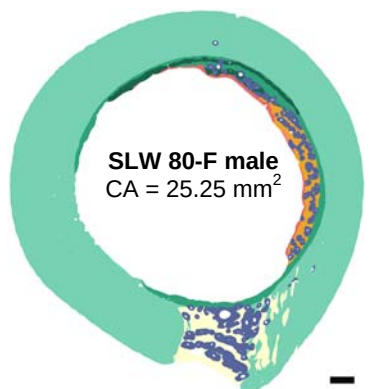
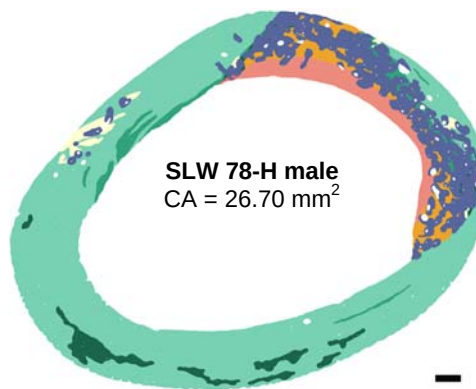
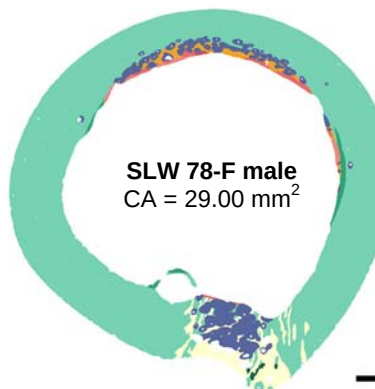
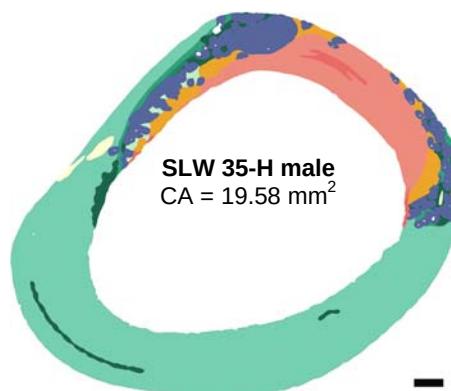
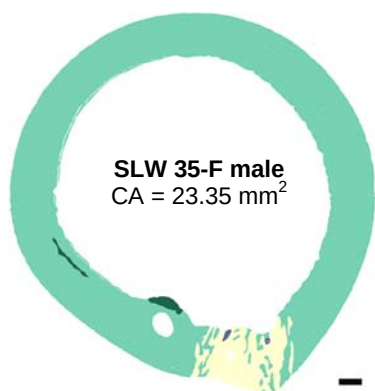
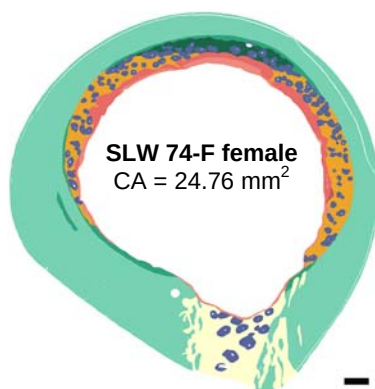
APPENDIX G-1 b. Tissue maps from *C. aethiops*: M1 age class.

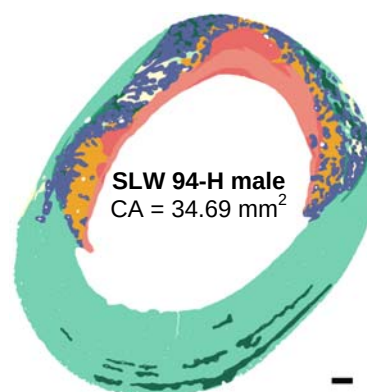
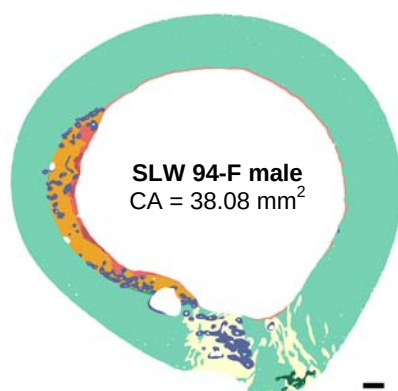
APPENDIX G-1 b continued. Tissue maps from *C. aethiops*: M1 age class.



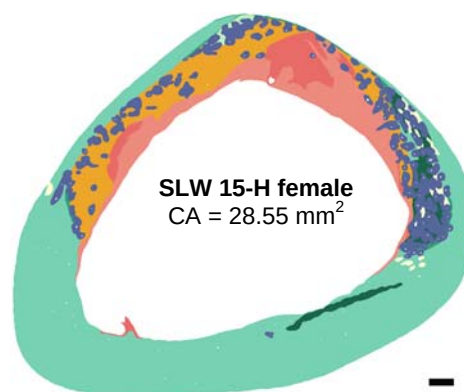
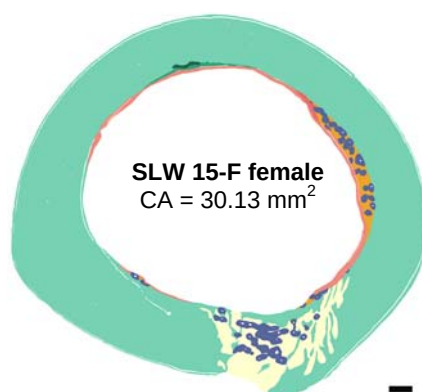
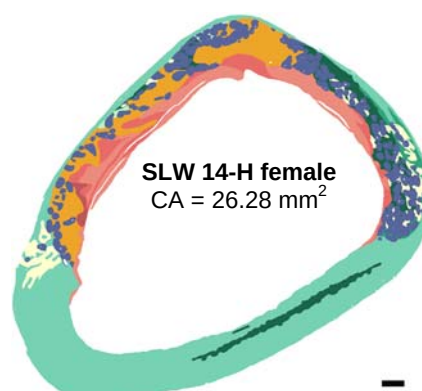
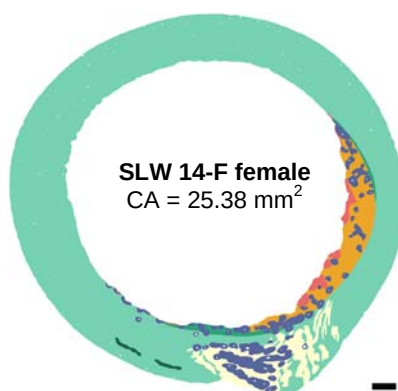
APPENDIX G-1 c. Tissue maps from *C. aethiops*: M2 age class.

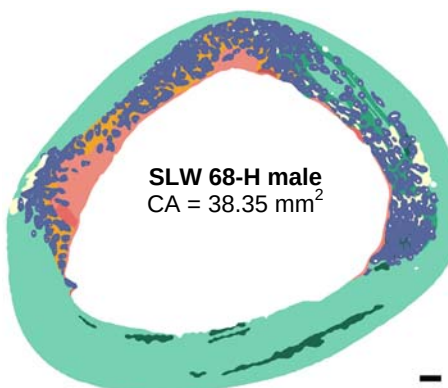
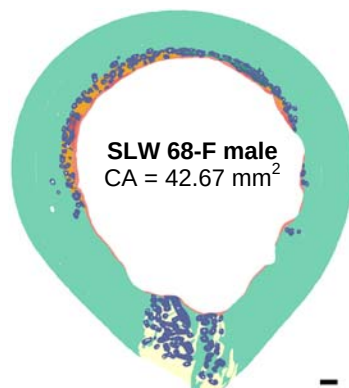
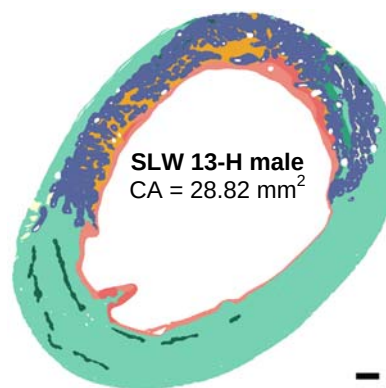
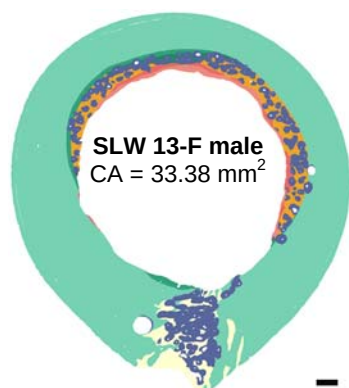
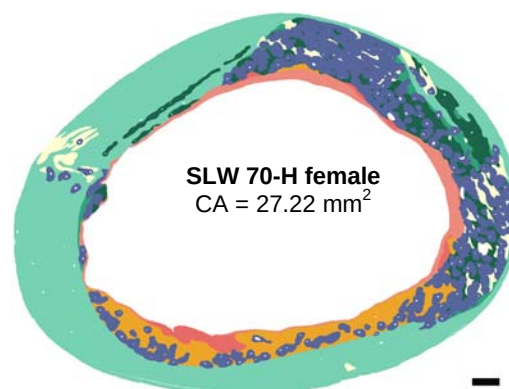
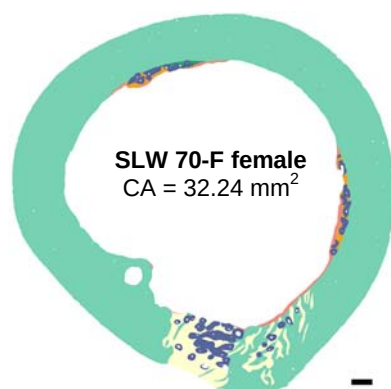
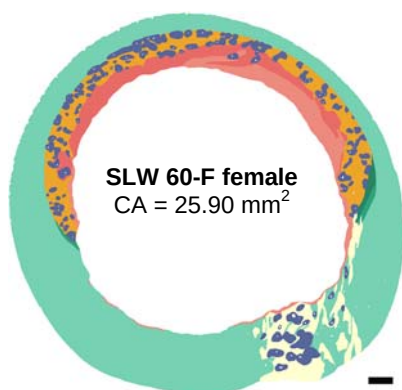


APPENDIX G-1 c continued. Tissue maps from *C. aethiops*: M2 age class.

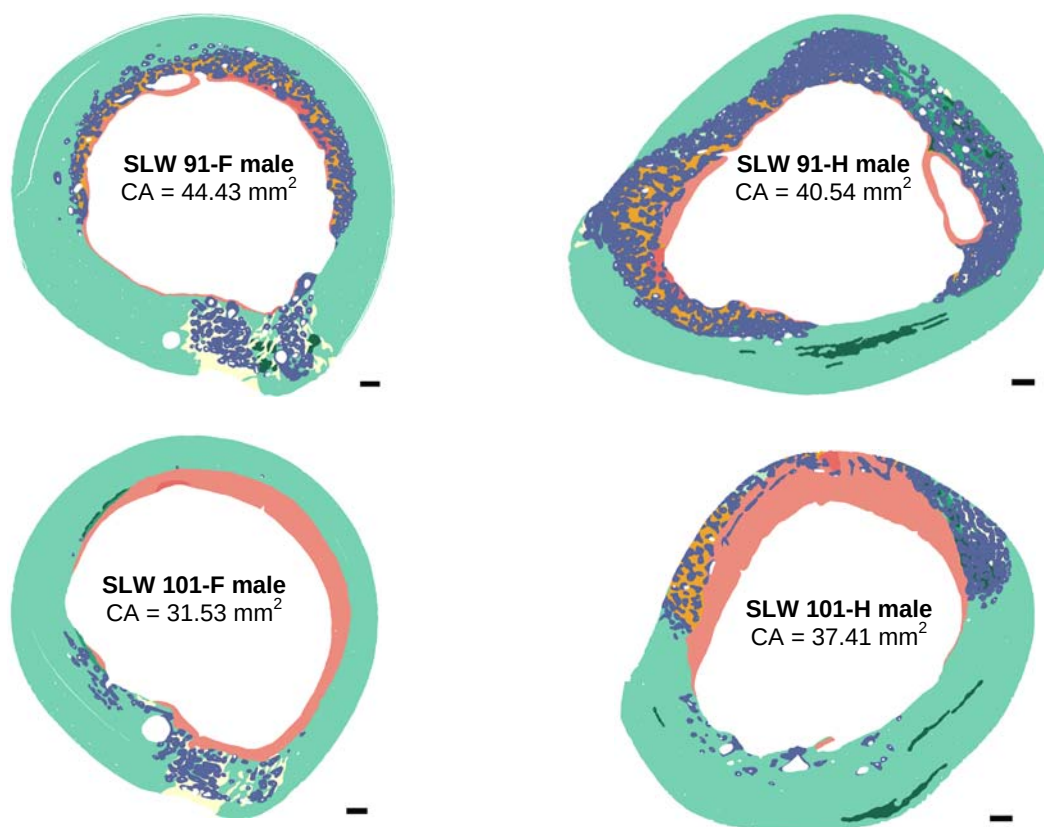


APPENDIX G-1 d. Tissue maps from *C. aethiops*: M3 age class.

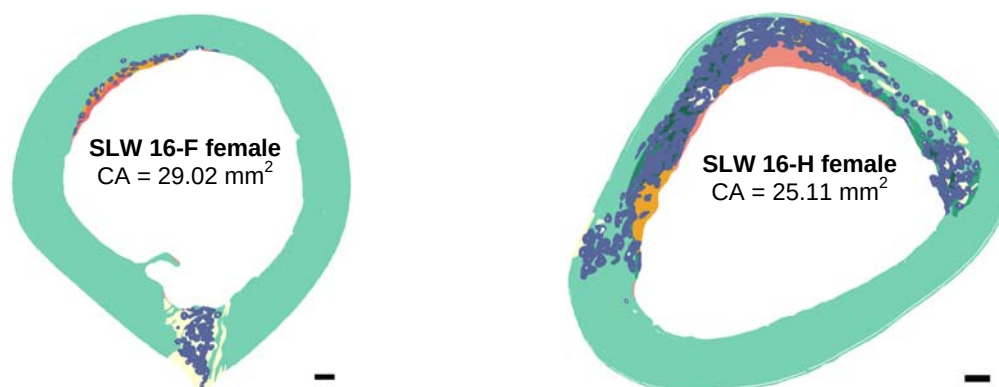


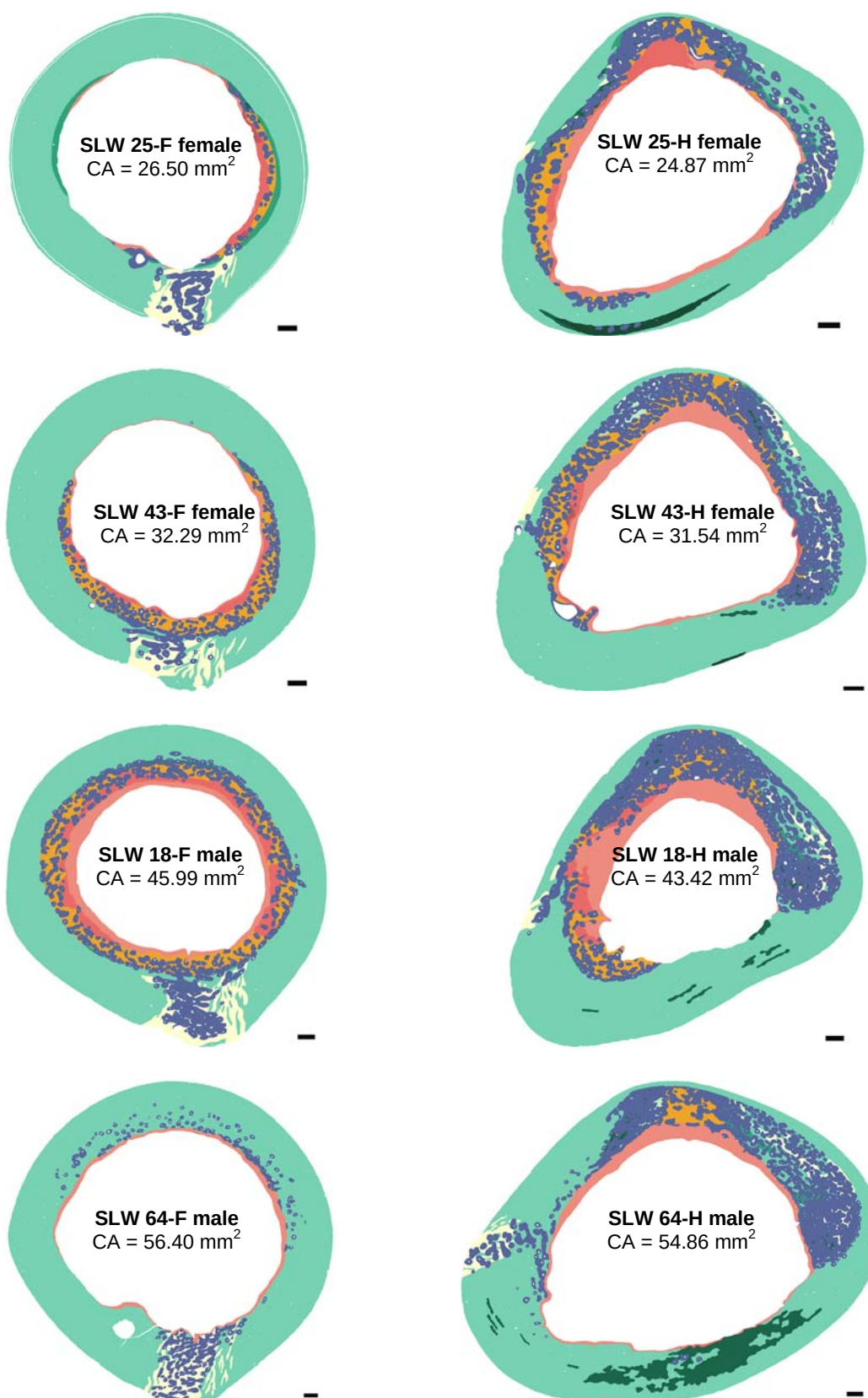
APPENDIX G-1 d continued. Tissue maps from *C. aethiops*: M3 age class.

APPENDIX G-1 d continued. Tissue maps from *C. aethiops*: M3 age class.

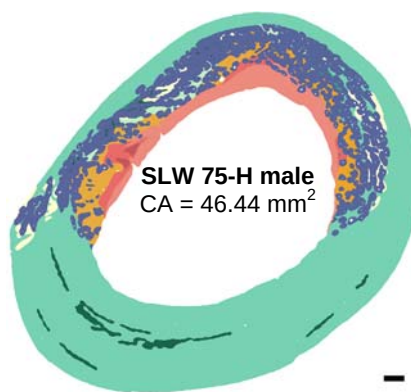
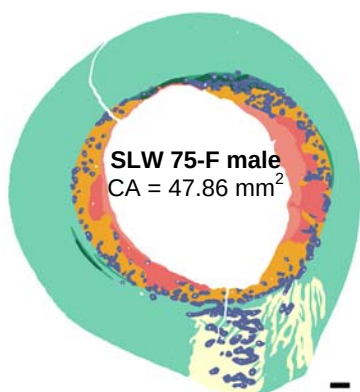


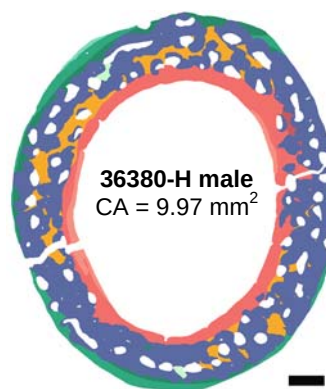
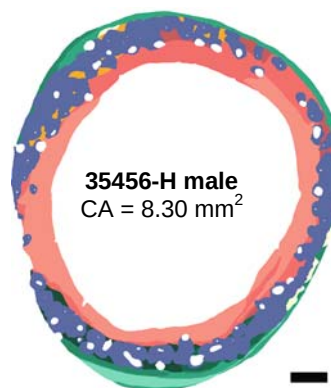
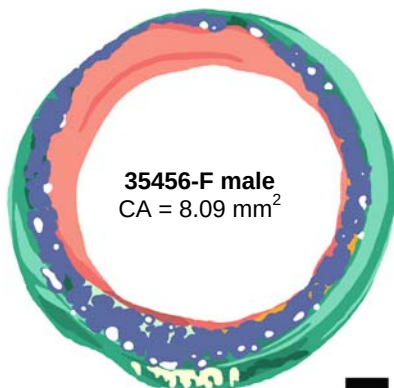
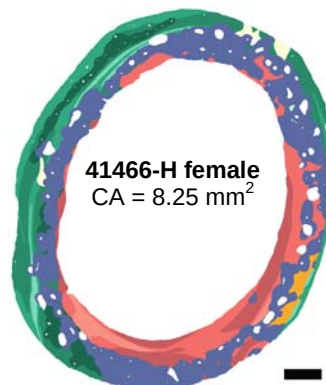
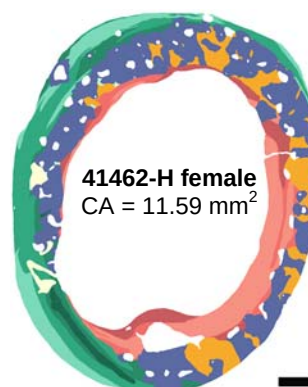
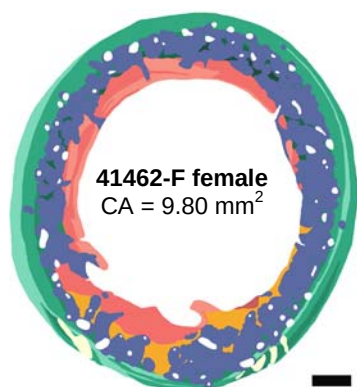
APPENDIX G-1 e. Tissue maps from *C. aethiops*: EPIPH age class.

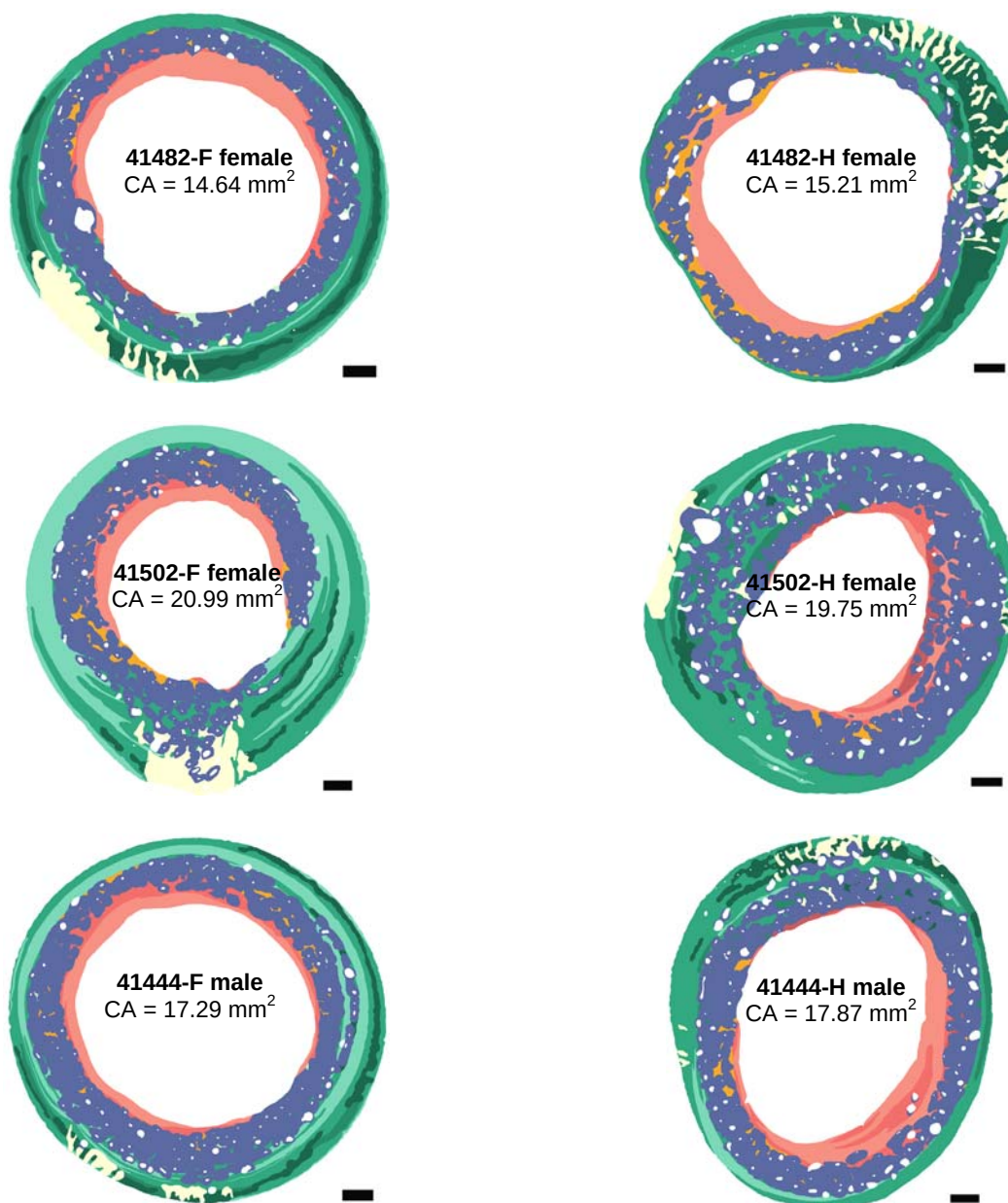


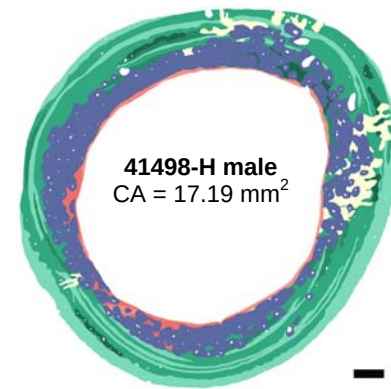
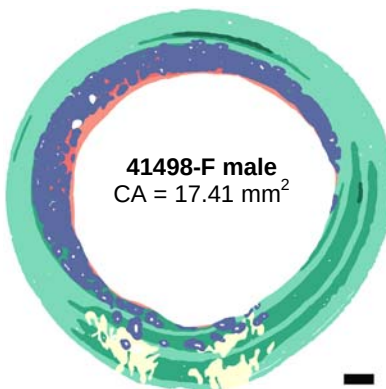
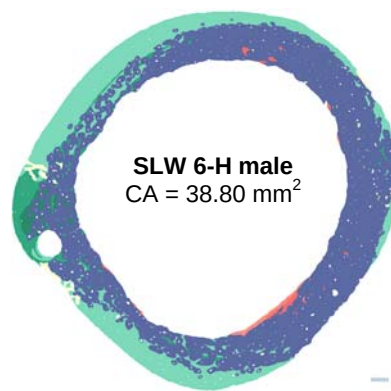
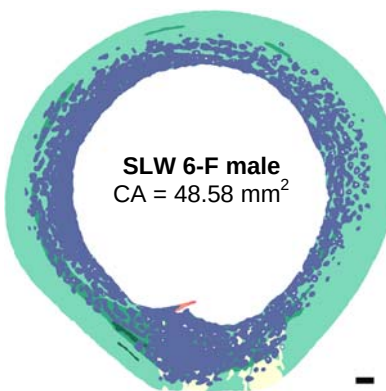
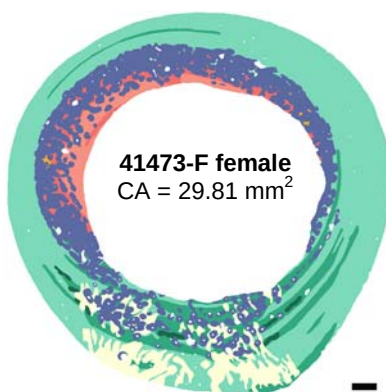
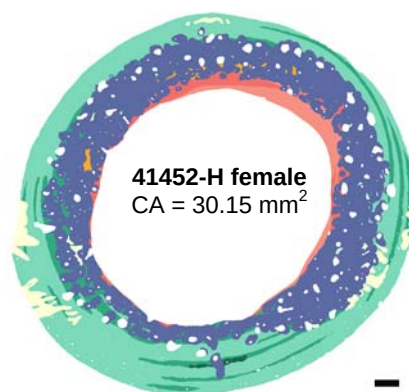
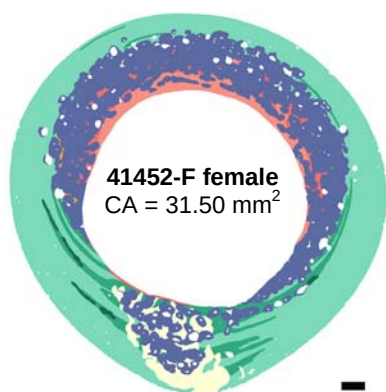
APPENDIX G-1 e continued. Tissue maps from *C. aethiops*: EPIPH age class.

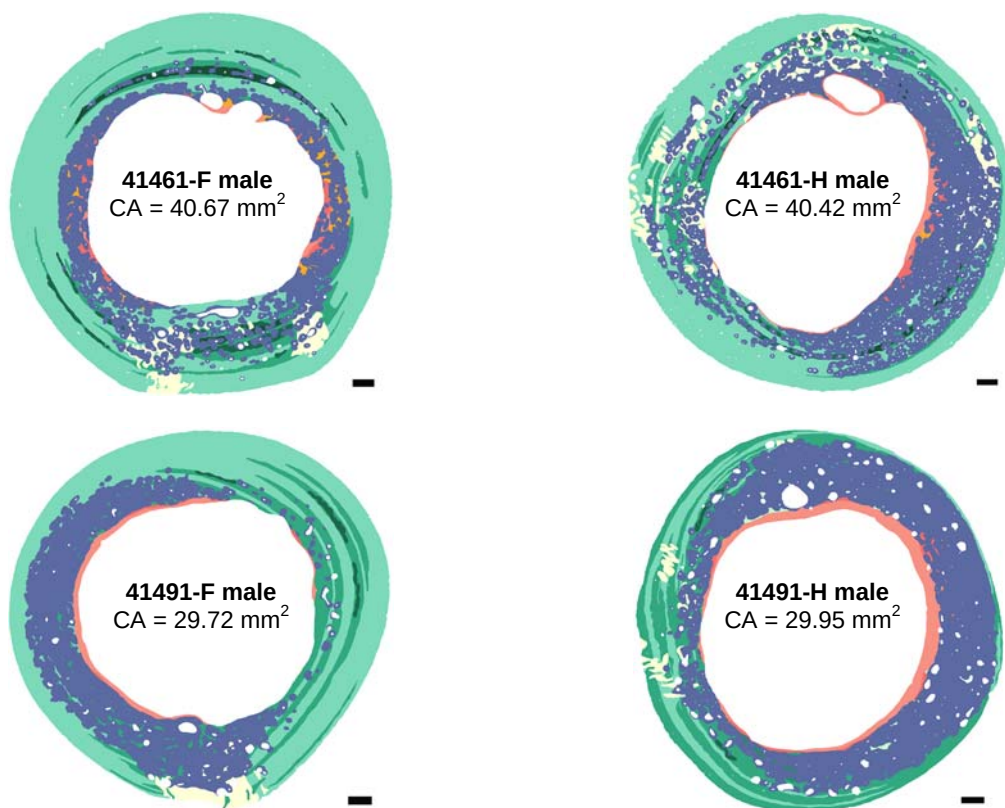
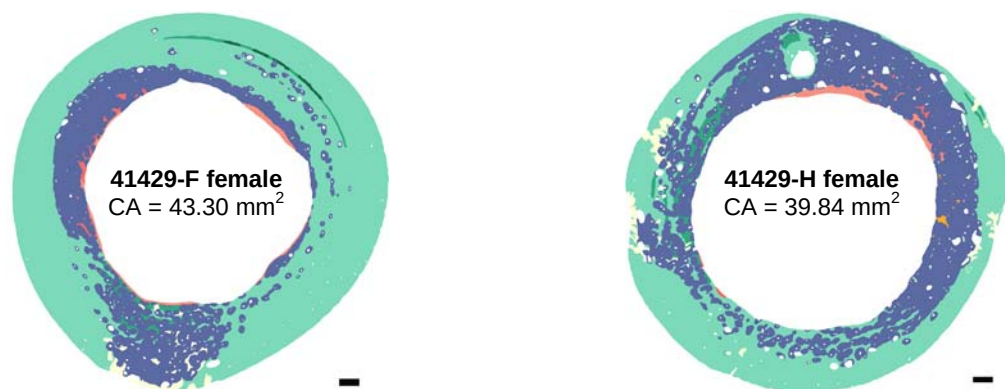
APPENDIX G-1 e continued. Tissue maps from *C. aethiops*: EPIPH age class.

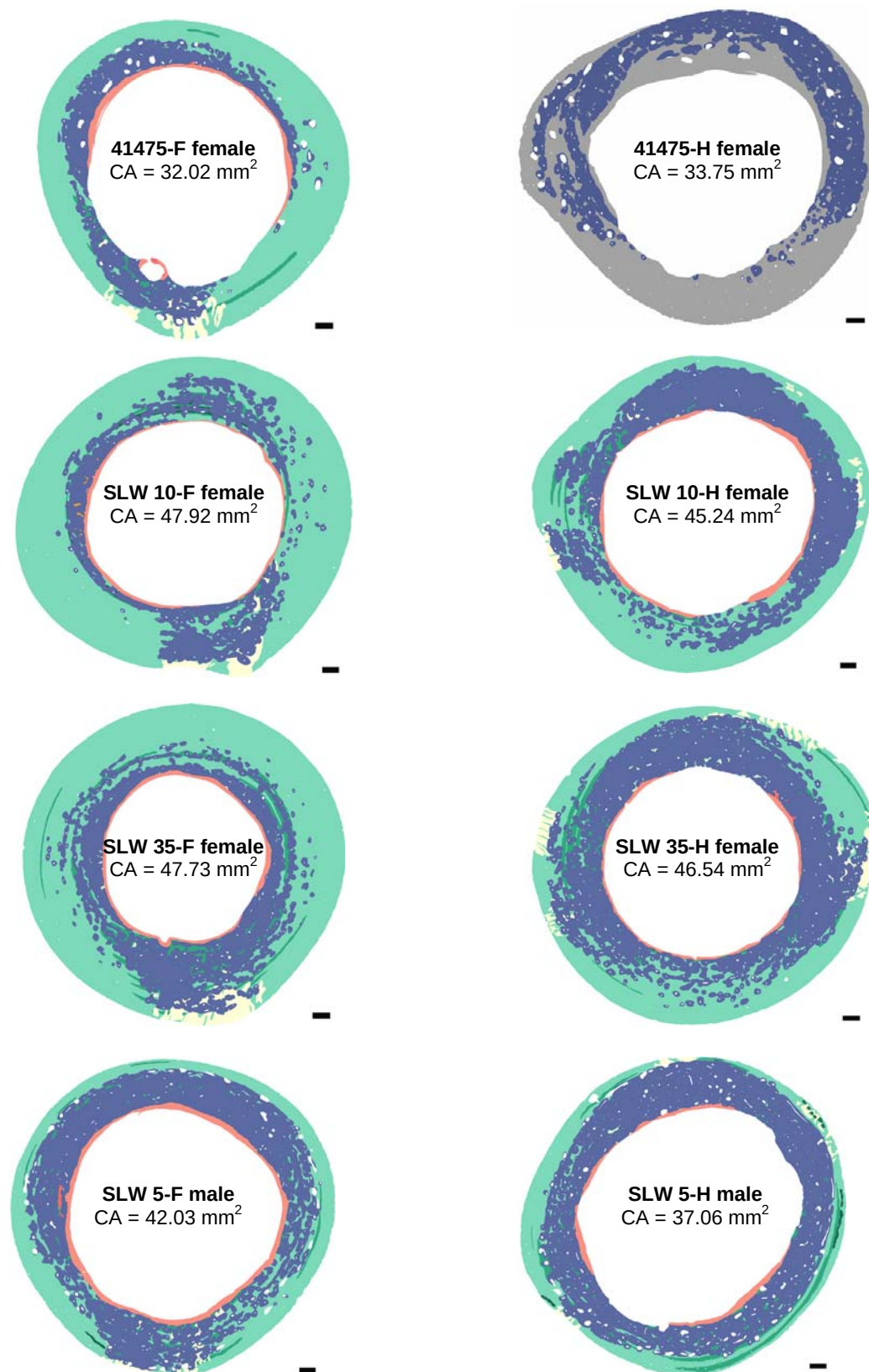


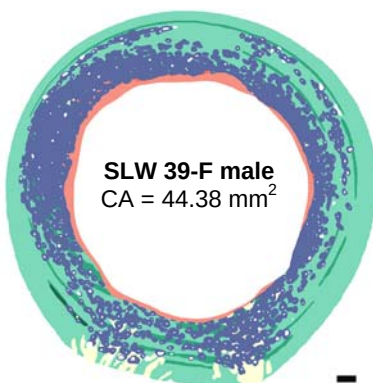
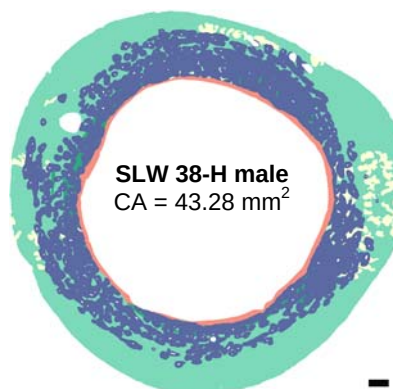
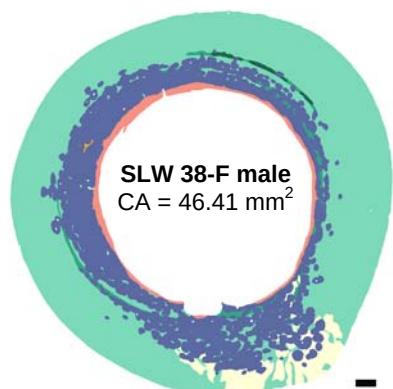
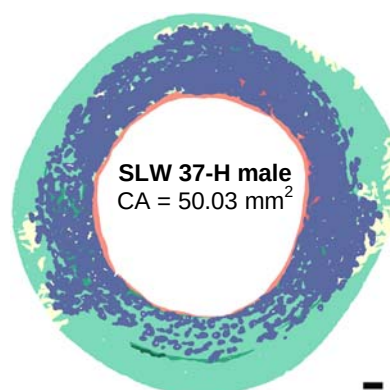
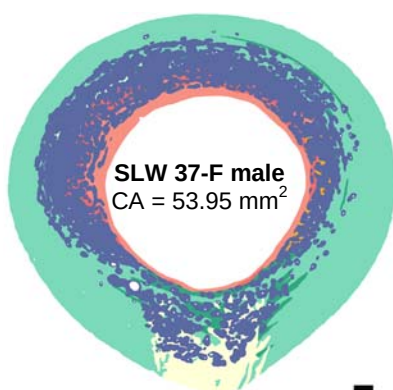
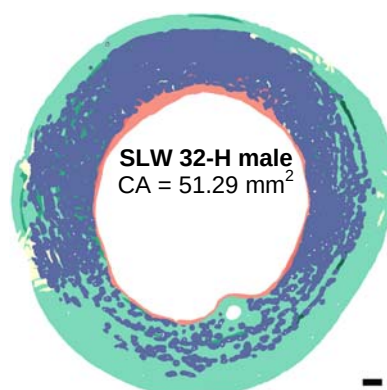
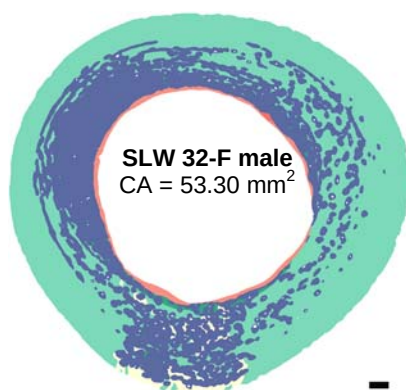
APPENDIX G-2 a. Tissue maps from *H. lar*: DECID age class.

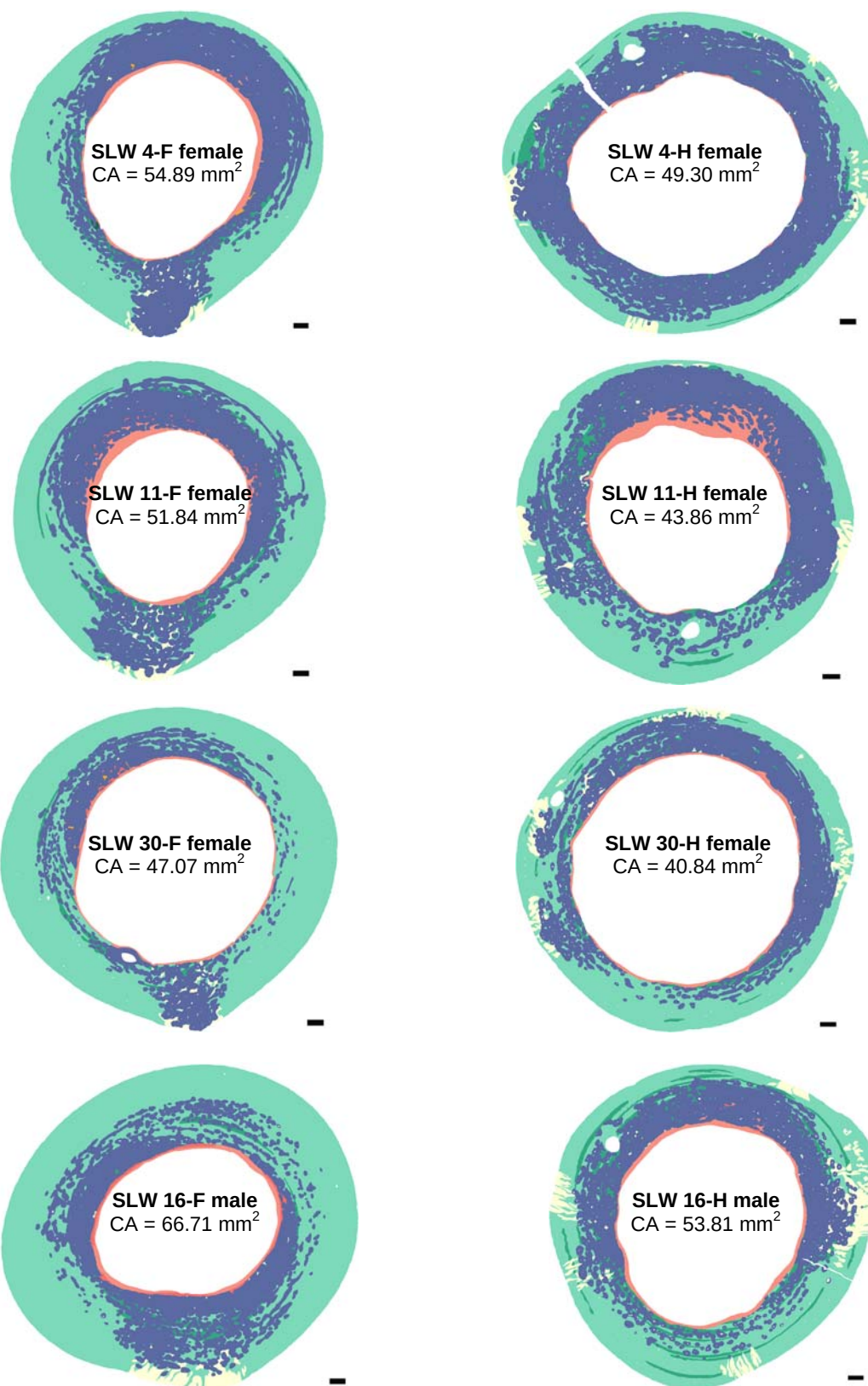
APPENDIX G-2 b. Tissue maps from *H. lar*: M1 age class.

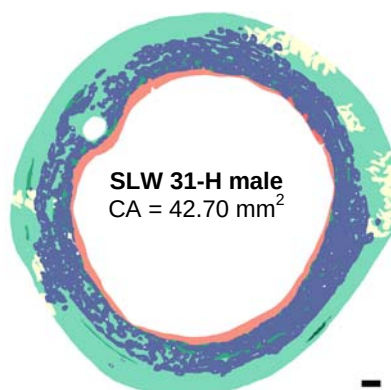
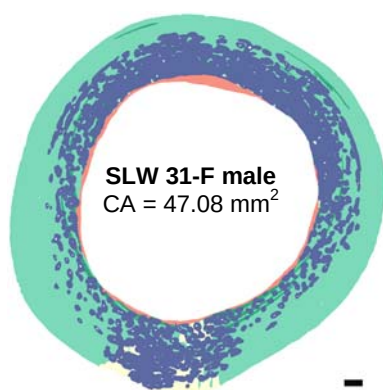
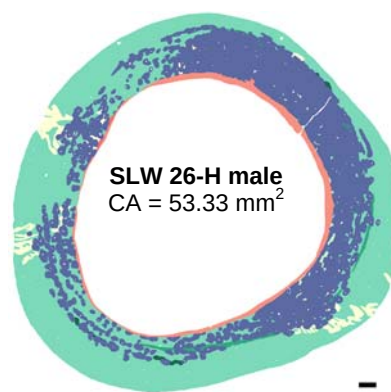
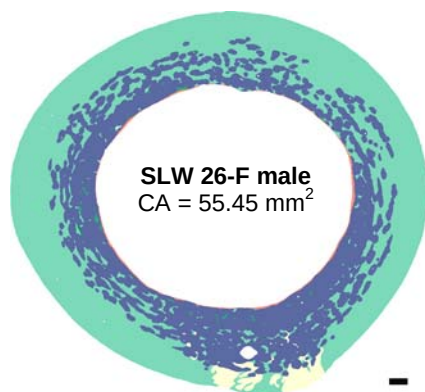
APPENDIX G-2 c. Tissue maps from *H. lar*: M2 age class.

APPENDIX G-2 c continued. Tissue maps from *H. lar*: M2 age class.APPENDIX G-2 d. Tissue maps from *H. lar*: M3 age class.

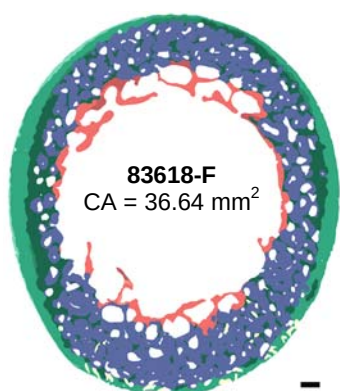
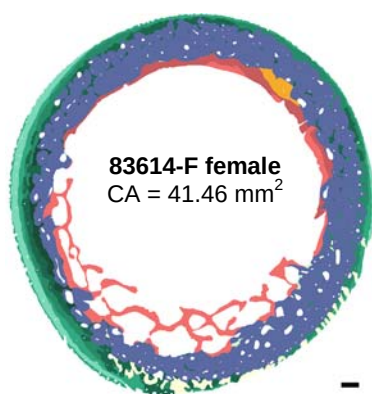
APPENDIX G-2 d continued. Tissue maps from *H. lar*: M3 age class.

APPENDIX G-2 d continued. Tissue maps from *H. lar*: M3 age class.

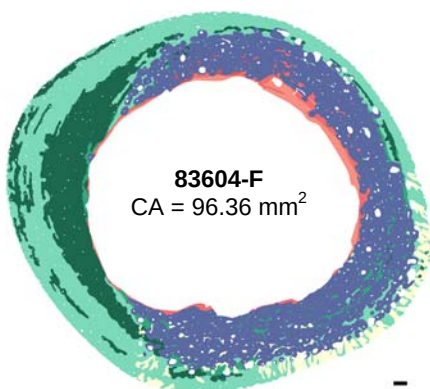
APPENDIX G-2 e. Tissue maps from *H. lar*: EIPPH age class.

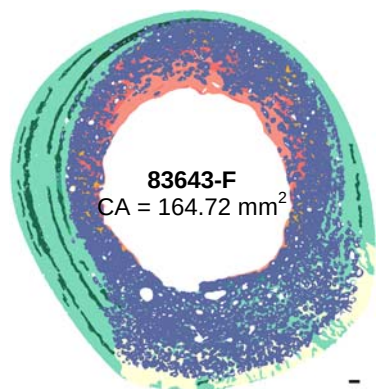
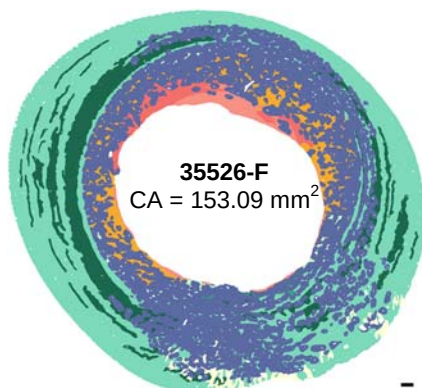
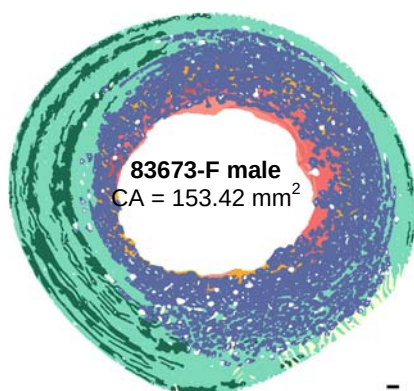
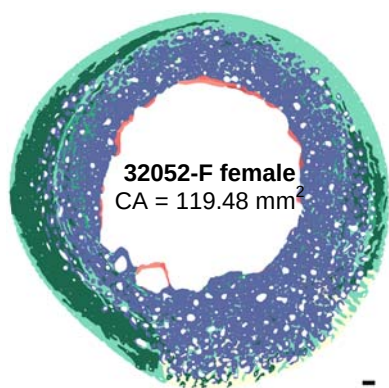
APPENDIX G-2 e continued. Tissue maps from *H. lar*: EIPPH age class.

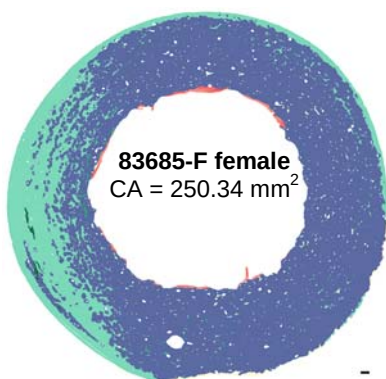
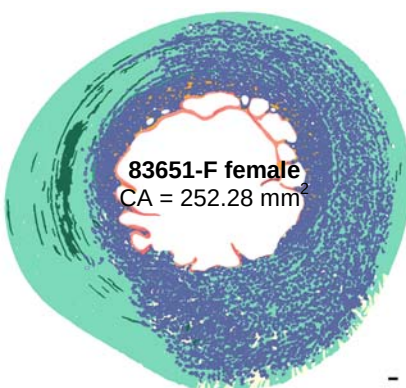
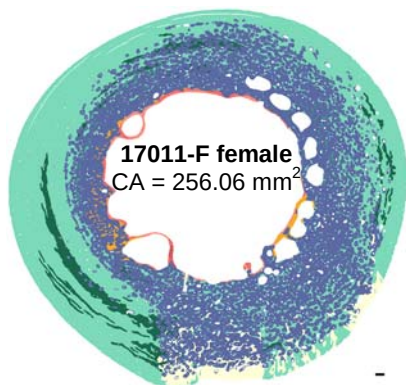
APPENDIX G-3 a. Tissue maps from *P. troglodytes* midshaft femur: DECID age class.



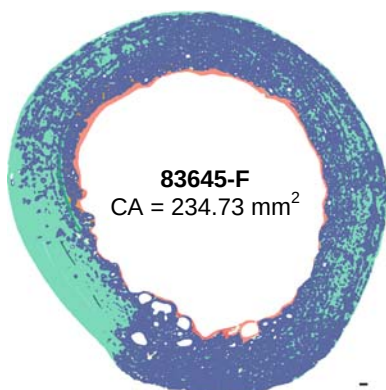
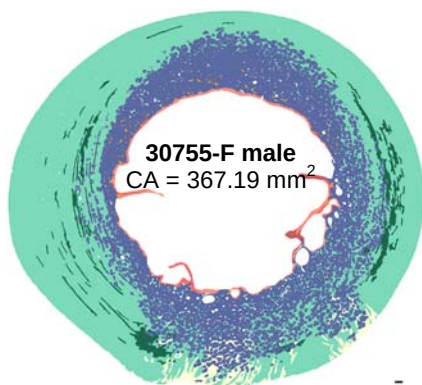
APPENDIX G-3 b. Tissue maps from *P. troglodytes* midshaft femur: M1 age class.



APPENDIX G-3 a. Tissue maps from *P. troglodytes* midshaft femur: M2 age class.

APPENDIX G-3 c. Tissue maps from *P. troglodytes* midshaft femur: M3 age class.

APPENDIX G-3 d. Tissue maps from *P. troglodytes* midshaft femur: EIPH age class.



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