HISTOMORPHOMETRY OF HUMERAL PRIMARY BONE:
EVALUATING THE ENDOSTEAL LAMELLAR POCKET AS AN INDICATOR
OF MODELING DRIFT IN ARCHAEOLOGICAL AND MODERN SKELETAL
SAMPLES

DISSERTATION

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ABSTRACT

During skeletal growth, long bones must change in size, shape, and relative position. This is accomplished diametrically by a process called bone modeling, which has been evidenced microscopically by patterned remnants of periosteal and endosteal bone distributions. It is the asymmetry of these distributions, or modeling drift, that accomplishes morphological change in the diaphysis. Until now, human modeling drift histomorphometry has received little attention. Previous research demonstrated a collection of specific histomorphological features could be used as a meta-feature, indicating the microscopic remnants of drift. This meta-feature, the endosteal lamellar pocket (ELP), is characterized by hemicircumferential lamellar orientation, primary Volkmann’s canals, and a relative decrease in the number of osteons compared to surrounding internal tissues. The current study provides a novel tissue level perspective, assessing human skeletal variation via quantification of ELP presence, position, and morphology in the humerus in order to discuss drift among individuals. Two distinct skeletal populations are compared: one archaeological, and the other, a modern control sample with known age and sex. Mid-diaphyseal, thin ground cross-sections are analyzed using custom point-count and hand-drawn techniques. Results provide: 1) an
evaluation of the use of endosteal tissue, specifically the ELP, as a summary of drift; 2) an comparative assessment of the two methods used in the analysis; 3) a comparison between the position of $I_{\text{max}}$, as an indicator of adaptation to mechanical loading, with the drift direction suggested by the position of the ELP; and 4) a baseline for variance in ELP characteristics against the background of periosteal and secondary bone distributions by region across subgroups generated by sex, age category, and population sample. Results indicate the ELP is a viable means of measuring and comparing modeling drift among subgroups. Both techniques work well under differing circumstances due to their individual strengths and weaknesses. The point-count technique, accomplished in a starburst pattern, is greatly aided by the use of a custom data-entry program for tracking multiple variables and becomes competitive with hand-drawn line assessments of ELP position when combined with frequency weighted vector analysis. However, for more simple studies interested in only primary ELP position and ara data, hand-drawn techniques are much more rapid. Important variance was found in the tissue distributions among subcroups, including females having significantly more primary tissue overall even when their lower Haversian area is taken into account. Modern individuals displayed more sexual dimorphism in drift than did individuals from an archaeological context. Finally, drift in the humerus was dependably posterio-medial in direction and significantly more laterally oriented than $I_{\text{max}}$. These data have far reaching implications. Completely new variables have been generated for the structured analysis of important variation in skeletal population samples, but also the importance of taking relative tissue-
age-at-formation into account becomes clear in this study. Efforts to account for drift in other applications such as those measuring, targeted remodeling, osteon type distributions, age-estimation, or stable isotopes could benefit significantly by comparing only tissues of equal ages or by applying an accurate correction factor for the given element’s local tissue-age variability.
DEDICATION

To my loving wife and colleague, Isabel, and the family that sustains us: our daughter Kaia, our son Orrin, and our parents: without you all, nothing else makes sense.
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Figure 65: The black solid arrows mark two different sets of modeling foci separated by an irregular trabecular spicule (potentially a remnant from an earlier growth period). If formation continued past this interruption these two foci could merge if timed correctly or the slower of the two would abut the faster. Thin-ground, undecalcified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt. Merged cross-polarized micrograph. Comprising images taken when primary lamellae were at 45° to normal x, y stage orientation. Scale bar indicates 300 µm.

Figure 66: A series of endocortical modeling formation phases present as adjustments to a much larger phase (past the lower field of view boundary). The white solid arrow marks the most recent phase, whereas the white lined arrows mark overlain fragments, indicating prior resorption took place before the final phase was formed. These phases are small enough to permit confusion with hemi-osteons, although the latter are typically only reported in trabecular bone. Black solid arrow denotes medullary surface. Thin-ground, undecalcified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt. Micrograph taken with red quartz cross-polarized filter (hilfsobject). Scale bar indicates 50 µm.

Figure 67: Two theoretically distinct lamellar phase microstructures depending on changing surface areas of apposition over time, represented schematically. A) A convex surface, viewed in transverse cross-section, is filled in. Each successive layer is larger in surface area than the last, requiring the activation of more and more previously dormant osteogenic cells from the membrane (top left). On a concave surface the same event produces a different lamellar architecture (top right). B) A convex surface, in which each successive layer’s area is, instead, smaller than the last, where fewer and fewer cells are actively forming bone (bottom left). On a concave surface the same event produces a different lamellar architecture (bottom right). These changes in surface area accompany
meaningful changes in some aspect of the formation signal that initiates or directs modeling activity. The size of the formation phase or its local micro-topography could also be a factor influencing surface areas of formation. The prevalence or meaning of this type of microstructural variation is unreported (Maggiano, 2011) ........................................281

Figure 70: A) Schematic of immature cortical (black) and trabecular (grey) regions destined for modeling alteration during long bone extension. B) Bone is resorbed at the metaphyseal periphery and within the medullary cavity, affecting both compact and trabecular tissue alike (white checkering). New compact tissue must also be constructed, both within trabecular voids during lamellar compaction, and at the periosteum via diametric apposition (grey bars). C) Even in the adult there remains evidence of these changes as the bone extends in length through endochondral ossification. Adapted from Enlow, 1962.................................................................304
LIST OF COMMON ABBREVIATIONS

PEM – Periosteal and Endosteal Membrane
BMU – Bone Multicellular Unit
BSU – Bone Structural Unit
ELP – Endosteal Lamellar Pocket
LCS – Lacuno-Canalicular Syncinctium
BLC – Bone Lining Cells
FOV – Field of View
ROI – Region of Interest
TCA – Total Cortical Area
A, AL, M, PM, PL, L, AL – The eight ROIs used in the starburst pattern corresponding to anterior, anterio-lateral, medial, posterio-medial, posterio-lateral, lateral, and anterio-lateral respectively
TCA – Total Cortical Area

*See Tables 1 and 2 for other variable abbreviations
CHAPTER 1: INTRODUCTION

1.1 Background and Research Aims

That human bone tissue “drifts” within the length of the diaphyses during skeletal maturation is a well-known phenomenon, called “osseous” or “modeling” drift (Frost, 1973; Enlow, 1962a; Streeter and Stout, 2003; Goldman et al., 2009; I. Maggiano et al., 2011, C. Maggiano, 2011). During this process bone is often formed on one side of the diaphysis and resorbed on the other; the process occurs on the endosteum as well, albeit reversed, such that the medullary cavity maintains its central position during growth (Figure 1).
Figure 1: Schematic representation of modeling drift in the femur.

Bone modeling accounts for the adult long bone’s diameter, morphology, and position, attributes important for skeletal biological applications but also for a baseline of understanding for bioarchaeological inquiry. Histology of ancient bone has been used to estimate age, sex, activity patterns, and health within ancient populations, all vital parts of reconstructing past lifeways (Larsen, 1997). Unfortunately, the measurement of modeling drift does not have a standardized method and is made particularly difficult by significant variation in microarchitecture (McFarlin et al., 2008; Goldman et al., 2003, 2005, 2009) and the fact that drift is defined by microscopic features but itself is a macroscopic process. Potentially for these reasons among others, drift has not often been the focus of investigations using archaeological material, and its variation among human populations or sub-populations is largely unexplored, particularly in the humerus. In addition, the degree to which drift direction and magnitude correspond to measures of
cross-sectional import (such as the axis of maximum resistance to bending) has not been satisfactorily evaluated. In short, without an understanding of bone microstructure at a tissue level, attempts at understanding how a bone achieved its shape or what contributed to growth or adaptational variation are limited, stunting the progress rate in many clinical and bioarchaeological applications of bone sciences.

Previous studies (Enlow, 1962a; McFarlin, 2008; Goldman, 2009) have described the general complexity of drift phenomena and noted the high variance seen in this aspect of development. However, until recently (Wanner et al 2007, C. Maggiano, 2011; Maggiano et al., 2008, 2009b, 2011; I. Maggiano et al., 2008, 2011a,b) no single unit or feature of drift has been proposed as representing or summarizing the process. The lack of a concrete unit of measure for modeling drift inhibits its investigation. This limit is not experienced in studying secondary bone tissues which have the benefit of including well-structured Haversian systems, summarizable by the quantification of osteonal bone structural units (BSUs). However, variable bone tissue microstructure might be, the same body of knowledge notes similarities and patterns in tissue level bone distribution (Enlow, 1962a; McFarlin 2008; Goldman, 2009). Several researchers have also encouraged the perspective that endosteal deposition is “lead” by periosteal activity which is more sensitive to growth factors and biomechanical stimuli (Gross et al., 2002; Jones et al., 1991; Lee et al., 2002; Meade et al., 1984; Srinivasan et al., 2002), a circumstance that could render it a better summary of net drift than periosteal tissue which must account for regional and global demands of adaptation and growth more
directly. There also exists the potential that younger-tissue-age endosteal bone could remodel more slowly than similarly aged periosteal tissues, permitting observation of endosteal remnants of drift well into the 5th decade of life on some occasions.

For these reasons the proposed research applies a new perspective to the analysis of microscopic human skeletal variation via rigorous quantification of endosteal bone tissue as a measure of growth and adaptation between modern and archaeological skeletal population sample. This is accomplished by considering recurring, predictable features of the endosteal cortex as a meta-feature for quantitative geometric analysis and simultaneous collection of histomorphometric indicators of tissue type, local tissue-age, and primary versus secondary depositional and resorptive activity. To accomplish this, two methods for quantifying endosteal bone (and its contexts) are tested: familiar point-count analysis and novel hand-drawn techniques (Maggiano, 2009b).

The predictable features of endosteal bone tissue associated with drift include is generally semi-lunate distribution around the inner cortex, “dense” hemi-circumferential lamellar orientation, decreased osteon presence relative to adjacent intercortical tissues, and radially oriented primary “Volkmann’s” canals (Figures 2 and 3).
Figure 2: Histological landscape of endosteal primary lamellar bone deposition during drift, emphasizing the radial “primary” Volkmann’s vessels, comparatively low osteonal presence relative to other internal tissues, and the osteonal perimeter that identifies its intracortical border. Scale marks 200 µm, 530 nm red quartz compensated cross-polarized microphotograph.
Figure 3: An even larger set of endosteally deposited primary lamellar bone phases that demonstrate the hemicircumferential pattern of deposition in comparison to the non-concentric periosteal deposition which can be seen at the top of the image. A) Medullary cavity, B) radially oriented primary Volkmann’s canals, C) arrest line denoting separate phases of endosteal bone formation, and D) the border between endosteal and periosteal primary deposition, highly obscured by osteonal proliferation. Scale marks 800 µm, 530 nm red quartz compensated cross-polarized microphotograph, femoral mid-shaft.

These drift indicators have been directly described and similarly explained by several authors (Enlow, 1962a; McFarlin, 2008; Goldman 2009) in the long bones of non-human and more rarely human primates. The general trend in progressive work on bone modeling began with early tissue level descriptions (Foote, 1916; Amprino and Bairati, 1936) and are now recognized as important indicators of tissue-age contributing to an understanding of drift (Enlow, 1962a). Now, however, a pattern can be seen as emerging
from drift observations that warrants a different type of consideration. C. Maggiano (2009, 2011) and colleagues (2009b, 2008, 2011) and I. Maggiano and colleagues (2011a,b) (also as Wanner et al., 2007), argue the potential for construction of an endosteal meta-feature that can summarize drift processes. This potential is thoroughly explored in the research reported here. As a meta-feature these endosteal structures have been termed the Endosteal Lamellar Pocket (ELP) and were characterized in the endosteal cortex of human femoral and humeral mid-diaphyses (I. Maggiano et al., 2008, 2011a; Maggiano, 2011). The distinction of the ELP as a feature of drift rather than the rough observation of all primary tissue emphasizes its predictable cross-sectional geometric distribution and facilitates its consideration as a quantifiable unit for angular comparison to other microstructural regions or to total cross-section axes of import. A consideration of all primary tissue simultaneously renders the assessment of quantifiable patterns much more complex. Of course, there is always the potential that drift is not summarizable in a meaningful way. The current study evaluates this precise issue in order to permit, if possible, the simplification of drift processes enabling comparisons between individuals and populations. Two separate techniques were developed and compared toward this end: 1) point-count assessment of tissue distributions in eight regions of interest (ROIs) arranged in a starburst pattern of tracks (anterior, posterior, medial, lateral, and the minor axes between); and 2) hand-drawn assessment of the ELP meta-feature using cross-polarized light and novel microphotographic imaging techniques accompanied by digital metric analysis. The position of the ELP, and consequently the
direction of drift was also compared to the direction of adaptation as indicated by \( I_{\text{max}} \), the axis of greatest resistance to bending of the shaft. In order to frame the ELP in its proper context, macroscopic measures and the qualification of quantification of total primary and secondary tissue distribution was also a major priority here.

Specific Aims:

A) Assess qualitative histomorphological changes associated with modeling drift through identification and comparison of periosteal and endosteal primary bone with secondary bone distributions about the cortex.

B) Provide and compare functional methodologies for the histomorphometric quantification of drift by employing the ELP as its indicator, measured by point-count and hand-drawn techniques.

C) Compare drift growth axes to those of major bending resistance as summarized by the angular position of \( I_{\text{max}} \).

D) Analyze variability in drift due to age and sex in a modern and archaeological sample.
1.2 Questions and Hypotheses

Although the data collected during this project is diverse, the current effort focuses on establishing a method and baseline of variance in the ELP as it relates to the overall process. Toward this end and to fulfill the previously mentioned research aims several research questions will be addressed:

Research Questions:

1) What is the histomorphological context of the ELP and, when compared with the data collected from the study, can it continue to be considered an indicator of general drift processes of the humeral cortex?

2) Point-count and hand-drawn line techniques are some of the possible methods available for relatively rapid, inexpensive, and accurate methods used for histomorphometry, but which is best suited for an analysis of the ELP in an effort to summarize drift?

3) How does drift information yielded by analysis of the ELP relate to other measures of cross-sectional change such as transverse diaphyseal geometry?

4) What is the basic variance in ELP presence, position, and size among subpopulations defined by age and sex in a archaeological and modern skeletal population samples?
The answers to these questions structure predictions and hypotheses as follows, each corresponding to one of the major aims of the current work:

Predictions and Hypotheses:

A) The histomorphometric context of the ELP is relatively complex due to the significant variance seen in tissue distribution during growth, but results indicate that the ELP can serve as a simple indicator of drift in the humerus. This prediction would be particularly well supported with the acceptance of the following alternative hypothesis:

**Hₐ**: Measures of drift considering only endosteal bone should achieve greater significance than those combining endosteal and primary measures.

B) The point-count technique is extremely well suited for countable variables, but due to its limitations in dealing with spatial and geometric data, hand-drawn techniques should assess ELP drift direction in a more visually accessible and statistically robust fashion. This prediction would be supported if there are clear differences in time efficiency between the techniques. The acceptance of the following alternative hypothesis would also lend it support:
\textbf{H}_A: Variability is higher and/or less patterned in area and angular ELP measures collected from the point-count technique in comparison with hand-drawn data.

C) Because \( I_{\text{max}} \) is an extremely important representation of resistance to bending in a given axis, ELP indicated drift direction should be extremely similar in its descriptive angle to that of \( I_{\text{max}} \). This prediction would be particularly well supported with the acceptance of the following null hypothesis:

\textbf{H}_0: There was no significant difference in angular position between the ELP axis of drift and \( I_{\text{max}} \) axis.

D) The basic variance in ELP position and size will differ significantly between members of subgroups defined by a) age, b) sex, or c) skeletal population of origin (modern versus archaeological). This prediction is suggested by the known and frequent effect that these groupings have on bone growth and adaptation. As brief examples: a) An age effect could be expected on ELP area in that the two juvenile age categories will have larger ELPs than the older age category due to modeling’s primacy during growth and endosteal resorption later in life; b) ELPs could demonstrate sexual dimorphism due to the delayed onset of puberty and extended upper body development of males.
resulting in increased stature and weight; c) Finally, modern populations apparently more affected by age associated endocortical bone loss could have fewer or smaller ELPs in comparison to the archaeological (less sedentary) populations. These predictions would be particularly well supported with the acceptance of the following alternate hypothesis:

\[ H_A: \] There will be significant differences (P<0.05) between ELP variables from individuals of differing a) age, b) sex, and c) different skeletal population samples.

1.3 Context for Research and Adopted Conventions

The work presented here is part of a much larger effort to illuminate tissue level changes in the human skeleton for application to bioarchaeological and skeletal biological inquiry. For this reason and for clarity, a declaration of the assumed perspective of this work is necessary, accompanied by explicit statements regarding notable conventions adopted here. This is particularly important due to the potential misconception that the ELP is a newly discovered feature of bone. However, it is very important to recognize and emphasize that this is not a new feature. The particular pattern of endosteal formation used to define the ELP has been noted for nearly 100 years (Foote, 1916). It has typically been referred to in older texts describing growth process
and has been used to contribute to a basic theory of skeletal biology (Enlow, 1962a; Frost, 1973), but has not yet been sufficiently examined in larger populations or statistically, due to several likely reasons: 1) its large size but microscopic identifiers, 2) challenges of nomenclature and tissue differentiation, 3) high microstructural variation, and 4) the absence of non-invasive means of measurement in archaeological samples and lack of younger tissue from organ donation sources.

The ELP is not microscopic. In fact, its original misidentification by the current author as a pathology was due to its brilliant unaided visibility in back-lit excisions. In most cases, however, due to at least some invasion of Haversian tissues, a microscope is necessary to fully detail the ELP intra-cortical perimeter. This circumstance makes it difficult to think of as a “feature” because familiar histological features, like the osteon, are much easier to conceptualize and view within a single field. 2) Quantification of histological drift patterns has been a challenge because the ELP’s intra-cortical border is obscured by remodeling and because the feature is large but requires a microscope to fully view. New techniques described in the current research will facilitate border discernment, not only making the measurement process more rapid, but rendering parameters explicit, easing replicability and reducing error. 3) In general, observations of bone histomorphology mention apparently high microstructural variability (Goldman et al., 2005; Chan et al. 2007), lending the impression that patterns exist but are not dependable. It is unclear how often this variation is over-estimated due to the partitioning of the cross-section into arbitrary “thirds” of cortical thickness that have
relatively little actual relationship to growth processes which are much more complex, ensuring new and old tissue could be adjacent in nearly any region of a bone cross section – but not in every region with random distribution. Previous studies have found the distribution of primary tissue to be quite predictable and easily readable (Enlow, 1962a; Frost, 1973; C. Maggiano et al., 2011; Maggiano, 2011; Ausk et al., 2012), particularly that of the endosteal tissue (I. Maggiano et al., 2011a). Before resigning to high variability as a reason to generalize drift processes, drift variability should be measured in a fashion sensitive to tissue age-at-formation. These contextual challenges inform the major aims of the research undertaken here: explore appropriate methodologies and basic variation in modeling drift.

Another important contextual note regarding this dissertation is that it is part of a much larger effort to study both growth and adaptation in bone, and to explore new methods of hypothesis driven testing applying results to bioarchaeological investigations at the archaeological sites involved. For the purposes of this work, only results from the Xcambó site in Yucatan, Mexico are considered. This is because other sites involved in the study, intended as test sites, do not contain dependable orientation information (anterior, posterior, medial, lateral) and are intended for use in application of technique rather than the invention of it.

Several important conventions are assumed here. It is important to know that their assumption does not indicate their complete acceptance by any means, but does avoid skirting issues to the detriment of continuity and efficiency of language necessary
for scientific consideration of concepts discussed here. Bone histological terminology can regularly be the subject of debate. Here the standard definitions used are borrowed from various works (Enlow, 1962a,b; Frost, 1973; Parfitt et al., 1987; Robling and Stout, 2003), and customized by applying a perspective focused on primary bone phase structure and trends across the entire cross-section and across population samples rather than individuals (Maggiano, 2011). This perspective whenever possible ignores typological description in favor of procession description.

The phrase “subperiosteal bone” or “subendosteal bone” as used in some quarters (see Goldman et al., 2009, as an example), are omitted for efficiency since “periosteal or endosteal bone” is completely synonymous – there is no “supra-periosteal or endosteal” bone for example so the modifier is unnecessary. In some cases distinction between certain terms is also unnecessary. For example, the difference between primary osteonal and primary vascular canals (both primary vessel with varying arrangements of associated lamina) is not a significant concern during an analysis such as this; however, that they are only present in specific tissues formed by a specific membrane, is (see Section 6.1.2). Resorptive spaces are more important to differentiate in discussions involving resorptive bays, trabecularized voids, and trabecular voids sometimes behaving in a fashion indistinguishable from the medullary cavity itself (see Section 6.1.2.4). Likewise, although some of the discussion regarding minimodeling, mixed minimodeling/remodeling, and hemiosteonal remodeling must be addressed here (see Section 3.2.2.1.3), the confusion is simplified within these pages by assuming that on large
surfaces activated during growth and drift and remote from tight trabecular void-space, modeling process account for changes encountered. According to convention (Parfitt, 1982, 2000), remodeling is still assumed to be coupled here, despite ample and growing evidence that remodeling is coupled only when it is “coupled” chronologically (dramatic expansions of resorptive bays in early growth and later in senescence, or the continued seem to show definitively that activation can instigate unmitigated resorption and does so regularly) (Goldman et al, 2009; Maggiano, 2011). The reason for addressing these concerns here is to clarify that the intention is not to prove terminology, it is to do science. In some cases terminological agreeance is beneficial to communication and detrimental to new ideas.

Other clarifications of intent: this study focuses on primary tissue distributions and modeling drift but to do so requires significant collection of data on secondary bone processes. Much of the data collected during this project awaits future analysis in forthcoming works that focus on porosity, drifted osteonal distribution, and field of view level spatial analysis. In addition, this study uncovers base-line growth and adaptive response in bone and as such makes little mention of potentially important dietary, pathological, or specific hormonal/genetic controls or influences on bone which are not within the current scope of study.

Finally, for reference accuracy and clarity: sections of the background material regarding bone modeling contained here were written for this dissertation before its publication in press, which happened to transpire before the dissertation was complete:
“Bone histology: An anthropological perspective.” (Maggiano, 2011). Therefore, in all relevant cases, material here references this publication either directly or at the end of a given paragraph. Any of this material is more properly referenced by citing the more publically available print rather than this dissertation.

1.4 Broader Implications

Histological examination of thin-ground bone sections has been successfully applied to many clinical, forensic, and bioarchaeological topics. The current research complements these efforts. It provides primary, exploratory data using basic theories of skeletal biology to generate new questions for research. Pilot studies have demonstrated that the ELP can be recognized as a histological meta-feature representing bone modeling processes (Wanner et al 2007, C. Maggiano, 2011; Maggiano et al., 2008, 2009b, 2011; I. Maggiano et al., 2008, 2011a,b) and that it can be quantified using the proposed methods (Maggiano, 2009b).

The histological study of bone growth and functional adaptation must sometimes homogenize compact bone structure across a section or remove factors introducing variation. Alternately, potential variability in microstructure could be so high as to confound identification of meaningful patterns. However, patterned regional variations in tissue structure, like the ELP, provide useful information regarding bone growth and mechanical adaptation that depend on the tissue’s age-at-formation. That age depends
largely on the cortical pattern of modeling drift and not all adjacent tissue in bone was formed at the same time; hence Wu and colleagues’ (1970) corrections for mean tissue age during remodeling analysis. However, if humeral ELP tissue from a 45 year old has a mean tissue-age-at-formation corresponding to the individuals 18th year of life, this discrepancy equally confounds some types of histological analyses and permits novel approaches.

Applications for modeling drift in long bones have been rare due to a lack of distinct features for inter-group quantification. This leaves a gap between our knowledge of bone macro- and micro-morphology that could inhibit current techniques or the development of new theoretical approaches. For example, the potential for developmental constraint on the position and frequency of various bone microstructural features provides a complication for various anthropological investigations of bone histomorphology. If differential tissue-age-at-formation is ignored then it cannot be accounted for as a control for further research regarding mechanical and physiological phenomena. For histomorphometric approaches, variables collected are not often separated by tissue-level structural patterns but are instead divided into arbitrary cortical “perimeters.” often thirds (outer, middle, and inner) – this practice could create confounding artifacts that artificially inflate regional microstructural variability, hiding trends and patterns.

However, considering tissue-age-at-formation and drift processes could be beneficial for new bioarchaeological analyses. For example, if the ELP has a predictable
appearance, and collects invading osteons more slowly than adjacent tissue, this facilitates age estimation greatly by providing even more histological data than OPD alone. Consider that OPD collected from the region once containing the ELP should hit an upper asymptote at a later “age” than typical cortical tissue, possibly permitting increased accuracy in estimating late adult individuals. Data collected in this manner also permits more detailed comparisons between individuals or populations in the onset and outcomes of growth spurts continuing adaptation in late-teen/early-adulthood that accounts for mature skeletal morphology. The ELP assists these efforts even in fragmentary remains using new variables like drift direction and magnitude that address the record of diaphyseal morphological change over time. Indeed the “record” kept by the ELP could even permit new forms of focused trace element or stable isotopic sampling using laser assisted optical mass-spectrometry equipment. For example, testing the ELP tissue of a 45 year old (formed during skeletal maturation) could provide a larger window into the individual’s living history, sampling young and older bone tissue sequentially. In short, investigating relationships among ELP presence, position, and morphology; and estimated age, sex, and bending strength in several population samples, offers new means by which to test skeletal variation and understand the human collective past.

Results of this type of work also have the potential to shape our understanding of boney response during growth and adaptation, in general. This is largely because clinical investigation is typically focused on pathological circumstance, particularly in older
individuals. Providing a baseline regarding boney tissue level behavior in children, teens, and adults could be informative for clinical applications. For example, the particular direction and magnitude of drift in youth could have important ramifications on the amount of bone lost later in life due to age-related bone loss, having direct consequence on fracture risk in older ages. In addition the position of the ELP as a summary of drift might not correlate with the angular position of cross-sectional geometric values, like $I_{\text{max}}$ that have direct bearing on the ability of the bone to resist bending in that axis. In this case the total phenomenon of bone growth and adaptation becomes more apparent despite its complexity. The implications of tissue-level bone histology can augment efforts currently investigating age-estimation, targeted remodeling, osteon morphotypes among others. This is largely due to the consideration that often current studies compare bone microstructures without taking into account their age of formation, falsely hiding patterns or inflating variation. Drift variables presented here can be applied to particular bioarchaeological questions at Xcambó as well, contributing new perspectives on the division of labor at this site that seems to indicate sexual dimorphism in physical activity patterns, or even on important subsistence and trade changes transpiring over the course of its occupation. Comparisons of drift data and element rotation, torsion, and their biomechanical effects can achieve explanatory power in fields as diverse as sports medicine and physical therapy. The potential for future applications of the current research is significant. Further analysis of the ELP bears potential in closing this gap and widening perspectives on human variation in bone growth and mechanical adaptation.
1.5 Topics Addressed

The basic context, aims and hypotheses, and broader implications have already addressed. Chapters 2, 3, and 4 provide a more detailed treatment of the background necessary for modeling drift inquiry and create a broad landscape for the interpretation results. Chapter 2 relates the basic cells and processes responsible for boney change and the physical, chemical, organ, and systemic factors that influence them. This is particularly necessary for setting the stage for later discussions on bone microstructure, tissue, and formation and resorption phenomena. Chapter 3 expands the scale of consideration regarding boney changes accomplished by sister processes: modeling and remodeling. In this exposition, the microstructural forms and functions of both processes are detailed, including comparisons of their structure, vasculature, and biological activity through various phases of development. The discussion closes with remarks on variation in bone microstructure, a topic returned to often in the discussions and conclusions. Chapter 4, an anatomical chapter, reveals the major musculoskeletal influences on the Humerus and its developmental and adaptational context. This context is related as often as possible to the element’s evolutionary circumstance in order to illuminate various unique aspects of humeral morphology and development that are otherwise underappreciated.
The following chapter, Chapter 5, begins discussion specific to the current research and has two goals: 1) providing background for sample studied and techniques employed, and 2) detailed descriptions of these techniques, generated variables, and their statistical examination. The archaeological and modern samples used in the study are characterized, as is the cross-polarization microscopy and cross-sectional geometry permitting their analysis. Overall the methods section of this chapter is separated into 4 phases representing the connection between each of the major aims of the work and resulting data: Phase 1, descriptions of drift histomorphology and variation; Phase 2, Point-count and hand-drawn methods separated to facilitate comparisons of the requirements, benefits, and limitations of each; Phase 3, comparison of drift direction and that of maximum bending resistance, represented by $I_{\text{max}}$; and Phase 4, comparisons ELP presence, area, and position among regions and subpopulations. Detailed treatments on methods here ensure results can be more easily understood, but more importantly, that they can be referenced during replication or extension of the study.

Chapter 6 records the results, qualitative and quantitative, of the current research in each of the four phases of analysis. This transpires through histomorphological description of formative and resorptive evidence in each tissue type which is accompanied wherever possible by exemplary microphotographs as figures. In other sections statistical results are accompanied by graphical representation to facilitate the simultaneous consideration of complex datasets and tables are used to relate requisite
quanta related to tissue distribution. Typically this data is represented in standardized form to permit comparisons that are independent of bone size, or total cortical area.

The discussion of results is found in Chapter 7 and is separated into the same four research phases used to organize the methods and results chapter, linking each back to the original aims and hypotheses for the work. Finally, in Chapter 8, these hypotheses are revisited and conclusive remarks are made regarding each. This section also includes directions for future research and statements regarding the implications of the work from a wider perspective.
CHAPTER 2: BONE CELL HISTOLOGY

2.1 Introduction

Bone cells of various forms are responsible for bone formation and maintenance, simultaneously satisfying the body’s requirements for ensured strength and flexibility of bone tissue, its adaptation to changing mechanical demands, and the storage and release of vital mineral ions. Despite difficulties in the identification of many developmental precursors, these cells can be grouped into four major categories when mature (or active): osteoblasts, osteocytes, bone lining cells, and osteoclasts. All of these cells come from two embryological tissues, mesenchymal stem cells and hematopoietic stem cells.

2.2 Osteoblasts

Osteoblasts are rarely dividing, columnar cells, easily identified by their eccentric nucleus and prolific golgi apparatus (Ham and Cormack, 1979). A preponderance of these vesicle-like organelles and density of the endoplasmic reticulum marks the cell’s active phase and contributes to its primary bone-forming capacity. This is accomplished through the apical (nuclear proximate) secretion of osteoid, a type-1 collagen-laden,
extracellular, organic matrix (Martin and Burr, 1989). Subsequent mineralization of this matrix, ossification (Ham and Cormack, 1979), envelopes collagen bundles in either loose and disorganized orientations (as in woven bone) (Ham and Cormack, 1979) or in alternating, dense and loosely packed appositional layers (as in lamellar bone) (Marotti, 1993), lending mature bone tissue its birefringent bright or extinguished appearance when viewed under polarized light. The microscopic constitution of the final organic and mineral matrix lends bone its dual properties: viscoelastic resilience and strength, respectively. It is possible that on a larger scale the alternation between densely and loosely packed collagen plys satisfies the same requirement for simultaneous resistance to bending and torsional forces and plastic recovery (Marotti, 1993).

In development, and during boney repair and adaptation, osteoblasts form from proliferated pluripotential mesenchymal stem cells through the osteogenic cell differentiation pathway. The osteogenic pathway is distinct from other connective cell pathways, those forming the precursors for cartilaginous, muscular, marrow stromal, tendo-ligamental, and adiposal cell lines, though identifying each precursor line and the growth factors, hormones, and markers differentiating them is challenging (Aubin, 2008). The eventual distinction of the osteogenic pathway from its osteochondrogenic precursors is due to specific chemical signaling paths initiated early during development. Bone morphogenic proteins (BMP) and other growth factors are involved in some phases of osteogenic cell differentiation (Chen et al., 2004). The resulting general proliferation of osteoprogenitor cells may be replenished by age-dependent self-renewal of parental cell-
lines, possibly lining bone surfaces (bone lining cells) (Aubin 2008). Osteoprogenitor cells are the parent to preosteoblasts which potentially differentiate into osteoblasts, osteocytes, and bone lining cells (Aubin 2008).

2.3 Osteocytes

Some osteoblasts break away from the osteogenic layer and remain behind, encased by loose collagen lamellae (Marotti, 1993) in cavities, called lacunae, which may permit only 100 nm of surface clearance for the cell. At this point the osteoblast has undergone several structural and functional changes and is referred to as an osteocyte (Martin and Burr, 1989). Osteocytes, though still retaining their golgi and endoplasmic reticular organelles, no longer produce osteoid (Martin and Burr, 1989) and do not divide (Ham and Cormack, 1979). They have formed pseudo-dendritic processes, extending into the boney matrix through ~500 nm diameter tubules, called cannaliculi (Knothe Tate, 2003).

In three dimensions, the lacuno-canalicular system (LCS) can be visualized as a vast pericellular fluid-filled network of osteocytes connected by gap junctions into a functional milieu, or syncytium (Donahue, 2000; Knothe Tate, 2003). Osteocytic positioning within this syncytium is perfect for the exchange of several potential cell signals originating from either endogenous mechanisms (active transport, osmotic and pulsatile pressure gradients, and hydraulic conductivity) or exogenous mechanisms (mechanical loading and acoustic and electromechanical energy) (Knothe Tate, 2003).
Although the specifics of osteocytic mechano-transduction are not known, the LCS is thought to propagate signals for the efficient removal of damaged bone material and perhaps the formation of new primary bone in response to site specific loading history of bone, such that it maintains its structural integrity with increasing activity (Taylor and Lee, 2003).

2.4 Bone lining cells

At the conclusion of bone formation osteoblast-like cells cover all bone surfaces and remain connected via the LCS to the osteocytic syncytium. These cells are referred to as bone lining cells (BLCs). It is unclear whether BLCs represent the mature surface morphology of osteoblasts, or if in fact they have adopted a less specialized morphology similar to osteoprogenitor cell lines (Aubin, 2008) and as such have temporarily repressed their osteogenic potential as evidenced changes in their internal composition (Ham and Cormack, 1979).

Since BLCs cover all exposed bone surfaces, they comprise the “osteogenic” layer of the periosteum and endosteum (sole contributing tissues of diametric bone growth) and Haversian canals (discussed below) (Ham and Cormack, 1979). The function of these nondescript cells is not well known. It can be appreciated, however, that due to the potential site-specificity of both remodeling and modeling processes, and due to the disconnect achievable between formation and resorption at bone surfaces (Boyde, 2003), BLCs occupy a unique position for potentially moderating any of these
processes. For example, according to the theory of Rodan and Martin (1981), BLCs are directly involved in the activation of osteoclast aggregation in response to parathyroid hormone (PTH). The presence of PTH (and perhaps other signals unrelated to calcium ion maintenance) induce BLC morphological changes, thickening the otherwise thinly spread cells and exposing the boney surface underneath to the resorptive process. Calcitonin’s secretion by the thyroid has much the opposite effect and shuts down osteoclastic activity. This process may be one of the avenues by which bone controls calcium and phosphate stores during times of metabolic need and explains how osteoclasts may respond to signals for which they have no receptors (Ham and Cormack, 1979). More detailed studies are currently investigating the differential response of bone lining cells to fluctuating or continuous levels of PTH (Kroll, 2000; Poole and Reeve, 2005).

So the function of bone lining cells can be protective, inhibiting resorption. Interestingly, other researchers suppose that bone lining cells and osteocytes are constantly signaling for resorption but are hampered by their connectivity to a syncytial LCS that represses this signal so long as resorption is unnecessary (Martin, 2000). A clearer picture of the major function of bone lining cells, and one far more in line with their primary position in layers of osteogenic surface membranes and other bone surfaces everywhere is provided by Chow and colleagues (1998). They have demonstrated that even after a single 5 min bout of tail loading, rat vertebra show BMP regulated, endosteal bone growth response, accomplished directly by the bone lining cells themselves. In
response to mechanical loading, bone lining cells show distinct morphological and
organelle changes, identifying them as osteogenic agents. No resorption and no cell
proliferation occurred at sites of growth suggesting that bone lining cells are merely
temporarily inactive osteoblasts until need arises (Chow et al., 1998).

2.5 Osteoclasts

Osteoclasts, bone resorbing cells, are extremely large (20-100 μm) and are
additionally characterized by a multinucleated core, prolific mitochondria, and a deeply
infolded, ruffled boarder at the cell’s base (Martin and Burr, 1989). The ruffled boarder
is the site of active bone destruction, a process that generates lacunae that typically
enclose the osteoclastic hemisphere of the cell. These small lacunae, called Howship’s
lacunae, mark actively resorbing bone surfaces with a distinctly rough and “scalloped”
appearance (Ham and Cormack, 1979; Martin and Burr, 1989). Bone mineralized matrix,
namely hydroxyapatite, is dissolved through acidification of enclosed surface area of the
ruffled border. The organic matrix is digested by several different enzymes, including
matrix metalloproteases and cathepsins (Väänänen et al., 2000). Freed calcium and
phosphate ions are absorbed via endocytosis at the ruffled boarder and transported across
the cell by vesicles and released into the increasing resorption area (Väänänen et al.,
2000).

Osteoclasts, once thought to derive from (or disassemble into) the same
osteoprogenetor cells leading to osteoblasts (Ham and Cormack, 1979), are now known
to arise separately via multiple fusions of monocylic macrophages. Osteoclastic cell differentiation requires RANK ligands, a nuclear activating factor, and a macrophage colony-stimulating factor (M-CSF). In the presence of RANK-L and M-CSF signals, fusion of monocytes results in the formation of the multinucleate osteoclast. Interestingly, an osteoblast-originated competitive inhibitor, osteoprotegrin (OPG), occupies the osteoclast RANK surface receptor, disrupting osteoclastic activity (Schoppet et al., 2002). This is the molecular basis for a localized separation between resorptive and formative processes.

2.6 Summary

All changes transpiring during bone tissue formation, maturation, and adaptation are governed by osteoblasts and osteoclasts that form and resorb bone respectively. At times these cells are arranged in predictably specific locations, particularly in the case of the bone multicellular unit (BMU) responsible for intracortical remodeling. At other times, however, this arrangement can be altered morphologically to permit resorption to outpace formation, for example (as is the case during rapid periods of growth that require removal of tissue and redistribution or during age-associated bone loss later in life). Modeling processes require less close associations of cells and local cellular activity than is seen in the BMU, instead, often organizing in patterns of formation that could activate across large expanses of the PEM membranes. Bone adapts to its physical loading
history through the sensitivity of the lacunar-canalicular Syncinetium which
interconnects osteocytes locked within the bone. This permits a previously quiescent
layer of bone lining cells to activate a formation or resorption phase with no previous
required preparation – a feat typically assumed impossible during BMU activity. The
result can be large scale changes in bone growth and morphological adaptation.
CHAPTER 3: MODELING AND REMODELING AS RELATED PROCESSES

3.1 Introduction

Bony change outside of the epiphyses occurs via two processes typically defined in juxtaposition. Modeling is responsible for all formation and resorption initiated by the periosteal and endosteal membranes. In contrast, remodeling ensures the bone’s material integrity is maintained by replacing old bone through resorption and subsequent formation at collections of cells called bone multicellular units (Frost, 1973). Together these related processes ensure that, during skeletal maturation, bone achieves a delicate balance of adaptive architectural strength and efficiency of mass that is maintained as long as possible after maturation. Variation in relative bone size and shape informs much of skeletal inquiry, whether medical, forensic, or archaeological. Therefore, a detailed knowledge of bone modeling is required to understand basic processes underlying features of diagnostic, identificational, or inferential import (Maggiano, 2011).

As discussed in Chapter 1, modeling activity is not easily conceptualized, in part because it is not easily measurable or even directly observable. Compact bone is an optically dense, dynamically adapting, mineral and organic tissue of remarkable structural and chemical complexity. We cannot watch bone histology as it changes and
so are left to infer process from resulting stratigraphic microarchitecture. In this regard observing modeling is even more challenging than other bone processes which have relatively easily defined units of deposition or feature characterizations, as is the case for bone remodeling for example. Conceptualizing modeling accurately may be elusive, but it is necessary to truly understand boney changes at the microscopic scale (Maggiano, 2011).

There has been significant contention and confusion regarding even the basic definition of the term, “modeling.” To develop a functional understanding, it is first necessary to 1) differentiate modeling as a sub-process of general growth and development, 2) recognize that the process is similar in all bones, whether they are formed endochondrally or intramembranously, and 3) distinguish clearly between modeling and remodeling (Maggiano, 2011).

Toward that end, the following section first clarifies distinctions between “bone growth” and modeling. A full treatment on the tissues involved and resulting boney structure follows, comparing the histology and vascularity of the two osteogenic membranes: the periosteum and endosteum. These are referred to throughout this dissertation as a combined unit on occasion: the periosteal and endosteal membrane, or PEM, particularly when differentiation is not necessary. After this structural background, the focus will turn to what modeling can accomplish: 1) diametric diaphyseal apposition (as a subcomponent of general bone growth), 2) metaphyseal reduction, 3) medullary extension and centralization, 4) lamellar compaction, and 5) mechanical adaptation.
However, formed bone must be maintained, repairing microscopic damage and ensure proper vascular supply. Therefore, bone remodeling is discussed next. Despite the seeming clear distinction between bone modeling and remodeling, some confusion exists due to historic ambiguity in the usage of terms and because small scale modeling and remodeling are difficult to differentiate. Therefore, special attention is paid to identifying these processes, and terminology is explicitly clarified. That they are separate processes, however, is not as useful a perspective as one emphasizing their synergy and overlap. This perspective is more useful for the current research which requires an understanding of both processes in equal measure to illuminate the phenomenon of bone modeling drift. Finally, the Chapter closes with a discussion on variability in bone microarchitecture based on various demographic, genetic, and life history circumstances.

3.2 Modeling

An important defining characteristic of modeling is its complete conceptual distinction from other aspects of long bone development, growth, and fracture repair. Modeling can be differentiated from these processes, in that it accounts only for activity at the periosteal and endosteal membranes (Frost, 1973) rather than within the physis or callus. Bone “growth” is a higher order and more general concept referring to overall increase in the size and/or mass of developing bone and transpires via two main
processes: intramembranous (non-epiphyseal) and endochondral (epiphyseal) formation (Maggiano, 2011).

But as a hard tissue, bone cannot grow via formation alone. If it did it would weigh too much to satisfy its structural and functional role in the mobile musculo-skeletal system. In addition, unlike soft tissue, bone cannot be moved in relative tissue-space-time after it is formed. Therefore, growth necessitates alterations of the bone’s shape through significant resorption in addition to net formation. Transpiring at the PEM, modeling satisfies much of this growth requirement. In general, it removes bone from the medullary surfaces and deposits bone diametrically, permitting an overall increase in bone size and local alterations, maintaining or adjusting bone shape during growth. The underlying mechanisms behind modeling are similar regardless of the bone type considered (Maggiano, 2011).

No cartilaginous precursor or “anlage” is necessary for primary bone formation during the development of intramembranous bones, such as the scapula or skull (Martin et al., 1998). Instead, formation takes place directly within or under mesenchymal condensations of differentiating osteoblasts attaching to primary trabecular spicules within the connective tissue itself (Fawcett, 1994; Scheuer and Black, 2000). The periosteum activates as the site of primary ossification immediately after its cellular condensation, differentiation, and maturation, thereby accounting for the continued growth of the bone (Fawcett, 1994, Scheuer and Black, 2000). This is also the site for
adaptation due to loading histories through mechanosensation and localized formation and resorption (Maggiano, 2011).

Endochondral bones form by a more complex, dual process. The bone is longitudinally extended via endochondral ossification at the metaphyseal plate, but is augmented diametrically by intramembranous activity at the PEM (Martin et al., 1998; Scheuer and Black, 2000). Periosteal and endosteal activity is similar in both intramembranous and endochondral bone development, but growth and adaptation via long bone modeling primarily occurs diametrically, whereas flat bone modeling is seemingly more dynamic (Maggiano, 2011).

The role of bone modeling during growth and mechanical adaptation is complex. Not only must it negotiate the dominant demand for greater net formation than resorption, it must do so while permitting some regions to resorb completely in order to achieve functional morphology. Through these means, modeling accounts for the eventual achievement of the bone’s axis of orientation, its curvature or shape, and its overall thickness (Frost, 1973). All this is performed by two membranes, the periosteum and the endosteum, which, for all their assumed similarity appear quite different histologically, function in different environments, are affected differently by mechanical and chemical stimuli; yet maintain a concerted effort, cooperating, even synergistically, in order to affect boney changes (Maggiano, 2011).
3.2.1 Form

This section describes the histological structure of bone modeling, detailing the generative tissues themselves, the excreted primary bony matrix, and its larger depositional pattern. Distinction is drawn between the periosteal membrane and endosteal membrane, in that the former verges on preforming more like an organ and is self-sufficient, whereas the latter is a single cell layer thick and cannot be considered independent from the hematopoetic matrix from which it separates the bone. Differential response to hormone and mechanical stimuli will also be addressed while simultaneously demonstrating the synergistic nature of net PEM activities. In addition, issues with identification of true bone structural units (BSUs) in modeling processes are outlined.

3.2.1.1 Membrane Histology and Vascularity

The periosteal membrane (Figure 4) comprises an outer fibrous layer and an inner layer of osteogenic cells called the cambium, firmly adhered to the bone surface via Sharpey’s fibers that extend deep into bone tissue (Tang and Chai, 1986). A three zone model has also been suggested by Squier and colleagues (1990), based on the functional separation of three zones: 1) an external region marked by mature fibroblasts and dense collagen fibers; 2) a region with increased vasculature, pericytes, and a more amorphous extracellular matrix; and finally, 3) tightly adhered to the bone itself, a thin osteogenic layer, packed with osteoprogenitor cells and osteoblasts (Maggiano, 2011).
Figure 4: A) Mature periosteal membrane of the humerus (bracket 1), demonstrating clear distinction between the outer fibrous layer (bracket 2) and the smaller, darker osteogenic cellular layer (bracket 3). Note the most recent primary bone deposition has occurred in two thin phases (white arrow). B) Mature endosteal membrane of the humerus (white arrow). Top margin shows marrow lipid tissue (black arrow) and marrow space (black lined arrow). Note a cement line between Haversian tissue (below white lined arrow) and the most recent endosteal primary formation phase (above white lined arrow). “A” and “B” demonstrate a quiescent bone surface, as evidenced by the lack of unmineralized matrix. C) Fetal (6 mo. gestation) metatarsal bone collar demonstrating cellular and tissue level changes associated with osteogenesis at the periosteum, including columnar osteoblasts (white arrow). Periosteal deposition of woven bone transpires via significant secretion of unmineralized matrix (osteoid) (black arrow). Note the targeted intracortical resorption at internal void surfaces facing the endosteum (bottom of image) as demonstrated by the position of osteoclasts (black lined arrow). D) Endosteal formation in the same bone as “C” via columnar osteoblasts on one side of a trabecular spicule, while the other side appears newly resorbed with one remaining osteoclast (black lined arrow) and Howship’s lacunae left behind as evidence. All images are generated from paraffin embedded, decalcified, thin transections, stained with hematoxylin
and eosin. Scale bar indicates 50 µm for “A” and “B.” taken at 100x magnification; “C” was taken at 200x magnification; “D” at 400x. Images courtesy of Lisa Lee (Maggiano, 2011).

Fibroblasts and their excreted matrix, most dense in the easily discerned, external layer, lend the membrane its particular strength and exert surprising force on the bone itself via membrane insertion on the physis (Forriol and Shapiro 2005). Though the membrane does not completely enclose the epiphysis (Jee 2001) it resists 80% of the total force necessary to surgically remove the epiphysis (Forriol and Shapiro, 2005). Pericytes in the second zone are less well understood but could be involved in osteogenesis, perhaps even in supplying osteoprogenitor cells (Diaz-Flores et al., 1992), as evidenced by their higher concentration in the more osteogenic periosteum and decreased presence in the endosteum (Brighton et al., 1992; Allen et al., 2004). The histomorphology of the osteogenic layer changes based on the activity of the constituent cells. In dormancy, these osteogenic cells are elongated; while in actively forming bone they adopt a more cuboidal shape, arranged as a simple stratified epithelium while secreting osteoid (uncalcified extracellular matrix) (Ellender et al., 1988; Chow et al., 1998) (See Maggiano, 2011).

Reports are varied in their interpretation of similarities between the periosteum and endosteum. The periosteum and endosteum are histologically similar according to Shapiro (2002), but Hohmann and colleagues point out that the periosteum is densely innervated and contains lymphatic vessels (Hohmann et al., 1986). Still others emphasize
their differences more strongly, labeling periosteal osteogenic tissue as fibroblast-like and 
in deep periosteal layers, whereas BLCs comprise the endosteum (Martin et al., 1998). It 
would seem that the periosteum is a more histologically complex membrane than the 
endosteum, whether currently depositing osteoid or not (see Figure 4). Not only are the 
membranes distinct histologically, but their environments are completely different. The 
periosteum is constrained and interrupted by tendons, ligaments, and fibrocartilage, 
whereas the endosteum is awash with hematopoietic bone marrow (Jones et al., 1991). 
The periosteum accounts for the majority of blood flow to the bone and simultaneously 
achieves nearly all its venous drainage (Brookes, 1971; Simpson, 1985). Periosteal 
vessels create a complex network with fewer anastomoses in the diaphysis compared to 
the metaphysis (Crocker et al., 1970). On occasion, periosteal vessels can even affect a 
full cortical penetration to the medullary cavity (Reinelander, 1972). In contrast, at least 
in the adult, the endosteum seems to penetrate bone tissue radially via Volkmann’s canals 
which connect Haversian systems laterally within the intracortex (Enlow, 1962a; 
Maggiano, 2011; I. Maggiano et al., 2011a).

The periosteum and endosteum are also differentially affected by mechanical and 
chemical stimuli. For example, the periosteum is well known for its capacity to induce 
formation in response to skeletal loading, particularly when supernormal loads are 
experienced dynamically, separated by resting or recovery periods (Mosley et al., 1997; 
proposed a model in which pressures on periosteal surfaces, by adjacent muscles for
example, can impede bone formation or induce bone resorption and affect bone cross-sectional shapes. Conversely, the endosteum has shown far reduced or non-existent formative adaptation in response to mechanical loading (Gross et al., 2002; Jones et al., 1991; Lee et al., 2002; Meade et al., 1984; Srinivasan et al., 2002). Likewise, periosteal tissue is apparently more sensitive to pharmacological stimuli (Midura et al., 2003). Basic sex hormones affect each envelope differently. In general, periosteal formation is positively affected by androgens and negatively affected by estrogens (Turner et al., 1990). Alternately, endosteal estrogen stimulation generates bone formation during puberty (Martin, 2003), which reverses with decreasing estrogen levels (Yao et al. 2000, 2001). Venken and colleagues (2006), however, call attention to the complexity of these pubescent boney changes, specifically in that boney response is not only hormone specific, but also dose dependent. Even the general lifetime function of the two tissues are opposite according to some authors who regard formation as the net function of the periosteum and resorption as that of the endosteum (Epker et al., 1965; Stoker and Epker, 1971). Despite these differences and despite their clear physical separation within bone tissues, the periosteum and endosteum are capable of extremely fine-tuned cooperation during growth and adaptation; without which, bone’s compositional resilience and capacity for remodeling alone would insufficiently counter the loss of strength suffered by inappropriate morphology. In the healthy, active individual, modeling is initiated and directed by a unique genetic and mechanical developmental history, accounting for most of what we recognize about a specific bone’s morphology and function. This unique
history is an important source of histomorphological variation and records itself stratigraphically within the microarchitecture of a bone (Maggiano, 2011).

3.2.1.2 Primary bone histology

In general, modeling formation at the PEM occurs either in rapid deposition of woven bone with less well-organized collagen content; or as more slowly forming, lamellar bone with more regularly organized collagen (Martin et al., 1998), though much can be gained from considering a continuum existing between the two (Martin et al., 1998). Giraud-Guille (1988) identified two different types of alternating collagen fiber orientations in lamellar bone: orthogonal and helicoidal. Other researchers noticed that “pairs” of layers exist with either dense or loosely bundled collagen fibers (Marotti, 1993). Regardless most of this work has been limited to osteonal lamellar tissue. Whether important differences exist in the ultrastructure of primary and secondary lamellar bone collagen is unknown (Maggiano, 2011).

Individual lamellar sheets are fairly uniform in thickness at approximately 2-3 µm in cross-section (Ascenzi et al., 1982; Reid, 1986) but of unknown dimensions along other axes. This is due to trade-offs between observable volumes and sufficient magnification and resolution. Unfortunately, only transections permit clear observation of PEM lamellae, rendering measurement in three-dimensions impossible. Light microscopy reveals their stratigraphy, however, detailing localized growth and adaptive
processes at the histological scale. A single lamella (pl. lamellae) is a pair of layers (Martin et al. 1998) each with differing collagen orientation (typically one bright and one dark under polarized light, see Section 5.4.2) (Johnson, 1964), though on occasion the terminology is loose (“lamella” can refer to only one of the 2 µm sheets in the pair). Lamellae result from calcification of osteoid (average osteoid seam, 15 µm) (Johnson, 1964). Osteoid is layered upon existing mineralized bone surfaces by osteoblasts activated from preosteoblasts present and potentially resupplied within the PEM (Martin et al., 1998, Aubin, 2001). Total calcification of an osteoid seam may take roughly 10 days and result in 3 lamellae (each with two 2.5 µm sheets) (Johnson, 1964). During their formation lamellae encase some of the osteoblasts from the osteogenic membrane that produce them. These cells then adopt a new morphology, identifying them as osteocytes, locked in small oblong cavities between lamellae called lacunae (Martin and Burr, 1989). Most often these lacunae are present within rather than between lamellae, specifically within those identified as “loosely” constructed by Marotti (1993). Extremely small crack-like fissures, or canaliculae (~500 nm in diameter) (Knothe Tate, 2003), emanate from lacunae, connecting osteocytic processes to form the lacunar-canalicular system (LCS) (Figure 5) through which osteocytic processes intercommunicate (Knothe Tate, 2003; Taylor and Lee, 2003) (See Maggiano 2011).
The LCS is a complex network, or syncytium, acting as a means for transmission of chemical and/or mechanical signals, which, as part of compact bone’s mechano-transduction system, are vital for healthy bone growth, maintenance, and adaptation (Donahue, 2000; Knothe Tate, 2003; Taylor and Lee, 2003). Part of this system includes the BLCs covering most bone surfaces. Aubin (2001) outlines the complex cell lineages of the osteoprogenetors and describes the important role of BLCs in membrane activity. According to Martin (2000b), one of the potential roles for lining cells is protecting bone surfaces from a constant resorption signal, typically dampened in healthy, active tissue. To permit resorption these cells must somehow pull away from the bone’s surface, exposing it to resorption, though this is one of many theories regarding bone
mechanotransduction awaiting further study (Martin et al., 1998). Osteoclastic destruction transpires via mineral acidification and organic enzymatic digestion of the surface area enclosed by the cell’s ruffled border (Väänänen et al., 2000) which limits the scale of resorption to small regions, leaving a “scalloped” surface due to Howship’s lacunae (each typically < 20 µm) (Maggiano, 2011).

In order to discuss the microstructural variability induced by modeling activity, we must first become familiar with terminology used to identify PEM origin bone types. The terminology associated with primary lamellar bone architecture can be difficult to negotiate, especially when the human case is portrayed as usual or representative of vertebrate bone, in general. It most certainly is not. Human cortical bone, along with that of other primates, displays important differences from other animal bone, particularly in regions formed by the PEM during modeling. Locke (2004) encourages the use of the word “laminar” to define typical PEM apposition, rather than “lamellar”. First, referral to bone as “lamellar” does not separate primary and secondary bone formation (for example both modeling -primary- and remodeling -secondary- apposition is lamellar). Second, human compact bone structure is not the norm. The vertebrate norm is often appositional sets of 4-20 lamellae, called laminae (Locke, 2004) that are layered on top of one another and contain porous horizons formed by the regular entombment of vascular networks, at one time associated with the formative membrane (Locke, 2004). Laminae display dramatic variability in their spatial relationships with one another, and in the degree or orientation of vessel incorporation within each layer. This leads to various additional
tissue descriptors, including “plexiform” and “fibro-lamellar”. Plexiform bone (Martin and Burr, 1989) is similar to laminar bone except that it is less concentrically organized, repeatedly folding back on its self (Figure 6). Fibro-lamellar tissue has more randomly distributed primary vascular voids, which are later filled in by lamellar bone. Compare these to the even pace of appositional lamellar formation in a large reptile (Figure 7) (Maggiano, 2011).

Figure 6: Periosteal woven bone deposition in the laminar fashion with plexiform vessel inclusion in the perinatal horse foal femur. White arrows in all images denote the periosteum; black arrows, the vascular
void or canal; and black lined arrows mark bridging woven struts providing support for the newest of the forming external laminae.  B) In this young individual (same as “A”) vessels are still within many layers of large unfilled voids prior to boney compaction.  C) Laminar bone exhibiting compacted plexiform structure in the 18 mo. old Sinclair Minipig tibia. The black arrow (expansion) marks a previously periosteal vascular network now deep within the cortex. Note that entombed vessels are dominantly circumferential but with short connections between generations of vascular sheath (black arrow in expansion). The black bracket indicates one full layer as described by Currey (2002), bordered by feint light lines of woven bone. Confocal laser scanning micrograph of archaeological bone fluorescence. Images “A” (100x magnification) and “B” (40x magnification) are microphotographs of paraffin embedded, decalcified thin-sectioning treated with hematoxylin and eosin stain; “C” is a 40x magnification microphotograph of an undecalcified thin-ground section containing visible, though feint, fluorochrome labeling. Images courtesy of Steven Weisbrode (Maggiano, 2011)

Figure 7: Left) Fibrolamellar bone tissue from the goat pericortex. Longitudinal primary vessel canals (black solid arrow) are surrounded by woven bone (white solid arrow), while internal compact structure appears more laminar, enclosing vessel networks (white lined arrow). Confocal laser scanning micrograph of archaeological bone fluorescence. Scale indicates 100 μm. Right) Alligator pericortex demonstrating regular concentric lamellar apposition (black solid arrow) as well as the occasional inclusion of longitudinally oriented primary vascular canals, surrounded by highly mineralized woven bone (black lined arrow). Micrograph taken in white light transmission. Scale indicates 100 μm. Image courtesy of Y. Castro, D. Vanmali, and T. Dupras (Maggiano, 2011)

3.2.1.3 Types of primary bone and associated vasculature

Much of what distinguishes PEM primary tissue types depends on the organization of lamellar, vascular, and woven bone regions. Currey (2002) well-
describes laminar, plexiform, and fibro-lamellar bone from this perspective. In general, the formative layer is separated from the surface of the bone by struts of woven bone spanning a highly vascularized space upon which the newest layer is formed. Within these vascular voids, lamellar bone is laid down more slowly. Sometimes woven scaffolding can form so quickly that there are several tables of unfinished lamina upon one another and only deeper intracortical tissue displays more mature lamellar microstructure. To illustrate meaningful differences between these bone types, Currey (2002) cites a simple perspective outlined by Castanet and colleagues (1996), in which the relative speed of formation at the membrane determines structural variance in the mallard duck: the fastest formation is accomplished by circumferential laminar formation, trapping nearly entire generations of vascular sheaths; and slower formation is accomplished by fibro-lamellar or primary osteonal tissue, which captures vessels more rarely and particularly seems to favor longitudinal vessels (Maggiano, 2011).

This model would place much of human PEM bone in the slowest category of formation, one where vessel entrapment is even more uncommon. This is because human periosteal lamellar tissue typically traps few transverse vessels from the vascular sheath during osteogenesis and so cannot typically be referred to as laminar. However, rapid growth periods in humans can demonstrate laminar-like sets of pericortical woven or lamellar bone separated by longitudinal primary vessel horizons locked within “generations” of lamellar apposition (Figure 8). Even in the infant human, laminar-like human bone is typically limited to only one or two “layers” of the immature periosteal
cortex; this pattern exists rarely and/or briefly and never reaches the volume seen in other species (measurable in centimeters on occasion) (Maggiano, 2011).

Figure 8: Human infant pericortex demonstrating occasional inclusion of longitudinal vessels, as primary osteons (black arrow) as in “fibrolamellar” or “primary osteonal” bone. In this case there exists a single “pseudo laminar” layer within the forming (mostly woven) pericortex (black bracket). The black lined arrow denotes a resorption bay destined for expansion during endocortical resorption or infilling via BMU-based remodeling. The white arrow marks one of many Howship’s lacuna roughening the bay’s perimeter, evidence that resorption was active antemortem. Thin-ground, undecalcified transection of archaeological, femoral mid-diaphysis, Dakhleh Oasis, Egypt. Cross-polarized micrograph taken with 530 nm red quartz compensation. Scale bar indicates 200 µm (Maggiano, 2011)

3.2.1.4 Primary bone structural units

Unlike the BMU, PEM primary lamellar formation occurs independently of resorption. This is not to say that resorption cannot occur on the same surface as immediate subsequent formation (or vice versa), just that previous resorption is not a
requirement for formation. The rate of human periosteal apposition has only been measured in a few studies. Balena and colleagues (1992) report, periosteal formation rates reached an upper value of 4 \(\mu\text{m/year}\) as measured in transiliial bone biopsies from pre- and post-menopausal women (Parfitt, 2002). In later investigations of the ilium (Parfitt et al. 2000) it was found that the net periosteal apposition rate was 0.581 on the external periosteum, compared to an “inferred” net endocortical apposition rate of 0.154 \(\mu\text{m/day}\) on the inner endosteum. They report that this amounts to a total mean periosteal mineral apposition rate of 1.04 \(\mu\text{m/day}\) in children 2-20 years old (Parfitt et al., 2000). It is typically argued that the general formation rate for modeling is faster than remodeling (2-10 \(\mu\text{m/day}\) compared to 0.3-1.0 \(\mu\text{m/day}\), respectively) (as shown in a Table from Jee et al., 2007 providing data collected from Parfitt, 1983 and Jee, 2001). However, one should take caution in interpreting numerical comparisons that can at times report rates of apposition by a single layer of osteoblasts with rates of growth contributed by multiple layers of osteoblasts, as would be the case in measuring total diametric (PEM) changes, or even “single” membrane locations undergoing rapid plexiform growth, for example, where surface topography permits several teams of osteoblasts to contribute to growth along the same axis simultaneously (Maggiano, 2011).

Martin and colleagues (1998) provide an interesting discussion on modeling growth rates and their alteration with physical exertion by discussing four-point bending experiments of Forwood and Turner (Forwood and Turner, 1994; Turner et al., 1994). These authors found that in the rat, periosteal woven bone deposition in response to
mechanical strain seemed to be all or nothing while periosteal and endosteal lamellar deposition were both dependent on strain magnitude (Forwood and Turner, 1994; Turner et al., 1994). Martin and colleagues (1998) suggest this contributes a quantum-like aspect to modeling due to histological and functional limitations of periosteal and endosteal histology (Maggiano, 2011).

In addition to changing rates of formation, modeling activity affects variable magnitudes of bone volume. When the membrane is fully engaged in formation, lamellae can span nearly the entire PEM; generating “circumferential lamellae”. This leads to the familiar histological landscape of a long bone transverse cross-section (especially in preadolescents or young adults): PEM lamellae “sandwiching” more highly remodeled bone occupying the intercortex (Enlow, 1962a) (Figure 9).

Figure 9: The traditionally described histological landscape of young compact bone. Primary lamellar bone (black solid arrows) formed by the periosteum (lower left) and endosteum (upper right). White solid arrow marks remodeled Haversian tissue formed by BMU activity. This remodeling transpired in the older intercortical primary tissue and has now turned over a large area, replacing primary osteons with secondary
and increasing the general porosity of the region. Thin-ground, undecalcified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt. Cross-polarized micrograph taken with 530 nm red quartz compensation. Scale bar indicates 300 µm (Maggiano, 2011)

Garn (1972) found that the period during and immediately after the adolescent growth spurt was marked by endosteal formation continuing sometimes into the 4th decade of life, especially in males. After primary modeling is accomplished and skeletal maturation is complete, the periosteum may remain slightly active while endosteal bone formation ceases altogether (Lazenby, 1990; Ruff et al., 2006). Primary pericortex resorbs less (Martin and Armelagos, 1979; Kerley, 1965) and potentially is exposed to more remodeling than endosteal tissue. This could be due to increased transference of stress along the outside of bending bones (Heller et al., 2001; Van Buskirk, 1989; Kimura and Amtmann, 1984; Ruff and Hayes, 1983a; Pauwels, 1965), and potentially due to the increased tensile strength of primary compared to secondary bone (Vincentelli and Grigorov, 1985). This can result in significant areas of unremodeled primary endosteal lamellae, even in individuals approaching the estimated fifth decade of life (I. Maggiano et al., 2011a). After this age it becomes more likely that continued remodeling and/or age associated endocortical resorption could remove all remnants of primary endosteal tissue (I. Maggiano et al., 2011a; Maggiano, 2011).

Not all periosteal or endosteal modeling is achieved by full membrane formation or resorption. Modeling can also occur in smaller more amorphous or regional domains of varying cortical depths as observed in transverse cross-sections microscopically.
Consecutive and concentric lamellae that are at least partially uninterrupted are modeling BSUs and are referred to here as “phases.” borrowed from geology and archaeology’s term for a specific frame of stratigraphic deposition. In 1973, Frost used formation or resorption “packets” as a general descriptor for the same phenomenon. Frost (1964) recommends the term, “foci.” for sites of initial modeling apposition or resorption, a term also used here. A phase or focus can be modified by the descriptor “formation” or “re Biomech sorption” when necessary. Unfortunately, the same limitations discussed previously with regard to lamellar quantification, apply to modeling phases. Current techniques suffer an inability to follow either lamellae or their phases, to terminal ends in three dimensions. Features microscopic in one axis and macroscopic in another, locked within hard tissue, pose a great challenge for quantification. However, polarization microscopy is a useful aid in viewing lamellar stratigraphy (Bromage et al., 2003, Marotti, 1993, Boyde and Riggs 1990, Schultz, 2001; Maggiano et al., 2009a, 2011, 1. Maggiano et al., 2011a), as is thoroughly discussed in Section 5.4.2.

3.2.2 Function

3.2.2.1 Bone Growth and Modeling

In order to envision how small scale changes in lamellar orientation, apposition, and resorption account for modeling during growth and adaptation, we need to expand
our perspective, considering the concerted efforts of both surface membranes simultaneously. Over an individual’s lifetime, the function of boney envelopes is sometimes oversimplified. Growth typically requires periosteal expansion and endosteal resorption (Epker et al., 1965; Stoker and Epker, 1971). However, modeling cannot be this simple if the goal is to affect complex morphological change in an adaptive context. For example, according to Garn (1970), more than 25% of an individual’s total cortical thickness will be accounted for by the narrowing of the medullary cavity via endosteal bone deposition. Without concerted local modeling activity bones would be unable to alter their curvature or orientation with respect to other elements. In addition, longitudinal extension would fail to form an effective compact diaphysis; instead metaphyses would grow unaltered volumes of trabecular tissue. In order to account for necessary shape change during growth, the relative rates of formation and resorption activity must be altered from region to region along both the periosteum and endosteum (Maggiano, 2011).

3.2.2.1.1 Metaphyseal reduction

In addition to drift, modeling processes account for the shaping of bone during its elongation at the growth plate (Figure 10, white checkering). This occurs, in part, via metaphyseal reduction, Frost’s (1973) “necking down” or “metaphyseal drift system” where periosteal resorption reduces and smooths the diameter of metaphyseal tissue.
destined for eventual inclusion in the diaphysis (Enlow, 1962a; Jee and Frost, 1992; Rauch et al., 2001).

Figure 10: A) Schematic of immature cortical (black) and trabecular (grey) regions destined for modeling alteration during long bone extension. B) Bone is resorbed at the metaphyseal periphery and within the medullary cavity, affecting both compact and trabecular tissue alike (white checkering). New compact tissue must also be constructed, both within trabecular voids during lamellar compaction, and at the periosteum via diametric apposition (grey bars). C) Even in the adult there remains evidence of these changes as the bone extends in length through endochondral ossification. Adapted from Enlow 1962a (Maggiano, 2011)
In this fashion, the growing metaphysis undergoes periosteal resorption almost immediately following the formation necessary to stabilize the new tissue forming at the widening physis (Enlow, 1962a). Slowly, as the bone elongates, the flared diameter reduces to that of the diaphysis. Only the cessation of longitudinal extension and the dampening of modeling activity “freezes” the metaphyseal morphology, precluding continued large scale resorption at the metaphyseal surface. The resulting adult morphology is then free of course to alter through mechanically adaptive modeling, albeit with a reduced sensitivity to mechanical change in adulthood as reviewed by Ruff and colleagues (2006). Though this process was well described by Enlow (1962a) almost 50 years ago, very little work has been done quantifying or describing metaphyseal reduction in a detailed fashion. This is potentially due to a serious limitation of bone biology. Namely, that absent bone is much more difficult to measure than newly formed bone (Maggiano, 2011).

3.2.2.1.2 Medullary extension

The same limitation affects our understanding of a similar, often ignored process: medullary extension, where the cavity expands and elongates, ensuring optimum strength/weight ratios for the growing bone. This is accomplished both by intracortical and endosteal surface resorption and involves the resorption of trabecular tissue longitudinally and compact tissue diametrically (Enlow, 1962a, Garn, 1970). According
to most authors, this is the only important function of the endosteum. The loss of tissue on this surface over the lifetime of the adult is even significant, particularly in females where those 20-60 have been reported to lose roughly double the bone tissue, dominantly from endosteal bone resorption (Martin and Armelagos, 1979). (3) But this is not the only example of well-orchestrated, complex manipulation of both sister processes simultaneously (Maggiano, 2011).

3.2.2.1.3 Lamellar compaction

The process Enlow (1962a) termed “lamellar compaction” (Figure 10, B), light and dark grey stripes), is also very rarely researched from a modern perspective, in general (an important exception is Parfitt et al., 2000), but especially in long bones. Lamellar compaction is much easier to view in the “flat bones” and the rib because these elements maintain some number of medullary trabeculae throughout life, most of which are formed by prior external periosteal growth and subsequent expansion of resorptive bays (Parfitt et al., 2000). The process is often overlooked in long bones. However, compaction also occurs during their extension, in bone-space which was once trabecular, but with maturation must become compact (Maggiano, 2011).

In effect, lamellar compaction is the reverse of compact bone “trabecularization” by which the compact bone tissue takes on a trabecular appearance through expansion of large resorptive bays. These cavities are connected most commonly with age associated
bone loss, but are also present in abundance during dramatic periods of growth (Figure 11).

Figure 11: Infant cortical woven expansion at the periosteum (white solid arrow), is countered by dramatic endocortical resorption (marked by Howship’s lacunae roughening bone surfaces) both at the endosteal margin (black lined arrow) and in expanding, large, resorption bays (black solid arrows). Black solid arrows also denote loss of several “trabecularized” cortical connections. Thin-ground, undecalcified transection of archaeological, femoral mid-diaphysis, Dakhleh Oasis, Egypt. 530 nm red quartz compensated cross-polarized microphotograph. Scale bar indicates 300 µm.

Just as bone once compact must become spongy; spongy bone must become compact if the demands of growth are to be met. Why this process happens is suggested through experimental and computerized modeling data examined in an interesting work contributed by Tanck and colleagues in 2006, which argues lamellar compaction near the spongy cortex transpires via mechanical adaptation phenomena. Therefore it is possible
that lamellar compaction is actually a secondary effect of longitudinal growth (Maggiano, 2011).

The microscopic structure of compacted trabecular voids is difficult to interpret. In cross-section, Enlow describes this tissue’s lamellae as more disrupted and “whorled” (Enlow, 1962a). This is likely due to the morphological complexity of trabecular voids compared to the relative simplicity of peri- and endo-cortex surfaces and the ordered formation taking place in intracortical BMUs. As long as quiescent BLCs along trabecular void surfaces, like those of the endosteum, maintain their reactivation and osteogenic capacity, Enlow’s lamellar compaction provides a rapid and direct means of transforming trabecular space into compact tissue via modeling. This potential is suggested by direct observation in animal studies that show “uncoupled” formation due to mechanical loading (Chow et al., 1998) or intermittent PTH treatment (Dobnig, 1995) via reactivation of quiescent BLCs, necessitating similar studies on BLCs of endosteal compartments during growth. However, trabecular voids could also be filled by hemi-osteonal remodeling, if remodeling formation can outpace resorption – a phenomenon reported or discussed often (Martin et al., 1989; Jee and Frost, 1992; Frost, 2003; Parfitt, 2000, 2003) (Also see Section 3.3 for a detailed description of remodeling, osteonal and hemi-osteonal). This type of remodeling in trabecular is accompanied by a BLC layer of the surface remains intact as a “ceiling” above osteoclastic resorption and subsequent osteoblastic formation transpiring underneath (Hauge et al., 2001). However, this ceiling could be present during modeling resorption or formation as well. Exactly where the
defining line between modeling and remodeling lies, indeed whether there is a line or not, is significantly unclear in this specific case (Maggiano, 2011).

Much of this would depend on whether the endosteal membrane is significantly different from the BLC “membrane” encasing all internal bone structures, including large resorption bays or trabecular voids. Basic histology suggests they are similar, aside from the concentration and type of hematopoitic tissue within the void-space. Regardless of any histological similarities, however, trabecular struts should experience dramatically different local surface area mechanics (Ensrud et al., 1995; Szulc and Seeman, 2009) where these conditions could be optimal for the activation of BMU activity to avoid instigating loss of trabecular connectivity via standard osteonal remodeling (Parfitt, 2003). The issue of (or if) this trabecular modeling and hemi-osteonal remodeling could be differentiated histologically is a question raised by Jee and fellows (2007). They answer in the affirmative by supporting Frost’s (1988a, 1988b, 1989) usage of the term “minimodeling” to differentiate “packets” (here, “phases”) of bone formation on trabecular surfaces with no evidence of prior resorption due to the presence of “smooth cement lines.” or arrest lines (Jee et al., 2007). (4) The continuation or movement of the BMU could still be a problem within trabecular and endosteal environments alike, but, regardless of how trabecular spaces are filled, conceptually; physically, they must be filled, lest metaphysseal reduction expose the spongy bone surface (Maggiano, 2011) (See Section 3.2.2.1.3 for observations regarding modeling or remodeling internal void-space).
3.2.2.2 Mechanical Adaptation

For over half a century skeletal biologists have been re-inventing their perspective on bone biology, adding to their observations regarding cell type and function a more complete consideration of bone strength. Now they emphasize bone’s ability to sense its normal and supernormal loading and adjust, constitutionally and morphologically, to withstand them. In this new paradigm, sometimes called the Utah Paradigm (Frost 2001), modeling and remodeling occur in direct response to mechanical loading, and growth is necessary but not sufficient to account for bone features or their change over time. This perspective has carried us far, from an incomplete, over-focus on cellular activity and mineral homeostasis, to a more complete understanding of bone as a dynamic tissue, responsive to mechanical strain. The realization that modeling and remodeling can be affected by loading history approaches communal epiphany. But reservation is required in its employment, lest a new form of over-simplification, mechanical determinism, take over, obscuring other primary influences on bone (Maggiano, 2011).

The most critical determinants of a long bone’s mechanical rigidity are strength relative to size, cross-sectional shape, and composition (density and mineral content) (Martin and Burr, 1989; Biewener, 1992; Goldman et al., 2007; Sone et al., 2006). Bone tissue has the ability to adapt all three determinants to physical loading conditions. This has been demonstrated frequently through experimentation (Woo et al. 1981; Matsuda et al. 1986; Rubin et al. 1995; Mosley and Lanyon 1998) and in recent studies on athletes.
Structural adaptation is accomplished through a negative feedback system, which adds, resorbs, or replaces material, maintaining mechanical integrity (Frost 1964a,b, 1988c; Turner, 1999; Skerry, 2006). Although this phenomenological system, often called the mechanostat, predominantly focuses on localized mechanical strain, systemic factors are also influential, including genes, hormones, nutrition, and even climate (Frost, 1987; Turner, 1999; Pearson and Lieberman, 2004). Recent evidence even suggests that the brain may also have a direct role to play in apportioning response to functional adaptation (Rubin and Rubin, 2008) (see Maggiano, 2011).

The importance of mechanical adaptation for proper bone modeling is hard to overestimate and is only now being fully realized. Computerized modeling of mechanical factors in femoral bone growth and development has demonstrated evidence that bones are in some ways “self-designing” structures (Carter et al., 1996). In addition to the vital role even fetal muscular contraction provides, we also know that the onset of bipedal locomotion in early child development affects bone morphology and development in many ways (Haapasalo, et al. 2000; Gosman, 2009). In general, limbs that experience mechanical loading respond by changing bone shape and size. Limbs that have lost or never attained mobility, due to disorder or pathological condition, demonstrate highly predictable bone malformation or deformation (Schoenau and Frost, 2002). Since mechanical demands influence modeling, we can expect modeling activity
to continue after longitudinal growth cessation at around 20 years of age, despite well-known decreases in mechano-sensitivity of bone after maturation. This is precisely the observation of Garn’s (1972) radiological study, showing endocortical formation continuing into the 4th decade of life. Corroboration was provided by Sumner and Andriacchi (1996) who reported diametric growth continued into the 3rd decade during their assessment of cross-sectional properties of the humerus and femur. The potential cause for such a phenomenon is that muscular strength increases well into the 3rd decade (Parker et al., 1990), and the skeletal system must adjust accordingly bone (Maggiano, 2011).

Some non-human animal studies have investigated the mechanical effects of muscle activity on bone more directly by removing a necessary muscular or skeletal element surgically, and/or labeling bone with fluorescent markers for response observation. Epker and Frost (1965) related the work of Avis (1961) and Washburn (1947), studies in which the removal of certain muscles inhibits the formation of the mandibular angle and coronoid process, respectively. Mosley and fellow researchers (1997) have demonstrated that the ulnae of growing rats responds dramatically to physical loading by increasing the overall diameter of apposition, whereas active controls demonstrated only normal lateral modeling drift. More specifically, they found that at lower strains, overall bone formation decreased, rather than increased. Adaptive response was, instead, accomplished by a change in long bone shape via straightening of the diaphysis. At higher strains, total bone modeling increased, both by increased lateral drift
apposition and reversal to formation on opposing envelope surfaces as well, affecting a
general diametric increase in total cortical area. Similar studies on rat ulnae by Robling
and colleagues (2001) showed that even brief static loads could stunt modeling apposition
as well as longitudinal growth, and that this effect is proportional to the magnitude of
loading. They also found a positive bone growth effect from applied dynamic loading
(Robling et al., 2001). To summarize the net findings of similar research, it seems that
bone formation is stimulated by specific activity circumstances. Dynamic, supernormal
strains, with higher strain rates interspersed by periods of recovery are more osteogenic
than static, lower strain, low strain rate during sustained activity (Mosley et al., 1997,
Burr 2002, Fluckey et al., 2002). For more information see summaries of functional
adaptation experiments provided by Currey (2002), and Ruff and colleagues (2006), or
reviews covering classic experimentation on the subject (Nilsson and Westlin, 1971;
Jones et al. 1977) (See Maggiano, 2011).

It is important to remember, however, that “primary” influence of muscle activity
is not the only mechanical influence on modeling process. Body mass and height, or the
length of the bone can have their own effects in addition to differential physical activity
(Robling and Stout 2003). Some have argued that periosteal formation on occasion can
be caused mechanically by increased loads due to endosteal bone loss (Lazenby, 1990;
Beck et al., 2001; Carpenter and Carter, 2008). The “secondary” mechanical
environment can account for the unique morphology of the tibia as well. Carpenter and
Carter (2008) showed that static loading from the muscle bellies surrounding the tibia
could account, in large part, for its triangular cross-section. Similar static loading of the extra-periosteal environment likely accounts for the vessel or digital impressions visible on boney surfaces. For all these reasons the mechanical effect on modeling is both significant and complex and requires a detailed understanding of the primary and secondary effects of local mechanical environment bone (Maggiano, 2011).

3.2.2.3 Primacy

Modeling processes illustrate the perfect point for discussion on the often debated relative primacy of growth or mechanical adaptation in bone morphology. Theoretically, the concept stands: whatever bone can accomplish with no mechanical stimuli we can call development or growth. Whatever growth alone cannot account for requires mechanical stimulation (Maggiano, 2011).

Frost (2001) used a car analogy to represent the components of bone activity and their employment. Presented here is a reconfiguration of this illustrative analogy. To envision the role of growth and mechanical adaptation, we can compare two extreme points of view. In one, a driver would depress the gas pedal and speed off in a straight line until unfortunately smashing into the first impeding object of sufficient mass. A second driver of another mind seats himself and steers the wheel vigorously, shifting gears, engaging the parking break, the turn indicators, all while parked serenely in his driveway. Insisting on the dominance of either adaptational or developmental processes
in bone biology is like forcing one of these extremes upon a hapless driver (Maggiano, 2011).

Debate between prime forces typically inhibits a holistic understanding of important concepts like bone strength, form, or function. However, when the goal is to uncover and identify the mechanisms behind bone form and function, this separation must be made – despite challenges. By the previous analogy, despite insisting that a functional car be both controllable and mobile, some specialists should know: a gas pedal does one job, and a steering wheel, the other (Maggiano, 2011).

For example, if a “prime effector” for bone modeling is desired, it is probably growth. Whether bone adapts to mechanical loading is, by definition, secondary to whether the bone exists – albeit an important second. One of the first true assertions that growth is primary was the often cited “element swapping” experiments like those reported by Murray (1936). In these types of studies, embryonic transposition of limb buds, sometimes even with musculature removed, did not interrupt the formation of element specific morphology like diaphyseal curvature or even the femoral head or condyles (as discussed in Krahl, 1945). Modern evidence for this perspective can be found in Wong and colleagues’ (1993) finding that even without fetal movements required for normal development the primary bone collar still forms around the diaphysis early in development. Yet, sufficient evidence has also been collected to argue that fetal movements are vital for the achievement of functional morphology (Rodriguez et al., 1988; Hall and Herring, 1990). In a particularly well phrased argument for why we
shouldn’t view boney change solely through biomechanical eyes, Turner (2000) points to works that demonstrated the insensitivity of bone to mechanical adaptation when either growth hormones (Forwood et al., 2000) or parathyroid (Chow et al., 1998) are unavailable. He also reminds us that the positive osteogenic effects of Prostaglandin E2 (Akamine et al., 1992) and PTH (Ma et al., 1995) are completely independent of physical loading. To put it another way, that modeling occurs is attributed to growth and development; how it occurs is attributed in large part to mechanical (and other epigenetic) factors (Maggiano, 2011).

Attempting to directly measure the differential effects of growth and mechanical adaptation on modeling is challenging. One way to do that is to uncover the genetic sources of modeling variation. Hansen and colleagues (2009) recently attempted to do just that by measuring the heritability of femoral cross-sectional geometry in baboons. They found that genetic effects accounted for only about 15-23% of cross-sectional geometric phenotypical variance. Likewise, Volkman’s (2004) research group determined that genetics accounted for 2.9% to 15.4% of the variance observed for mechanical traits they measured in the rat femur (most of which remained significant after standardization by body mass). Robling and Turner (2002) point out that mechosensation is not without its own genetic component. For comparison, however, approximately 75% of human height is estimated to be heritable (Beunen et al., 2000, Liu et al., 2006; Silventoinen, 2003; Ulijaszek, 2001), providing yet another reason to
investigate modeling processes on their own merit, distinct from the process of long bone elongation (Maggiano, 2011).

3.3 Remodeling

The previous sections have detailed modeling as an aspect of bone formation during growth and development as well as mechanical adaptation. Once formed, however, bone tissue must be maintained. In some organisms (including humans), modeling’s sister process, remodeling, accomplishes this function – one shaping, the other reconstituting. The two sister processes would seem easily separable. As Parfitt (1983) and Jee and colleagues (2007) summarize, modeling and remodeling are separated by 6 or 11 (respectively) different defining traits, including speed of formation (modeling is faster) and microstrain activation (modeling requires ~2x the strain to stimulate formation). Unfortunately, despite general differences, easy distinction on a case by case basis is often elusive and some considerable confusion exists regarding which process accounts for which boney changes (Maggiano, 2011).

This complexity rests on an even older confusion in terminology. Primarily terminological issue develops from three main confounding issues. 1) Unclear distinction between periosteal/endosteal osteogenic envelopes and those formed and functional during remodeling processes. 2) The unfortunate assumption that evidence of prior resorption indicates remodeling (equating resorption and remodeling on certain surfaces
despite little or no evidence for BMU formation). 3) The historic (and even modern) clinical use of the word “remodeling” as a general term to describe nearly all boney changes during growth, healing, or in response to various stimuli. Often this leads to modeling processes being labeled “periosteal or endosteal remodeling” (e.g. Frost, 1964a,b), necessitating caution in the interpretation of some early literature. By the 1970’s, publications like Frost’s (1973), attempted to clarify the distinction between these modeling and remodeling but the distinction has unfortunately remained unclear in some camps (Maggiano, 2011).

Distinction can be drawn between “modeling” and “remodeling” both functionally and structurally. Modeling accounts for changes in bone size, shape, and position (relative to other tissues) via primary, bone formation and resorption at the PEM. It can result in dramatically larger volumes of deposited or resorbed bone tissue, sometimes encasing entire bone surfaces in sheet upon sheet of bone, or removing them altogether. In contrast, remodeling is the process of bone maintenance and repair, accomplished by bone multicellular units (BMUs), collections of osteoclasts and osteoblasts arranged in a particular structure. This multicellular unit penetrates existing primary bone via resorption at its leading end “or cutting cone” and concentrically and appositionally replacing it, from the void’s periphery, inward, with new lamellar bone. Often these secondary lamellae close around an accompanying vessel and nerve system at their center, forming a vascular canal (Martin et al., 1998; Parfitt, 2003). BMUs result in smaller bone structural units (BSU), referred to as Haversian systems, or “osteons”
(~200-350 µm) in intracortical tissue, and “hemi-osteons” (60 µm) in cancellous tissue (Eriksen et al., 1994; Parfitt, 2003). BMUs form in three sequential steps: activation (A), resorption (R), formation (F) at a single two-dimensional location. It is more difficult to remember, however that each BMU is simultaneously accomplishing both “R” and “F” in three dimensions (and potentially “A” as well, during branching events) (Maggiano, 2011).

PEM activity and BMU activity are also distinguished by the degree of “coupling” between formative and resorative processes. PEM activity requires no connection (in process or mechanism) between formation or resorption (Frost 1973; Martin et al. 1998). Therefore, according to convention, modeling could progress from “A” to “F” and subsequently reverse to “R” when necessary (or vice versa). This stands in stark contrast to BMU activity which appears “coupled” with formation closely following resorption in both time and space (Martin et al., 1998; Parfitt, 2000). Unfortunately, most observation of bone histologically is not in real time which can hide certain circumstances during development or disease that challenge the closeness of BMU coupling, ensuring that formation lags behind resorption significantly, leading to large resorptive bays. This phenomenon is sometimes called “trabecularization” of compact tissue, an occurrence blurring the lines between compact, trabecular, and endocortical tissues and therefore between osteonal and hemi-osteonal BSUs and those resulting from endosteal modeling events, necessitating caution in histological interpretation (Maggiano, 2011).
In short, convention as applied here suggests: referring to any process accomplished by the PEM rather than the BMU, requires term “modeling.” even when formation follows previous resorption. In addition, great care will be used here in the use of processual terminology, “periosteal” or “endosteal.” and positional terminology, “pericortical” or “endocortical”. Conflating these terms creates error and oversimplifies boney response, leaving important questions unasked. Confusion is also be averted here by using the acronym “PEM” to address the periosteal and endosteal membranes collectively; and by referring to bone tissue formed by these membranes by a cortical descriptor (i.e. pericortex or endocortex) when context permits (rather than only using these terms to refer to the current surfaces of the cortex, or as arbitrary “zones”) (Maggiano, 2011).

3.3.1 Form

Remodeling is best understood as a process by which bone constitution is maintained via secondary lamellar replacement by a particular patterned bone cell aggregate called the bone multicellular unit (BMU) (Frost, 1973), or remodeling units (BRU) (Eriksen et al., 1994). The BMU is a collection of bone resorbing and forming cells that ensures proper bone development and health by replacing immature bone (woven or fibro lamellar), or adult bone threatened by age, microtrauma, or other interruptions to the integrity of the LCS, with mature lamellar bone. Although typically bone science focuses on bone, it is important to remember that remodeling is vital to the
vascularity of bone and that on occasion this responsibility could trump typical “bone-centric” concerns. Remodeling is also the only way internal bone vasculature can change after primary ossification, suiting changing demands on nutrient supply during both developmental and adaptive efforts. Remodeling is accomplished through three general phases: 1) Activation, 2) Resorption, 3) Formation and 4), Mineralization (Martin and Burr, 1989).

Activation is the recruitment of osteoclasts and osteoblasts to the site designated for resorption and formation. Arguably this phase is the most important of the three. An increase or decrease in the activation rate dramatically affects the overall state of the bone, potentially increasing resorptive and formative rates multiplicatively with each new activation (Martin and Burr, 1989). During activation, osteoclastic cells are formed or activated and osteogenic cell precursors begin to mature into osteoblasts (Martin and Burr 1989). Because the BMU will move through solid bone far from the surface, vascularization of the cavity follows behind closely, supplying nutrients and necessary cell reinforcement.

The resorptive phase begins as soon as mature osteoclasts have collected at the activation site on the bone surface and begin dissolving the bone’s calcified and organic matrices. The rate of progression of osteoclastic resorption is roughly twice as slow in cortical, as it is in cancellous bone – metabolically, however, the cost is nearly equivalent (Eriksen et al., 1994). The histomorphology of resorptive and formative BMU regions are slightly different between these bone types, accounting for the variation in speed. In
cortical bone collected osteoblasts penetrate bone tissue as a “cutting cone” about 200-300 μm across (Martin et al. 1998). Behind this cutting cone is a resting phase where no bone is being additionally resorbed. This resting phase lasts for about 9 days (Martin and Burr, 1989), and results in the cement line, or reversal line observable at the boarder between BMU deposited lamellar structures.

After the resorptive space is fully formed and the resting phase has past, osteoblastic activity begins on the new surfaces left behind by osteoclasts. Osteoblasts are recruited constantly to replace those that remain behind, locked within lacunae to form the cellular component of the LCS. On the BMUs bone forming end or “refilling cone” osteoblasts work side by side occupying a surface of 15 μm each and do not move along following behind the cutting cone, but instead remain in their current position filling in the tubular resorption cavity from all sides, layer by layer (Martin and Burr, 1989). New osteoblasts must differentiate or activate behind the reversal line to continually refill the area carved out by the cutting cone. Formation alone takes about 90 days (Eriksen et al., 1994) and is based on the osteoblasts rate of osteoid secretion.

About 10 days after formation begins, calcification occurs due to hydroxyapatite mineral condensation within the osteoid and amongst the collagen fibers (Martin et al., 1998). During primary mineralization, in the first week, about 60% of the osteoid is mineralized. Following this, it takes about six months for mineralization to be completed (Martin et al., 1998). Incompletely mineralized osteons display different mechanical properties than osteons with completed mineralization (Martin et al., 1998).
Thus, each phase of remodeling activity is marked by specific precursor cells or mature osteoclasts and osteoblasts. Cortical BMUs are conical in shape (200 μm wide and 400 μm long), and travel through bone roughly longitudinally at about 40 μm/day in long bones (Eriksen et al., 1994).

The entire process, including the exponential decline in osteogenic rates towards the final stages can take up to six months (Martin, 2000a). Martin and Burr (1989) have offered several theories on why this is the case, but current research regarding osmotic pressure and pulsatile pressure gradients should also be considered in modern approaches (Knothe Tate, 2003). This consideration could eventually explain apparent pressure atrophy phenomena at bone-vasculature interfaces. While in the past, the processes of BMU resorption and formation were viewed as inextricably linked, or coupled, by signaling processes, i.e. the freeing of matrix components by osteoblasts may stimulate osteoblastic activity (Martin and Burr, 1989). The degree by which activation and formation are truly coupled, one of the major distinctions between remodeling and modeling processes, has been under reconsideration of late (Gasser, 2006). It is interesting that perhaps further illumination of signaling pathways could show that they are the same or similar for both remodeling and modeling (Boyde, 2003).

Resulting lamellar bone, left in the wake of the advancing BMU is sometimes referred to as a bone structural unit (BSU), but more commonly as a secondary osteon or Haversian system to distinguish them from the vascular supply of primary bone tissue. When viewed in transverse cross section, Haversian systems and their concentric
lamellae are reminiscent of a bulls-eye, with a vascular canal at the center marking the thoroughfare for BMU, and ultimately the neighboring cortex’s blood supply. Osteons are now recognized as more complex structures than originally conceived (Cooper et al., 2003; Tappen, 1977). A vast network of branching and conjoining osteons comprise Haversian systems that may run for several millimeters through long bone diaphyses (Cooper et al., 2003; Parfitt, 2002; Tappen, 1977).

Osteons have many types some of which could be important for understanding branching morphology, growth disruptions, or biomechanical influences. Particularly interesting are drifting osteons, present particularly in less mature bone. These osteons resorb bone more quickly than can be formed, in multiple directions, simultaneously (Stout, 1997). Formation appears limited to one side of the void surface, however, leaving what, in transverse cross section appears as a meandering osteon that is even capable of cutting back on tissue it has already deposited. Drifted osteons have not been linked to any known pathological circumstances, but what causes a disconnect between resorptive and formative rates and directions to be metabolically or mechanically useful is not clear.

BMUs in cancellous bone are not only, in some aspects, larger (>300 μm wide and many times as long), but also have a different shape (Eriksen et al., 1994). Whereas cortical BMUs are conical, cancellous BMUs appear semilunate in three dimensions – again with the widest part toward the front resorption edge (Eriksen et al., 1994). The depth of the resorption pit reaches about 60 μm and is filled by osteoblastic activity in the
same fashion as described previously but takes approximately 145 days to completely refill (Eriksen et al., 1994). The resulting BSU has sometimes been referred to as a hemi-osteon (Parfitt, 2003).

Despite the newly appreciated complexity of BMUs, for various research purposes, and because, theoretically, branching nodes mimic new activation events, the osteon is still treated as a distinct unit representing the A-R-F phases of remodeling processes. Likewise, the BMU is still viewed as a coupled process (Parfitt, 2003), despite circumstances where coupling is at least delayed if not completely absent during intracortical resorption on one (drifting osteons) or all sides of the bay (see Figure 11).

3.3.2 Function

The functions of remodeling are diverse. First, remodeling simply replaces immature bone and repairs damaged bone, maintaining a bone’s mechanical integrity. Second, it provides a way for the body to balance the concentration of essential minerals in the serum. Third, as a more evolutionary perspective, remodeling reduces fracture risk and therefore increases the organism’s chances for reproduction (Burr, 2002; Parfitt, 2002).

Controlling or directing influences on remodeling rates are many. This is partially caused by the complexity of the BMU itself. Each cellular member of a BMU may have at times differing sensitivities to various biochemical and mechanical signals.
The topic is also made more complex by the potential existence of two “types” of remodeling necessary to meet all above mentioned functions: “stochastic,” background, nontargeted, and random remodeling; or “targeted,” localized repair remodeling (Burr, 2002). Remodeling that helps to keep a balance in the concentration of minerals in the serum is more or less independent from location, as long as it does not disturb mechanical integrity. However, remodeling for repair and assurance of mechanical integrity requires site-dependent specificity.

Experimental animal studies show that remodeling can be activated by exercise programs (Lanyon et al., 1982). Mechanical loading is by far the most well-recognized stimulus for inducing targeted remodeling. Microfracture, cell damage/apoptosis, vacant lacunae, and mechanical strain causing local deformation have shown the ability to trigger remodeling processes in vitro. How these conditions actually activate remodeling is still not fully understood, however, most likely, effects on haversian canal wall stresses, changing local strain near osteocytes or damaged or disrupted osteocyte networks may trigger bone differentiation on bone surfaces (Burr, 2002). Remodeling rates have also been shown to vary based on population specific genetic controls that are only now beginning to be understood in rat models (Robling and Turner, 2002).

The answer to the second question, how much of all remodeling is targeted and how much is non-targeted, is even more difficult to find and relies on computer models, since experimental evidence is difficult to obtain. Burr (2002) summarizes that following these models, about 30% of all remodeling might be targeted.
3.4 Variation

Control of modeling processes is generally attributed to some mix of genetic and mechanical influences (Frost 2001). Microstructural changes due to modeling variation also depend on the species and bone element observed, as well as the individual’s sex, age, nutrition, and health. Therefore, it is unsurprising that high levels of microstructural variability can be seen within a cross-section, between cross-sections, and among individuals and populations. A thorough review of variability in bone growth and adaptation, especially the effects of nutrition and disease, is beyond the scope of the current discussion which seeks only to provide a general overview of major contributors to modeling variation. How this variation plays out on the microstructure of modeling phenomena is comparably unknown (Maggiano, 2011).

Some generalities can be made, however. The primary contributor to modeling variation is age. Remodeling is quite well understood as an age relative phenomenon. In human adults, osteonal remodeling replaces an average of about 5% of compact bone each year (Martin et al., 1998). The remodeling rate is especially high in children, reduces in young adults up to about the age of 40, than rises and falls again at about 60, triggered by hormonal changes due to menopause (Martin et al., 1998). This is true for modeling processes too, particularly because modeling is one of the mechanisms responsible for growth. No male will ever look so different from a female that their age
becomes an unimportant contributor to modeling processes, regardless of their respective levels of health and activity, or their population of origin. If more bone exists at the periosteum of a male than a female, the difference is unimportant if *nothing* is known regarding their relative age (Maggiano, 2011). This is largely because the most important side-effect of age is increased body size and muscle strength and because modeling variation is initiated during periods of growth (largely before body size would plateau due to maturation).

Fortunately, we rarely have *zero* relative age information. But, this is much more important than merely stating that the volume of bone affected by modeling is time-dependent. In addition, we can expect the interaction contribution of age to be stronger than those of other sources of variation. For example, the strength of a sex-effect on modeling depends on age. Even more telling, the strength of an interaction effect between sex and skeletal element variation is also age dependent. Add to this the significance of differences in tissue-age-at formation and stratigraphy between even immediately adjacent microstructures, and age and time associated stratigraphy quickly become the most important considerations for interpreting modeling changes in bone (Maggiano, 2011).

The sex of an individual also has both primary and secondary effects on modeling processes. It affects hormone stimuli and growth timing, and the total muscle volume by which physical strain is exerted on bone. But sex can also affect access to food, the frequency and type of physical exertion, and exposure to disease, from one population to
another, limiting potential growth and adaptive response through modeling (Maggiano, 2011). Several researchers have found significant differences between populations in skeletal sexual dimorphism in general (Burr et al., 1990; Cho et al., 2006) and specifically regarding various cross-sectional measures related to modeling processes (Martin and Atkinson, 1977; Ruff and Hayes, 1983a; Feik et al., 2000; I. Maggiano et al., 2008). Sexual differences also exist in that PEMs are affected differently by hormonal circumstance. Estrogen mediated bone loss and gain in females is an area of significant research focus due to its links with osteoporosis risk later in life, which could be related to setpoint changes in the mechanostat (Martin, 2003). Garn’s study of the human metacarpal showed endosteal deposition associated with menarche and increases in late puberty, where the same bone is removed during the period after menopause (Garn, 1970). In addition, the remodeling rate has been found to increase in females at certain ages due to cortical thinning (Kaptoge et al., 2003; Russo et al., 2006). Even, pregnancy, parity, and lactation have been found to have effects on bone turnover (Agarwal and Stuart-Macadam, 2003).

Although ethnic variance is difficult to separate into genetic (ancestry) and epigenetic effects, bone mass also varies significantly between populations (Heaney 1995) – a phenomenon that has also been reported in cross-sectional geometric data collected on the post-classic Maya and medieval Germans (I. Maggiano et al. 2008). There is no reason to expect that this sexual or populational variation (especially when collected from diaphyseal cross-sectional geometrics) should be absent from data
collected on modeling microstructure. But *how* this dimorphism occurs could be variable and is relatively unexplored via histomorphology (Maggiano, 2011).

To complicate matters more thoroughly, all these effects can be specific to the element, affecting individual bones differently or even causing variation between sections taken from the same element (Chan et al. 2007). Attempting to discern between these effects on bone modeling microstructure can be extremely challenging. Attention to experimental design must be meticulous, controlling for as many of these variables as possible, when experimentation is possible, that is (Maggiano, 2011).

3.5 Summary

Modeling is the process by which bone grows intramembranously in order to accomplish long bone diametric growth, morphological adaptation, and relative changes to orientation with regard to the rest of the skeleton. The periosteal and endosteal membranes (PEM) activate formation and resorptive phases where necessary; this can transpire in small or large scales (the entire periosteum for example, during growth and development). The two membranes work in different ways, in part because the periosteum is external and is a more developed, “true” membrane, whereas the endosteum consists of only one thin layer of bone lining cells, not unlike the layer surrounding all internal bone surfaces.
The PEM accomplishes several different types of alterations to long bone structure, including: diametric growth, medullary expansion, metaphyseal reduction, and lamellar compaction, without which extending bones would fail to achieve optimal size or weight. Bone growth and shape change requires not only the redistribution of tissue but its microstructural reconstitution, switching from trabecular to compact and vice versa throughout the bones development.

Remodeling on the other hand is the process accounting for bone turnover, maintenance, and transpires in both stochastic and targeted modes – ensuring that tissue is kept healthy and redesigning the vasculature simultaneously. The results of this process are branching networks of Haversian systems, or secondary osteons that are formed when intracortical resorption is followed closely by formation that encases vessels and/or nerves in the inside of its bulls-eye-like structure. Remodeling can transpire on boney surfaces as well although descriptions are typically limited to trabecular bone and differentiation between such “hemi-osteons” and small modeling events, or “mini-modeling” is difficult if not impossible.
CHAPTER 4: HUMERAL ANATOMY AND BIOMECHANICS

4.1 Introduction

The humerus (Figures 12 and 13) is easily one of the most interesting long bones in the human body. This is largely due to several unique aspects of the human arm that it owes to ancestry and derived features related to carrying activities and bipedal locomotion. First, this section outlines this unique anatomical construction and provides a context for discussion on the specific humeral anatomy that follows. In addition to general humeral anatomy, the effect of three angular aspects of the bone is discussed: 1) humeral developmental rotation, 2) humeral torsion, and 3) the carrying angle. Finally, the basic biomechanic motions of the element are examined with special attention to arm movements involving muscles that insert at the midshaft and along the diaphysis itself.
Figure 12: The Anterior (left) and posterior (right) perspectives on basic humeral anatomy showing muscular attachments and boney features of interest. (Grey, 1918; public domain)
4.2 General humeral anatomy

The upper arm is divisible into 5 regions that account for movement of the humerus and thereby the gross mobility of the upper limb itself: pectoral, clavipectoral triangle, scapular, deltoid, anterior and posterior arm, cubital, and posterior elbow (Moore et al., 2011). The boney constructs of this system include the humerus itself, the clavicle, and the scapula; and anchorages on the sternum, ribs, skull, vertebra, and even the illium. Two major joints directly affect humeral mobility: the glenohumeral, and elbow joints (although the complex articulation of all prior mentioned elements are highly contributive to range of motion, especially the scapula) (Figure 13).
The glenohumeral joint (ball-in socket) is the joint in the human body with the least boney contact or encasement, lending it the widest range of motion (Aiello and Dean, 2002), and necessitating the most soft tissue carriage bracing of any joint (Moore et al., 2011). Direct contact occurs at the scapula’s glenoid cavity, and the indirect
stabilization of the acromion and coracoid process muscular systems. Those muscles and others passively anchor the proximal humerus in place even when they are not in active contraction. All told more than three major muscle systems are involved in stabilization and mobilization of the humerus (the major pectoralis muscle from the anterior axioappendicular system is also involved). These systems include: 1) the superficial posterior axioappendicular system (extrinsic shoulder: trapezius and latissimus dorsi), 2) the deep posterior axioappendicular system (extrinsic shoulder: levator scapulae and rhomboids), and 3) the scapulohumeral system (intrinsic shoulder: deltoid, teres major, and rotator cuff – supraspinatus, infraspinatus, teres minor, and subscapularis) (Moore et al., 2011).

The distal aspect of the element terminates at the elbow joint (hinge), and is responsible for anchorage of muscles involved in flexion of the lower arm and its rotation (pronation). Important origins here include the coracobrachialis, brachialis, triceps brachii lateral and medial heads, and, more distally the epiphyseal origins of the anconeus, brachioradialis, supinator, pronator teres, and common origin for forearm flexor muscles (Moore et al., 2011).

Although all these muscular forces transmit through the shaft of the humerus, several muscular effects on the shaft apply directly. The mid-diaphysis of the humerus is proximate to muscular insertions of the deltoid and coracobrachialis, and origins of the lateral and medial heads of triceps brachii, and the brachialis (Moore et al., 2011). The largest muscle with the least direct effect on the humerus (having no origin or insertion
there but potentially applying significant primary or secondary strains) is the biceps muscle which originates at the scapula’s corocoid process and supraglenoid tubricle, presses it long head into the intertubercular groove, and inserts distally at the radial tuberosity and forearm fascia via bicipital aponeurosis (Moore et al., 2011).

In fact, of all muscle mass held in the middle of the upper arm, the biceps and triceps make up roughly 80%, despite between all their heads possessing only two origins on the humerus: the lateral and medial triceps heads that spiral around the right humerus in a right-handed fashion (clockwise proximally and counterclockwise distally). It is perhaps important here to also note that this spiral is matched by the path of the axillary nerve as well as the radial nerve and deep artery (profunda brachii) pressing into the posterior humeral shaft to form the radial groove. Both of these major nerve and artery pathways originate on the ventral thorax and yet as they pass through the axillary region of the arm they transfer dorsally wrapping around the posterior humerus and continuing along the anterior face of its distal aspect.

4.3 Biomechanics

In addition to basic humeral anatomy, it is necessary to briefly outline each of the element’s major movements. Elevation of the humerus (flexion/abduction) and lowering it (extension/adduction) involve many muscular contributions. This can be easily envisioned during the process of climbing which, in addition to humeral movements
requires the raising of the trunk in humans – necessitating in addition to the shoulder girdle, the actions of remotely originating latissiums dorsi, pectoralis major, and trapezius muscles. This same action is also important for downward and forward motions of the arm such as using an axe, and for the crawling motions necessary for swimming. This example, involving the actions of muscles of the head, neck, spine, chest, and pelvis, well illustrates the engagement complexity of the upper limbs.

Maximum overhead humeral flexion, requires scapular rotation, and particularly engages musculature inserting along the anterior face of the humerus (Aiello and Dean, 2002). Likewise, extension also involves the scapula, and muscles inserting along the posterior humeral aspect. Abduction is accomplished by muscles lying over the shoulder in the coronal plane, whereas adduction requires mooring between the medial humeral face and the thorax (Aiello and Dean, 2002).

Targeted elevation of the humerus, flexion or abduction, is primarily accomplished via the deltoid, which in abduction has assistance from the supraspinatus (Aiello and Dean, 2002; Moore et al., 2011). The major pectoralis muscle aids the deltoid in flexion, but not at all in abduction. Of the downward humeral rotators (achieving extension and adduction), only the latissimus dorsi, the coracobrachialis and the teres major (though this last is superior to the midshaft) interact with the humeral shaft directly (aided by proximal insertions of the infraspinatus, subscapularis, and teres minor (Moore et al., 2011).
Humeral rotation is effected medially by the teres major and subscapularis; and laterally by the teres minor and infraspinatus. All of these effects are largely in place above the midshaft. Humeral lateral rotation is what promotes extension of the abductive range, and also is important for the external rotation of the throwing motion (Hamill and Knutzen 2009). Elbow flexion requires the brachialis, biceps brachii, and brachioradialis (Aiello and Dean, 2002). The brachialis is the dominant flexor; whereas the biceps can act in both flexion and supination (Moore et al., 2011). Biceps supination is most powerful when the hand starts prone (such as when breaking a stick held horizontally in front of the body). Elbow flexion is simpler and relies on the triceps brachii stabilized by the anconeus (Aiello and Dean, 2002). Pronation is assisted by only the anconeus (Moore et al., 2011) and so weakly affects the humerus.

How these motions affect the midshaft of the humerus is complex due to various types of direct and indirect forces mentioned here. Namely muscular interactions anywhere in the discussed region can exert forces on their locations of origin and insertion, as well as in transmittance through bone. But it is also important to take into account the physiological limitations put on the bone by its environment. According to Carter and colleagues (2002), the adjacency, size, and activity of non-attaching muscular elements also constrain shape properties of bone – in their case the tibia specifically. This is another way that muscular activity can affect the bone. In the humerus (Figure 14) this could apply in several meaningful ways to its midshaft cross-sectional geometry.
Figure 14: The total cross-section of the upper element just below the midshaft shows the position of soft tissue relative to the bone. Unfortunately this edition has a vague (incorrect) geometry for this portion of the typical humerus which should have a more flattened anterio-medial aspect (upper –right) which in later editions of this text is corrected (Gray, 1918; public domain).

4.4 Evolutionary context for arm use in humans

Several unique aspects of humeral anatomy become readily apparent through a comparison of the element between humans, apes, and other mammals. To start, all non-human primates have two additional muscles in their shoulder: the atlanto-clavicularis (typically clavicle elevator, and sometimes scapular rotator) and the pectoralis abdominis
(arm flexor) (Aiello and Dean, 2002). In general, humans have a smaller ratio between power and lever arms in brachialis but much smaller biceps (Knussman, 1967). Many of these changes have to do with the humerus decreasing its load bearing biomechanical demands.

According to Ashton and Oxnard (1963), for example, the propulsory muscle group mass in the human arm is less than that of ape cousins (particularly quadrupeds), while human arm-raising muscles (including the deltoid) are far greater in relative mass. This is likely a retention for use manipulating objects in front of the body. Perhaps toward that end, the deltoid insertion is further down the humerus in Homo than it is in any other primate (Inman et al., 1944), bringing its local effects close to the midshaft along an insertion that wraps from anterior to postero-lateral aspects of the diaphysis.

In some cases, the human form as a hybrid of apes. For example, the function and anatomy of the trapezius and rhomboids is quite similar to suspensory locomotors, like gibbons. However, the latissimus dorsi in humans is similar to the great apes in that it attaches to the iliac crest providing direct transfer of weight from front to hind limbs during climbing (Aiello and Dean, 2002).

In others, the human form is unique as is demonstrated by adaptations for tool manipulation. These adaptations are primarily focused in the wrist and interdigitation of phalanges. However, the humerus must also be considered as an element important for determining the position of the entire element and as the focus for mobility issuing from the shoulder girdle. Most important of the humeral adaptations (genetic and
developmental) permitting tool manipulation in the context of bipedality are its lateral rotation and position on the back and side of the ribcage, its twist or “torsion.” and its instigation of the carrying angle that deviates the forearm laterally from upper arm. All of these traits are greater in humans than in any other primate (Krahl and Evans, 1945; Knussman, 1967; Aiello and Dean, 2002; Larson, 1988).

4.5 Humeral rotation, torsion, and carrying angle

In humans humerus meets the thorax at the glenohumeral joint at near 90º dorsal rotation (flattening the scapulae along the coronal axis) compared to the typical mammalian body-plan (Aiello and Dean, 2002; Larson, 1988). This has been called its “rotation” and is effectualized during the 2nd month of development in utero (Krahl and Evans 1945). In non-primates humeral developmental rotation is absent, producing the typical orientation on the front of the chest, to either side of the base of the neck and sagittaly oriented scapulae. Often the difference between this rotation and humeral torsion has been confused or torsion neglected in many anatomical texts according to Krahl and Evans in 1945 – an assertion borne out by a summary scan through many modern anatomy books.

Humeral torsion is typically referred to the degree of medial rotation in the distal joint facing relative to the proximal (Krahl and Evans, 1945; Knusssmann, 1967; Aiello and Dean, 2002). On dry bone measurements, Krahl used the anterior aspect of the
proximal head and the bisection of the inter-epicondylar mass to construct the angle when viewed from a superior position down the shaft. He found the average human degree of torsion to be around 73°, that it differed between populations, that it increased with age until maturation, that stronger individuals had more torsion, and that the bicipital groove functions as a summary of torsion when only the proximal aspect is viewable (Krahl, 1976). In his 1976 publication, Krahl summarizes many aspects of humeral torsion, including its causes. He makes the argument that close examination of humeral torsion across species (from primates to fishes), supports that it may result from two contributive forces: a primary (genetic) effect and a secondary (developmental) one. The secondary effect could begin simultaneous to humeral rotation in the 2nd month of development and Krahl reports (1976) its increase until skeletal maturation. Most of this developmental change takes place before age 8, but continues changing significantly thereafter (Edelson, 2000). This developmental process is argued to transpire at the proximal epiphyseal plate, due to the superior position of lateral rotating musculature relative to the more inferior placement of medial rotators under the prox epiphysis (also cessation of the phenomenon seems timed to the fusion of this physis) (Krahl, 1976).

The modern means of humeral torsion measurement are diverse and particularly important for modern sports-therapy, biomechanics, and clinical investigations where non-invasive measurement systems abound. Today the angle is typically expressed in degrees of “retroversion” (the angle between the distal bisection of the epicondyles and
the proximal bisection of the humeral head apex and the greater tubercle (Hill et al., 1989; Crockett et al. 2002).

However it is formed or measured, humeral torsion in humans is unique. Compared to any primate, human humeral torsion is the greatest (Krahl and Evans, 1945; Larson, 1988; Aiello and Dean, 2002). Larson pointed out in 1988, that the degree of humeral torsion in humans may be an adaptation for tool manipulation rather than seeking its roots in suspensory or knuckle-walking locomotion. Her reasoning is particularly understandable for lesser apes who have low torsion as an adaptation for brachiation – but is not sufficiently robust enough to necessitate parallel evolution of the trait in great apes and humans (between whom torsion is more similar). Particularly Larson noted the importance of humeral torsion for supine lower arm dexterity and efficiency (1988).

This notion has been regularly studied in sports medicine and physical therapy in application to arm mobility (Chant et al., 2007) and force or velocity (Escamilla et al., 2002) (in many sports that use over-arm motion (baseball, swimming, tennis, etc). For example, 40% of racket velocity in tennis players is attributable to internal humeral rotation alone (Elliott et al. 1995). In another broader study, Whiteley and colleagues (2009) examined 217 volunteers, concluding that greater retrotorsion was found in over-arm athletes than non-athletes, dominant versus inactive limbs, and to a greater degree in throwers than in swimmers. Specifically, side difference was widely variable and significant (~12° on average in throwers, ~6° for swimmers). Differences between
swimmers and throwers here was argued to result from the greater action of larger muscles involved in swimming (pectoralis major and latissimus dorsi) in comparison to throwers who have more involvement of the subscapularis muscle (one theorized to account for torsion) (Whiteley et al., 2009). In other studies Whiteley and coauthors (2010) have attributed peak performance during throwing to both youth throwing activity and genetic predisposition (as measured by differences between throwing and non-throwing subpopulations in their non-dominant arms). This occurrence is a testament to the fact that simple inference cannot be attributed only to activity and mechanical loading but also to genes, particularly in traits as variable as humeral torsion.

Although predisposition may be a factor, biomechanical measurements linked to a finite element growth analysis models show how “twisted growth” along the humeral diaphysis (in mass) should be expected particularly in response to the moment of maximum external shoulder rotation rather than contact with the ball (Taylor et al., 2009). These authors attributed torsion to pectoralis major and latissimus dorsi, while loading of the deltoid and pectoralis during ball contact only resulted in more simulated bone growth at these insertions and along the anterior face of the distal half of the diaphysis and didn’t involve the medial aspect of the proximal half at all (Taylor et al., 2009). The pattern of deposition for both phases of motion examined is, however, torsional in general and in agreement with sites where new bone apposition along the shaft could account for retrotorsional change in orientation between the distal and
proximal humerus. This observation goes against Krahl’s older assertions that the shaft might not be involved in achieving torsion (1976).

Humeral torsion is accompanied by another uniquely human trait of the upper arm: the carrying, or valgus angle. This feature informs mobility and structure at the elbow, which is more restricted in range of motion in humans than in great apes (though humans still have more flexion than most other primates (Aiello and Dean, 2002).

The carrying angle is constructed by lateral lean to the lower arm along the coronal plane, relative to the long axis of the humerus. Asymmetry at the trochlea (Hamill and Knutzen, 2009), particularly the comparatively superiority its medial aspect, and/or the superiority of the trochlea with respect to the capitellum (Zuckerman and Matsen, 1989) creates the angle in the ulna, and is measured as the degree of lateral deviance from a straight angle continuing from the humerus. Agreement cannot be made on measurement methods due to competing limitations in clinical, radiographic/CT, and dry bone analyses. Therefore carrying angle has been measured in various ways (Van Roy et al. 2005). According to (Hamill and Knutzen (2009) (and not surprisingly, especially when the range of measurement techniques is considered), its variation is considerable (10-25º) and is widely known to differ by as much as 10º between the sexes (females range from 15 to 25º). Van Roy reports results from six different studies showing this wide variance and an average of 12º in males, 16º in females. Zampagni and colleagues (2008) agree variation is considerable, but report the range is between 11 – 14º, and note that they found no difference between the sexes potentially due to decreased joint laxity in their
athletes. They also confirm measures from past authors (Morrey et al., 1976; Van Roy et al. 2005) that suggest that elbow flexion decreases the carrying angle to less than 3º (linearly or three-dimensionally, respective to these groups of colleagues), and note that even slight variation in degree of flexion produces significant differences in statistical comparisons between groups (Zampagni et al. 2008).

In pronation, the carrying angle disappears – a feature important for the palm forward climbing and brachiation grip to be used during weight transfer (Youm et al., 1979; Aiello and Dean, 2002). Functional employ of the carrying angle can take advantage of several sources of maximization, including: supination in the forearm, extension of the elbow, and lateral rotation in the shoulder joint (Van Roy 2005). In humans, Aiello and Dean (2002) argue it has a role in tool manipulation and points out that outside of the apes, the absence of carrying angles limits the pronation of the lower arm to 90º, in comparison with the maximum in all primates of 156º in humans (using data from Knussman, 1967 and O’Conner and Rarey, 1979).

In lifting and carrying a load with both arms in front of the body, the carrying angle permits a wider total grip. Carrying loads cannot be the only consideration, since the real issue seems to be carrying loads bipedally. Without the carrying angle objects carried at a full extension of the elbow (e.g. Bucket, suitcase) compete with the carrier’s center of gravity (Van Roy, et al. 2005). This should cause trouble especially during gait and when the near leg is load bearing. Few authors address the potential benefits or costs of the carrying angle during throwing. It can be imagined that the carrying angle adds to
the peak of external rotation, despite partial flexion; however, it also necessitates its own
reduction prior to release at the terminal phase of internal rotation. Without this
reduction in carrying angle during this motion, the forearm deviates from the throwing
path defined by shoulder movement. Perhaps this is even the origin of the phrase
misnomer, “it’s all in the wrist.” No information was found regarding another potential
cost of the carrying angle: its impediment to most object manipulation which happens to
take place in front of the body and at the sagittal plane.

4.6 Summary

The humerus is part of one of the most complex joints in the body, providing the
largest range of motion. As such, the biomechanical demands on it are diverse. Most
notably, however, the effect of the Anterio-lateral insertion of the deltoid during various
extension movements and the more anterior and distal origin of the brachialis, engaged
during elbow flexion could be important for understanding demands on midshaft
morphology. This includes direct demands determining the axis of resistance to habitual
loading but also potentially informs the direction of drift if ligamental interfaces interrupt
periosteal deposition. Another important local anatomical consideration is the force
applied to the bone surface by muscle bellies, in this case the biceps brachii and the
triceps “sandwich” the bone, potentially influencing cross-sectional shape locally,
accounting for the anterior “ridge” or seam that is made apparent by its comparatively
flattened anterio medial face.
The human humerus has two fewer muscles in the shoulder than our primate relatives, and the degree of humeral torsion seen in humans is uniquely high, insuring the elbow faces anteriorly or even anterio medially rather than more laterally as in other primates. In addition, the carrying angle is uniquely significant in humans. These features are known to be highly variable throughout human populations and between the sexes. Particularly the degree of humeral torsion has been linked to throwing prowess in that a more lateral facing elbow permits longer back rotation during throwing motion.
CHAPTER 5: METHODS

5.1 Introduction

There are four main methods of procuring samples for studies in human skeletal biology. These include harvesting bone through biopsy, cadaver research, modern mortuary or past archaeological population samples. The current study focuses on the latter two, including analysis of curated contents of unclaimed graves from the Xoclan cemetery in Yucatan, Mexico, and well-preserved skeletons from the archaeological site of Xcambó. The reasoning for this sample selection is discussed in detail in this chapter, as is the macroscopic means of data collection, both osteometric and angular, and the thin-ground sectioning technique. Also described here are the necessary microscopic equipment, software, and techniques used for the two main phases of research: point-count and hand-drawn analysis, as well as the means of cross-sectional geometric measurement.

In the final section of this chapter the statistical method will be outlined, explicating approaches used for the comparison of collected datasets. Generally, the pattern of variance in ELP area and position will be assessed within and between the two methods used. Of particular interest is whether both datasets produce similar patterns
relating to the amount and direction of drift as revealed by primary bone histomorphometry. In addition, the statistical analysis will determine the strength of relationships between ELP position and the cross-sectional distribution of other microscopic features or the total cross-sectional geometry itself – testing, for the first time, if drift direction and axes of mechanical adaptation are similar. These efforts will be applied to comparisons among individuals as well as subgroups defined by population or demography.

5.2 Sample Selection

In general, the invasive nature of work requiring microscopic analysis of bone ensures that clinical sources of material will be rare, even in circumstance where biopsy is possible or desired. What tissue can be gained in this context is not only rare but is also misrepresentative as a sample source since they have overwhelming bias in favor of older individuals or the infirm. Likewise, the same limitation affects modern cadaveric samples, which have the benefit of accompanying life history and health information, but due to modern successes in lifestyle and health treatment, tend to be quite old. In addition, for both of these sources, one might find the time it would take to collect samples from donated sources to be prohibitive since access to the tissue and accompanying life history information is not given easily and harvesting is limited by available material.
Because biopsy or cadaveric, sample sizes are typically small and younger individuals are not well represented, archaeological projects permitting invasive techniques, like thin ground sectioning, become extremely valuable. This is one of the major reasons for the use of this source of bone in the current study. Archaeological samples offer several completely unique benefits to primary scientific investigation of bone biology. Variation in human behavior, cultural circumstance, environmental exposure, and resource access was much higher among non-modern populations in comparison to today. In addition, the mortality structure of these populations is more normally distributed with more young individuals included. This circumstance is ideal for the current investigation which targets tissues formed during growth processes and has as a major focus of inquiry, the genesis periods of large primary bone deposits. In addition, although drift variance and the existence of diverse age-at-formation tissues in the cortex should be quite important for anatomical and clinical scientific applications, it is potentially even more useful in a bioarchaeological setting where the need for inferential features of import is great. Hidden variance supplies a trove of potential new techniques or realizations impacting our understanding of past human life ways.

Unfortunately, the number of archaeological samples open for histological evaluation is extremely low. This is understandable for two major reasons: 1) archaeological human tissue is rare and histological techniques are destructive (even those using certain forms of μCT often have sample chambers smaller than the element in question and necessitate extraction); and 2) archaeological tissue is old and chemically,
mechanically, and microbiologically degraded, obscuring or obliterating the very 
structures of interest. However, microscopic techniques offer an entire new suite of 
investigative techniques that are increasingly of interest for the study of past lifeways. In 
addition, bone unearthed and curated even in opportune circumstances still wastes away 
in its new environment which typically fails to prevent microstructural digenesis. 
Histological preparation, however, preserves this level of information indefinitely 
(Schultz, 2001). Therefore, after prolific macroscopic analyses and before microscopic 
structure is obliterated, increasingly, curators of archaeological samples see fit to extend 
their studies to include histological analysis. These are the circumstances under which 
access to the Xcambó material has been granted, offering two-fold benefits to inquiry: 1) 
insights on biological and behavioral contexts of human activity at the site, and 2) unique 
perspectives in bone development and population level variation due to better 
representation of juveniles and young adults than modern sample sources.

For these reasons and because of the wealth of archaeological data available on 
the site, the larger ELP project relies heavily on analyses from a skeletal population 
sample from, Xcambó, Yucatan, Mexico (AD 350-700). Although the intentions of the 
current study are focused on basic analyses of primary bone biology, eventually the 
methods and results reported here will be applied to generate a better understanding of 
life at Xcambó. For example, during the Late Classic period, Xcambó seems to have 
experienced an economic shift that altered its role in the regional trade system in the area 
(Sierra Sosa, 1999, 2004; Tiesler Blos et al., 2004). It began as a salt production center,
but archaeological evidence suggests during this time period it could have adopted a more administrative position as a commercial port. Depending on how significant these economic changes were for inhabitants at the site, the day-to-day activities, routines, and circumstances for health could have altered in response. Evidence from cross-sectional analyses already preformed (I. Maggiano et al., 2008) supports this possibility, demonstrating through decreased robusticity and changes in sexual dimorphism that indeed physical activity patterns seem to have been changing significantly during this time period. Later applications of results from the current work would augment these studies and permit yet another rout for hypothesis driven investigation into past life at this influential Mayan port.

The Xcambó skeletal material is also considered a suitable launching point for reasons involving efficiency and accessibility. Due to previous research on Xcambó bioarchaeological contexts, including muscle insertion and cross-sectional geometric data collected on adults (Wanner, 2007; I. Maggiano et al., 2008), comparisons can be made with different time periods or subpopulations from the larger population sample. In addition, the site contains numerous juveniles. Children and adolescents were a vital inclusion, illuminating the periods of rapid growth thought to be causative of the ELP as a feature (C. Maggiano et al., 2008, 2011). By including these younger individuals, the study hopes to mimic as best as possible, longitudinal observations on growth and adaptive processes, despite that it uses a non-living population. These data augment the
applicability of findings to prior research and can be later bent towards answering larger questions regarding the bioarchaeological context at Xcambó.

Xcambó human remains considered for inclusion in this study consisted of 192 men, women, and children (many of which have bilateral partners for comparison). The sample is of mixed sex with similar representation and includes individuals with estimated ages of between 4.5-65. The lower age cut-off was used due to the unique histomorphometry of children under roughly 4.5 which is difficult to compare with children having larger bones and demonstrating a more mature gate. Likewise, the higher age cut-off was used due to the prevalence of reduced cortical thickness as a dominant feature of individuals past their 6th decade, a phenomenon transpiring via endosteal resorption that with increasing age would render endosteal origin tissue largely absent. Of that total 192, only some of which had humeral elements suitable for analysis.

Having addressed the benefits of using an archaeological sample for a study such as this, it is also important to understand its limitations. Diagenesis is a problem even for well-preserved skeletal sample, like the one from Xcambó. The particular geography and climate of the northern Yucatán Peninsula provides an environment quite capable of preserving human bone for hundreds or thousands of years. This is due, to the prevalence of salt flats and mangrove dotted badlands, and also a soil type that along with vegetative decomposition produces a zone of high alkalinity. The distinction between bone preserved in these soils and those in the organic humus nearer the surface is stark. Several bones bear the evidence of this preservation system. One femur, in specific had
been half settled in the preserving matrix and half exposed to less bone friendly environs, producing a dramatic difference in preservation in the medial half of the diaphysis compared to the lateral. The medial “face-down” side of the bone demonstrates near-biopsy quality bone preservation, while the opposing side is chalky and under microscopic scrutiny contains no collagen whatsoever and disruption to basic microstructure.

However, since relatively well-preserved bones can be highly diagenically damaged microscopically, the true preservation quality for this collection of materials was unknown until microscopic observations could be made. The criteria for inclusion in the current study were rigorous. Included humeral samples demonstrated excellent preservation in all regions of interest; that is, no data was missing due to diagenic disturbance. Individuals with one or two unreadable regions were marked separately for inclusion in other forms of analysis to be pursued at a later date. In addition, any individual demonstrating clear local pathology or intra vitam fracture was removed from the study.

After preparation of the bone transections (see Section 5.4.1) roughly half of the total collected young individuals (estimated ages 4.5-19.5) were excluded due to diagenesis based on the previously mentioned strict requirements. This was to be expected in that, in general, smaller bones suffer poorer preservation, microscopic or otherwise. Even after these strict criteria, in total 59 humeral elements remained for histological analysis. This group was separated into three subcategories by estimated
age: 4.5-12 (n = 12), 13 to 19 (n = 7), 20 to 65 (n = 34). Age distribution of the two populations (Figure 15 and 16) is not perfectly matched as Xoclan’s individuals more likely to contain only one member for a given age and have a lower $N$ overall than at Xcambó. These age categories were selected due to the several reasons.

Figure 15: Age distribution for all aged individuals at Xcambó compared to Xoclan
Categories help to combine near ages, incorporating some of the inevitable error in age estimation without decreasing the ability to refer to important age-related differences. The three categories are structured as they are in order to mark meaningful biological and cultural thresholds in development. By roughly age 4.5 the gate is mature and sufficient cortical bone exists for study, by age 12 to 13 the average adolescent will be entering puberty and beginning a period of rapid change in body size and proportion, and by roughly age 20 epiphyseal fusion has taken place in most individuals, corresponding to achievement of mature longitudinal dimensions. This permits evaluation of primary bone tissue distribution over time periods referred to in this document as “childhood,” “adolescence,” and “adulthood.”
Another limitation of using archaeological tissue is that, age must be estimated. The ages estimated for Xcambó individuals have been deduced through the use of a multivariate method taking into consideration many different age indicators depending on the rough age of the individual. Details on the specific age-estimation techniques used on the sample can be found in I. Maggiano and colleagues, 2008. Likewise sex determination is impossible in the youngest individuals in this study and, in adolescents and adults must be made based on various indicators also outlined in the same publication.

However, characterizing drift variance using the ELP requires security that the phenomenon is not limited to this archaeological site and that the demographic trends in the analysis are verifiable. For this reason the current research includes a modern, known-age and known-sex skeletal sample from unclaimed individuals recovered from Xoclan, a Yucatán cemetery. Many of these individuals are also connected to documentation detailing the cause or circumstances of death. Several were removed from the study for other reasons including localized periostosis, osteomyelitis, and one individual that was the only age outlier. The included males and females were represented by left humeral elements, demonstrated suitable bone preservation for histological techniques, and were older than 19 years of age at death, for a total of 76 individuals used the study. The current study’s skeletal population samples do not permit comparison between modern and archaeological context youths. This is a problem that was, unfortunately, unresolvable due to the fortunate rarity of accessible children’s
remains in modern cadaveric or mortuary contexts. However, even with this limitation, the inclusion of the Xoclan sample still provides an important modern context control for age- and sex-based comparisons of ELP variability among adults.

5.3 Macroscopic Data Collection

The methods outlined in this section are by no means representative of the total analysis for any one individual bone or skeleton from Xcambó. The osteology and archaeology at this site has been extensively studied by others and published elsewhere (Sierra Sosa, 1999, 2004; I. Maggiano et al., 2008). Only the measurement techniques necessary for the current research are detailed here. Also for the sake of efficiency, measures made on elements not included in this study are only briefly mentioned here, since they are not applied in any way to finalized data but lend impression to the completeness of records that were kept prior to cutting.

5.3.1 Osteometrics

Before processing samples for microscopic investigation, the bones used in this study were measured in various ways. This was done, not only because variables such as humeral length, diameter, or retroversion are potentially informative regarding any trends in histological data, but also because preserving as much information as possible prior to
destructive techniques is a high priority. For example, long bone length is known to contribute to increased bending moments important for cross-sectional geometrics (Ruff et al., 1991) and potentially ELP size or position. These measures also can be used to support previously existing analyses, and contribute to the records required on site.

A record sheet for each individual was kept that focused only on the elements in question rather than the entire individual’s preservation (this larger skeletal perspective is readily available if ever it is needed). Each sheet contains identification information, including: registration number, site of origin, the project name, the burial number (*entierro*, marked “E” on slides), and the catalogue number or box number (*caja*, marked “C” on slides). General notes were kept on the elements (humeral and/or femoral) collected from each individual and a diagram was filled in to demonstrate the completeness of the elements in question.

For each collected humerus the following data were collected: maximum length, midshaft circumference, minimum diameter at the midshaft, maximum diameter at the midshaft, the minimum perimeter anywhere on the diaphysis. Similar measurements were made on femora collected for later studies, including: maximum length, midshaft circumference, physiological length, anterio-posterior diameter of the midshaft, medio-lateral diameter of the midshaft, anterio-posterior subtrochanteric diameter, and medio-lateral subtrochanteric diameter. An osteometric board was used for determining lengths, a tape measure for circumferences, and digital calipers for diametrics.
5.3.2 Angular metrics

Since the ELP is a remnant of modeling drift and modeling drift account for the achievement of adult bone size, shape, and orientation, the degree of elemental torsion was also a measure of interest. Most typically referred to as “antetorsion” this measure reflects the degree of “twist” in the elbow compared to the shoulder joint axis. Most often this measure is reserved for living clinical casework and utilizes CT or xray scans. In this case, a rough method was derived for comparing relative torsion between elements by locating dependable landmarks on both epiphyses and assessing the angle of divergence in their orientation in the following manner (Figure 17).
Figure 17: Method for comparative measure of humeral torsion. Plasticine used at either bone end, A) proximal, B) distal, to summarize the orientation of the epiphysis. Angles were recorded on graph paper as noted.

First, plasticine was used to affix a straight section of thick wire to the most inferior aspect of the distal humeral epiphysis, bisecting the medial and lateral epicondyles in the widest axis, thereby summarizing the estimated angle of the distal aspect of the humerus as it joins the elbow-joint. Next, a similar wire marker was placed on the humeral head, again with two plasticine nodes: one at the “apex” of the humeral head (made large enough to permit clearance across the superior aspect of the humeral head), and the other
bisecting the superior view of the greater tubercle, summarizing the estimated angle at the glenohumeral articulation. Finally, a platform was necessitated in order to gain hands-free positioning of the element on the osteometric board at a “flat” plane using the distal wire as a reference. This platform was constructed using two popsicle sticks. One was affixed transverse to the orientation of the bone at the posterior aspect of the distal metaphysis using a larger ball of plasticine at the superior margin of the coronoid fossa. Another was placed at the posterior aspect of the proximal metaphysis, again level with the wire marking the distal epicondylar bisection. A sheet of graphing paper was taped to the stationary, vertical wall of the osteometric board. The head wire of the humerus was placed flat against this paper, providing a guide along which to draw the angle with a colored pencil. Each line was given an identification number, permitting several individuals to be recorded per sheet of paper. Afterwards, a protractor was used to measure the angle of each drawn line in degrees, providing the relative total torsion between proximal and distal epiphyseal orientations for comparison. The femoral relative torsion was measured in a similar fashion for inclusion in other studies along with the femoral bicondylar angle. These angular measurements measurement were not available for samples that were previously sectioned due to time constraints and the fact that they had already been reconstructed.
5.4 Microscopic Preparation and Data Collection

The idea to consider patterned, endosteal bone deposition as a “thing” fit for measurement became first apparent as an observation made with the unaided eye. Prior to any preliminary reports on the ELP, it was viewed quite easily (and accidentally) while checking for scratches on excisions during my first attempts at thin-ground sectioning. Light from behind the excision was passing more easily through a particularly large ELP on the first such occasion, illuminating it more readily than surrounding and more opaque tissue. At the time, many colleagues were of the opinion that it might be some type of pathology. During these observations, it was clear that backlight and a hand-lens was an insufficient means of viewing the feature or discerning what had caused it. Subsequent microscopic viewing during the sanding phases of slide preparation drew attention to the feature as a regular occurrence in bone from the wider skeletal population sample. The bone in question was from The Dakhleh Oasis, Egypt. Initial microscopic observation of these pristine samples eventually lead to the first collaborative effort to identify, describe, and report the histological structure of this endosteal bone formation as a metafeature, and its prevalence in the Xcambó population sample (Maggiano, 2011). To go beyond these types of observations, some novel microscopic efforts had to be made in order to formulate a technique for quantification of the ELP as outlined below, all of which requires a microscope.

Typically optical microscopes are useful for the study of transparent objects that themselves are microscopic in scale. One of the difficulties in dealing with bone on a
histological scale is that it is anything but transparent. Considerable amounts of effort are required to reduce a large chunk of resilient, opaque, dense mineral and organic matrix to a sliver between 20 and 100 microns thin so that light can shine through, illuminating hidden structural information, providing a preserved stratigraphy of biological processes transpiring over many years or even decades of a lifetime long passed. The first challenge of this project, was the same as for any effort to understand non-decalcified bone microscopy: preserving microscopic structure, anatomical orientation, and origin/identification contexts, while hack-sawing, bathing, plastinating, vacuuming, trimming, slicing, sanding/polishing, affixing, sanding/polishing again, and cover-slippping numerous extremely precious archaeological bone samples. For this reason the following methodological descriptions begin with the thin-ground sample preparation equipment and technique used in the study.

As challenging as this process can be, an even more vexing concern for this project was the fact that modeling drift (or even primary bone structure in general) has no standardized means of quantification (Maggiano, 2011). This largely results from scalar paradox. That is, modeling drift can only be defined microscopically, despite being macroscopic in scale. Perhaps this is one reason more attention has not been paid to the meta-structural level of bone microarchitecture, despite its importance in revealing the growth history of the bone. The ELP has only newly been suggested as a meta-feature important for analysis (Wanner et al 2007, C. Maggiano, 2011; Maggiano et al., 2008,
2009b, 2011; I. Maggiano et al., 2008, 2011a,b). As such it is necessary in this study to provide several lines of evidence by which to identify and quantify the region.

For this reason the following method describes unique adjustments made to point-count, and hand-drawn-line techniques in order to facilitate measurement of modeling drift variables and the ELP in specific. Though the point-count technique is nearly as old as microscopy itself and is familiar to microscopists in general, the particular technique employs a customized method of standardizing the point-count sampling of the cross-section that necessitates detailed review. In addition, the hand-drawn-line technique seems quite simple, using software to analyze the area and geometry of boney microscopic features outlined by hand. However, due to low contrast issues at low magnification, and the dramatic need for a much larger field of view, several new techniques are used in this study, employing red-quartz compensated polarizing microscopy and the 45º image acquisition technique. Detailed descriptions of these techniques follow.

5.4.1 Sample Preparation

Thin-ground sections collected from juveniles at Xcambó, like those previously collected at Xcambó, were prepared as suggested by Schultz and Drommer (1983) and Schultz (1988) via the following extraction, plastination, and grinding phases. Some deviations from procedure were necessitated due to unique lab settings and time
limitations. Namely extractions were taken with intent to prepare one slide rather than two. In addition, humidity was a formidable factor in sample preparation, despite efforts at climate control in the laboratory (including ample air-conditioning, dehumidification machinery, and moisture absorbent powders).

In fact, over 45 samples were destroyed during an unfortunate partial flood of the lab caused by a hurricane (Figure 18).

Figure 18: Samples that fractured due to hurricane borne humidity.

These samples were left un-cover-slipped in attempt to expedite the preparation process and literally exploded from the absorption of humidity by the bone whilst held in place permanently by the plastination and affixing medium, Biodur®. It is therefore strongly recommended that the following protocols be followed precisely to avoid such mishaps.
If it is necessary for some reason to avoid cover-slip the sample, the following preventative measures are vital: store only in climate controlled circumstances, avoid temperature swings that are even temporary in duration, seal individual slides in Ziploc baggies or seal the slide box with moisture absorbent silicate or powder products inside. Since these measures are not suitable in certain environs, it is preferable to cover-slip the samples as described below. After cover-slippering, thin-ground samples are much more resilient to humidity or other types of fracture and are protected completely from further digenesis.

In addition, some chemicals used in the following protocol are minor skin and eye irritants. Gloves, masks, and goggles are recommended for every phase of the preparation process but especially during the use of Xylene, the dry cutting or sanding of the bone or preparations, and the pouring and mixing of the Biodur components. At least one lab member on location had an uncomfortable allergy to the Biodur hardening solution that was kept in check by limiting exposure. Proper ventilation, particularly during xylene bathing and vacuum pumping the samples is also a necessity.

5.4.1.1 Extraction

A two centimeter transverse extraction was taken from the mid-diaphysis, as measured by the bisection of the total length of the humeral element. This region was chosen because of its standard use in long bones as the area summarizing mechanical
forces distributed by the local muscular system. The humerus does contain a rather large muscular attachment in the midshaft vicinity and can change its contour when robust, the that of the deltoid. However, for the purposes of this study’s cross-sectional geometric interests the midshaft is still assumed to summarize meaningful loading information and is used in part for this reason, as it has been used in other studies (I. Maggiano, 2008; Sumner and Andriacchi, 1996). In addition, the midshaft was selected for the purposes of summarizing modeling drift of the element. Other studies underway assess drift along the length of the diaphysis to determine intra-element variation, therefore this study is content to represent the individual using the midshaft as is most convenient and conventional (and less destructive to valuable archaeological bone than multiple extractions).

Where epiphyses were not available due to the young age of the individual, the total diaphyseal length was bisected to determine midshaft position. In cases of post mortem fracture or missing fragments, the element’s contralateral partner was used to measure and assess the location of the midshaft. Bones missing small portions of their distal or proximal epiphyses were included in the study only when the tuberosities or olecranon fossa was at least partly visible, such that the terminal ends of the diaphysis could be assigned by estimation. When necessary, seriation of several similarly aged individuals aided greatly in these efforts, as well as the use of other measurements as needed. Bones that were visibly extremely poorly preserved were not excised to avoid unnecessary destruction unlikely provide histological data.
Extractions were made only after macroscopic measurements were taken and the diaphysis was clearly marked in the zone of extraction for anatomical position, anterior and posterior, and around the complete diameter at three locations: the midshaft itself, one centimeter above it, and one centimeter below it. Complete diametric marking is recommended as a method to ensure a precise transverse plane excision is made. Cuts were made through the entire shaft using a 12’ long, 3’depth heavy duty hacksaw for larger bones and a much smaller higher tooth count hacksaw for the smaller bones. Not switching between the two when necessary would have resulted in the mere weight of the larger hacksaw breaking the fragile juvenile bones. Conversely the larger models make much more rapid and clean, straight cuts. A vice was used to safely secure the bone, padded with cloth folds to ensure vice did not damage the bone. The vice was secured only as much as absolutely necessary to keep the bone from shaking or twisting (Figure 19).
Figure 19: Padded vice grip on an extraction due for continued processing of eight longitudinal sections along each of the ROIs used in this study (these wafers were taken for comparison with other osteonal forms to develop an accurate perspective on light micropic appearance of osteons and drifters, sliced from several perspectives while maintaining geometric and orientation information.

This method of extraction never caused incident, fracture or abrasion and was much preferred to the use of a Dremel® with 1 ¼’ cut-off wheel, due to the latter’s small size and tendency to burn bone that is highly preserved. The Dremel® was, however, useful for cutting juvenile bones that had questionable collagen content, were poorly preserved, or seemed unusually fragile. When this technique was used, only low power was supplied to the device to avoid it kicking the sample back and breaking it. Once sectioned, proximal orientation was marked in pencil immediately in two locations: the proximal cut face itself (three times) and on the anterior mark on the outer cortex. Excisions were then weighed before logbook entries were created for each of them.

To prepare the bone for plastination, a bath of xylene was used to clean the bone and to provide an evaporate which will pull the plastination fluid through the bone pores
during vacuum pumping. The xylene was poured into a Pyrex® glass tray to a depth that will sufficiently submerse the bone excisions. Then wire framing was immersed into the tray in order to cordon off zones for each excision to rest inside. A notebook diagram for each bathing routine was kept to ensure sample identification during the xylene bath process. As a secondary precaution, sticker labels were made for each sample and affixed to the outside of the glass tray, each, corresponding in perimeter position to one of the regions marked by the wire frame. These wires kept bone excisions from sliding out of position due to the wave action of the xylene during transport to and from the fume hood. Only then were the samples added to the bath one to each of the wire-designated regions that corresponded to both the drawing and the sticker. Great care was made to ensure that the proximal face of the excision always faced the surface of the xylene and the anterior faced the “top” margin of the tray (away from the person holding the tray), again as a secondary means of permitting habit to reinforce maintenance of anatomical position. It was found that xylene neither affected pencil nor permanent ink marks (some bones were too porous or fragile for pencil marks to be secure). The bone was left to soak for at least one day before it could be plastinated.

5.4.1.2 Plastination

Plastination, or embedding, is vital to the process of generating undamaged thin-ground bone samples. Avoiding this step in the process generates tissue with more
fractures induced by the preparation process and fails to protect the bone from diagenesis as effectively as in the case of plastination. Particularly for fragile archaeological bone or trabecular regions, this method of protection is a must. The plastination fluid used for all samples in this study is Biodur®, a well-known substance that gains access to even microscopic pores (lacunae and canaliculi) under ideal circumstances and avoids some light artifacts present otherwise during epifluorescent microscopic analyses (Maggiano et al., 2006; Schultz, 1988; Schultz and Drommer, 1983). The down-side of using this plastination solution is that it takes much longer to cure than methyl methacrylate or other branded embedding techniques. Many Biodur mixtures exist for different applications. In particular, the current work used E12 resin and E1 hardener, mixed in a 10 g to 2.8 g ratio, respectively. The mixture must be stirred very gently by hand for upwards of a 15 min. to ensure proper solution is formed and to avoid generating bubbles as much as possible.

Flexible plastic ice-cube trays were used to house the plastination fluid and individual bone excision when possible. When excisions were too large for this technique, up to three were placed inside disposable aluminum trays. In both cases, identification of the excision was made apparent not only by drawings of the distribution or layout via diagram, but also by direct sticker labeling of the container. The plastination process began with pouring the mold shape to half full, then settling the bone in its proper position: marked proximal face down. This position is preferred due to the tendency for vacuuming to raise obscuring bubbles to the top of the embedment, keeping
the marks from being visible. Likewise, care was taken to assure the anterior mark was as close to the side of the given container as possible so that it could always be seen in the embedded block from the outside. After correct positioning, the remaining Biodur was poured into the medullary cavity slowly and allowed to spill over the bone until the bone was completely immersed.

The plastination molds were placed into a large vacuum chamber which was depressurized carefully by hand to avoid exploding the bone with expanding bubbles. When sufficient negative pressure had been achieved, such that no additional visible bubbles rise to the surface, a metal stirring palate was used to remove the bubble film before returning the chamber to depressurization and leaving the samples overnight (typically 7-12 hrs). Subsequently, the trays were removed (incidentally in tropical conditions at this point the samples are already hardening, something unheard of in the author’s personal experience in temperate climates), and placed in a secure location. The curing time varies with the climate. In temperate regions with low humidity samples sometimes take 3 to 5 weeks to cure fully depending on the size of the embedment. In more tropical climates however, samples were cured on occasion within two weeks.

5.4.1.3 Cutting and thin-grinding
Cutting embedded blocks was achieved using a Low Speed Isomet® geological saw (Figure 20), equipped with a micrometer arm and a diamond grit blade (set at 225-300 rpm depending on the sample thickness, slower for bigger).

![Low Speed Isomet® geological saw](image)

Figure 18: Low Speed Isomet® geological saw. A pair of these was used to make slicing wafers from excisions more rapid.

The blade in this system is water cooled which also transports particulates neatly into the water basin. For maximum efficiency two of these machines were run simultaneously, cutting wafers that were 1 mm thick. Occasionally, especially for large bones that were embedded in wider containers, many preparatory cuts had to be made through the embedment (not the bone) to cut the block to size for the slow-speed saw. This was accomplished, when necessary, by the same means as described previously, with hacksaw and vice.
Next, the cut wafers were marked proximally and anteriorly to maintain orientation and were sanded and polished on their distal facing surface, 2400 grain to 4000 grain before polishing on rawhide touched with gold polish. Polishing was best achieved using the index and middle finger to apply even force along the back (proximal) side of the wafer. Placing the surface at an oblique angle to overhead lighting reveals when the polish is sufficient in that it is glassy clear and has no visible scratches. After this, the wafer was ready to be affixed to a glass microscopy slide. A liquid syringe was used to deposit two droplets of Biodur to the glass. Placing the wafer gently down with the finger on its center, gentle circling motion exudes any bubbles formed. The wafer was then lifted and a “clean” deposit of Biodur from the syringe placed again underneath it. Another series of gently depressed circling and the wafer is in position: proximal facing up and anterior and posterior clearly marked. Excess biodure is removed with an X-acto® Blade. These affixed slides are then labeled, placed on top of popsicle sticks (or any other spacing object that helps to avoid any spillage from adhering the slide to the tray), and allowed to cure for an additional 5-7 days.

After affixed wafers have cured sufficiently, the second round of sanding and polishing begins. First, the anterior and posterior line must be transferred to the glass slide itself. To ensure that the mark is not easily removed the glass was sanded with 500 grit paper to roughen its surface outside the anterior and posterior perimeter of the bone. A ruler placed carefully steadied the hand which drew two straight lines, one marking he anterior and one, the posterior; the imaginary intersection of which passing roughly
through the bone’s centroid. This time much more thickness must be removed during sanding, so 500 grain and 1000 grain sandpaper were used to achieve approximate thickness. Verification of sample thinness at 70-100 by micrometer measurements was performed at 5 points across the sample plane, but this alone was not sufficient. Previous studies using three-dimensional confocal laser scanning microscopy (Maggiano et al., 2006) found that the actual thickness of measured samples varied from micrometer measurements due to the unknown amount of plastination fluid under the sample during affixation. In that study, several samples measured to 70 μm were actually confirmed as thin as 30-40 μm using the laser. Considering this issue and unique trouble with humidity encountered in some samples, optical methods for determining sufficient thinness were also used in addition to micrometer measurements. If, under certain circumstances the strict measure was forced, the sample would be ground away long before achieving the desired measurement. In order to finalize the surface and remove large scratches left by the high grit paper, water was added to much finer grit paper (1200 to 1400 grain) upon which the sample is placed face down and manipulated in a circular fashion. This was best accomplished gently using two fingers or a suction cup once the fingers become too swollen or lose their print friction from abrasion. Labels must be replaced after this phase of the preparation technique due to water damage and eroding on the sandpaper.

By far the most challenging portion of this process is the sanding. It takes gentle experience to know how much bone is removed with each pass and avoid destroying the
tissue entirely or unevening the surface irreparably. The tropical conditions produce some unique challenges as well. The most notable is that on rare occasion bone wafers may buckle symmetrically while curing such that after hardening the outside perimeter of the bone is slightly elevated from the glass with respect to the wafer’s center. When this happens, even the most skilled and steady hand will remove tissue from this perimeter much faster than the bone’s interior, risking damage or unevenness. Appropriate curing and climate are the only precautions that avoid this issue but not entirely. No means of mechanical steadying of the wafer is possible because Biodur is incredibly strong once hardened and anything used to clamp down the wafer would frequently stick to the wafer and glass dangerously. The only solution for this circumstance is to tear off minute pieces of sandpaper and sand only the interior region of the bone until sufficient thinness is achieved there (sometimes taking several additional hours to do so).

Upon completion of this phase, the sample only needed cover-slipped using the syringe of Biodur in a method similar to the slide affixing technique previously described. Great care was taken at this point to not introduce bubbles and so many repetitions of drops and circling massage were sometimes necessary. It was important to constantly clean the area while preparing the samples as excess Biodur can cure quickly when thinly applied and affix instruments or glass where it was not intended. Its excess was also much more easily removed from the slides when wet than after it has hardened. One final trick was found very useful in cases where bubbles seem impossible to avoid during cover-slipping. Place the cover as usual over the Biodur and circle, but at the area
proving problematic lay an extra small drop of Biodur on the slip margin before letting up the pressure. In this way, if negative pressure forms, it will draw this excess Biodur under this slip in only this region and in only the amount necessary, rather than pulling air underneath and creating bubble trouble.

All elements were reconstructed using pencil line demarcations for proper orientation and calipers for proper spacing (Figure 21).

Figure 19: Reconstruction on the Xcambó and Xoclán material after excisions and slide preparation.

Microscopic samples were stored in slide boxes, arranged in numerical order according to identification labels. Throughout the subsequent phases of the project a master spreadsheet was kept to denote which samples had passed through which phase of
analysis. In addition, color-coded stickers aided in marking samples for various actions and facilitating multi-tasking during the investigation.

5.4.2 Polarization microscopy techniques and equipment

Polarized-light microscopy takes advantage of bone’s ability to refract light at multiple indices. This property, called birefringence, is attributed to the presence of collagen in bone; collagen orientation (Boyde and Riggs, 1990, Bromage et al., 2003), and/or bundled fibril density (Marotti 1993) have both been implicated in generating this effect. Even the local contribution of fibrillar mineral versus non-associated mineral matrix appears to affect birefringence (Turner, 1995). According to Weiner and colleagues (1997, 1999) the ultrastructure of lamellae is much more complex, involving five sublayers, each with progressive rotation in collagen orientation. Spiesz (2011) has offered a modern quantitative analysis of collagen’s ultrastructural contribution to birefringence, measuring collagen’s average orientation to the longitudinal bone axis at 27º, corroborating Turner’s measurement of 30º (1995). These variations in collagen presence, density, and orientation all affect the resulting polarized image, rendering polarization a sensitive technique for various uses in an examination of bone (Maggiano, 2011).

“Cross-polarization” refers to the effect of using two linear polarizing filters oriented perpendicularly; the first is called a polarizer and the second, the analyzer
Theoretically, this orientation should cut out all light passing through both filters (the first cutting out the vertical and the second, the horizontal waves, for example). However, the reason the second filter is called the “analyzer” is that its function is to reveal any light wave disturbance between itself and the polarizer. So, incident, non-polarized light is shone through a polarizer, limiting the directional vibration of the wave set to one axis. Instead of next passing through the second filter, cutting out the other axis, and dimming the light completely; the bone adds axes of vibration to the light again through collagen’s birefringence. Now as the light enters the analyzer and is again linearly polarized, light still passes through, emanating from structures bearing certain organizations of collagen molecules. Therefore, the cross-polarized image of bone is seen as white light illuminated lamellae, bright where there shouldn’t be brightness if it weren’t for collagen’s birefringence. This is why the background, or off-sample space, is black in cross-polarizing light microscopy. The general use for polarization microscopy in bone is to assist the perception of structural detail.

There is a potential cost for its application, however. It is likely that if polarization of bone provides information, it will affect count data during comparisons between studies using polarized light and not those that do not. Seminal efforts in bone histological age estimation, for example, were not explicit regarding their use of polarized light. Its employment though is becoming more common, in part due to its many advantages (Schultz, 2001; Bromage et al., 2003; Skedros, 2009; Maggiano et al.,
Standard white light transmission typically gives more vague impressions of lamellar structure through the orientation of secondary structures such as osteocytic lacunae or canaliculi. This leaves room for the concern that polarized light analysis of bone may yield higher counts of some features, such as osteon fragments, however, no study could be found demonstrating this. The cautious interpretations in this study are not directly affected by this liability since they do not depend on comparisons polarizing and non-polarizing studies, in the same way age-estimation or other applications might.

The benefits of polarization far outweigh this slight cost for the current research. Cross-polarization microscopy contributes to nearly every aspect of bone microscopy in one way or another. For example, since birefringence is dependent on collagen (the mineral component may contribute but is not necessary as is evident by the fact that decalcified bone polarizes in a fashion similar to calcified bone), it can be used as a quick visual determinant of the degree of diagenesis. It is even theoretically possible to use this technique to construct a scale against which to determine bone preservation (Streeter, 2008). This is because disruption or destruction of the bone’s organic structure will destroy the birefringent effect. It can also be used to facilitate distinction of peri- and post-mortem changes (Schultz, 2001). The ultrastructural distribution of bone mineral and collagen has also been related to local material strength properties at several different scales of analysis (Gebhardt, 1905; Ascenzi and Bonucci, 1967, 1968; Bromage et al., 2003; Skedros, 2009). This last effect is of particular interest for bioarchaeological
applications seeking to understand differences in the physical loading history of a given bone in a skeletal population.

Perhaps most importantly for the current work, polarized light emphasizes lamellar orientation and aids observation of fine changes in lamellar microstructure, undetectable with white light microscopy (Schultz, 2001; Maggiano et al., 2009a; Maggiano, 2011). Especially visible are changes in lamellar orientation, either with or without disruptions to the structure of individual lamellae. Polarization also emphasizes reversal lines, or cement lines, due to their tendency to cut across lamellae which are particularly vibrant in polarized light. These changes are often unreported or tersely discussed in literature, where circumferential lamellae are treated generally as if they are contiguous and concentric – which is not the case (Maggiano, 2011). Since, specifically the non-circumferential nature of ELP lamellae (with respect to the current bone margins) are of special interest here, identifying bone that was deposited by the endosteum long before its time-at-death position.

Another benefit of using polarization is the ability to differentially emphasize lamellae by their structural orientation through false coloration achieved with a compensating filter. This study used, almost exclusively, red quartz compensating polarization in order to separate positive birefringence into two sets of colors: yellow to orange and blue to green (in this technique the cross-polarization artifact the same reddish purple as the sample background, and manifests exactly the same as without compensation) (Weber, 1927; Schultz, 2001; Maggiano et al., 2009a). In this way, even
when lamellar tissue is disorganized due to containing woven bone or micro-diagenesis, the orientation of the tissue is clear. Small scale alterations in orientation, when differentially colored become more noticeable and assist in “reading” the bone.

In addition to increasing viewable lamellar detail, cross-polarization microscopy offers other unique benefits to this study – benefits that surprisingly come from its most infamous weakness. When a birefringent substance’s optical axis is at a 45° angle to the polarizer, maximum brightness is permitted penetration; when it is parallel to the polarizer it is extinguished (completely darkened). Typically this is a nuisance in bone microscopy. Since bone lamellae are curved, regardless of the orientation of the bone sample a significant amount of birefringence will be extinguished (theoretically half of the total birefringence would be lost in a completely isotropic field of view). In completely circular lamellar structures like the osteon, this artifact is shown in its entirety (in all four zones that are not 45° with respect to the polarizer and analyzer orientation), and has been referred to as resembling a “maltese” cross (Hancox, 1972; Boyde et al., 1984) (Figure 22).
This cross is stationary (always facing Cartesian primary axes), even when the sample is rotated, permitting illumination of any region but never all regions simultaneously. Newer techniques, employ circular polarization (the addition of two quarter wave plates, “sandwiching” the sample within the space defined by the polarizer and analyzer). This setup avoids avoid interference artifacts completely, allowing all positive birefringent illumination in a field of view, perfect for some intensity based quantification techniques (Boyde et al., 1984; Martin et al., 1996; Bromage et al., 2003; Skedros, 2009).

In the case of the current study, however, the maltese cross became an asset so circular polarization techniques were avoided. The artifact affects primary lamellae as well, but at a much larger scale, given the gentler curve of the bones diameter compared to that of an osteon. It is this scalar difference between the osteonal artifact (ever present at low magnification) and the primary tissue artifact (which can be avoided during photography)
that further increases contrast between secondary and primary tissue. The specifics of this new imaging technique are covered in detail in Section 5.4.5.1, below. For all these reasons, this project makes extensive use of polarization microscopy as a means of generating contrast between microstructures, both for observing and imaging the bone. These benefits can only be achieved, however, with the correct equipment.

The microscope used for this investigation was a wide field standard optical microscope by Olympus®, model BX51 (Olympus America, Inc., Center Valley, PA), outfitted with 10x ocular and variable objective magnifications (4x, NA; 10x, NA; 20x, NA; 40x, NA). Basic digital imaging was accomplished using the Spot Idea CMOS 3.0Mp camera system and Spot Advanced® software and a Dell 64 bit Desktop with 6 GB of RAM. Image data was stored on the local drive, a transportable drive, a secure backup external drive, and on the laptop used during the study (when memory permitted or during analyses).

Cross-polarization was accomplished in the following manner: the analyzer had its position in the nosepiece module’s U-TAD adapter, whereas the polarizer was in a disc tray just above the light source (these are oriented at 90° to one another, using standard calibration techniques suggested by the manufacturer). The U-TAD adapter was also the interface for a red-quartz compensating filter which Olympus refers to as U-TP530nm (and sometimes as a 530 nm “tint” plate). A circular stage was used for exploratory or observational analysis, whereas the tracking X:Y stage was preferred for
point-count methods which required longer tracks of consecutive view, and for imaging for reasons discussed below (See Section 5.4.5.1).

5.4.3 Cross-sectional geometric data collection

The concept of cross-sectional analysis of long-bone diaphyseal geometry is based on the I-beam principle, which recognizes that the further a beam’s mass is distributed from its center in the direction of bending, the greater its strength in resisting the forces applied. In application to skeletal biology, the I-beam principle’s mathematics simplify a bone into an ellipse to identify some aspects of its strength. For example a circular tube is equally resistant to torsion, compression, and tension in any bending axis, whereas an ellipsoid tube is more resistant to bending in the axis that distributes most tissue furthest from the centroid. The math was first developed for moments of inertia, dealing with the mass of a three dimensional object, but these are converted in cross-sectional analyses to moment areas and target a specific region of the long bone diaphysis.

Mid-diaphyseal transections are used in most cases since muscular strain constitute the primary load source (Pauwels, 1965) and the simultaneous effects of muscular systems typically peak at the midshaft region of long bones (Biewener, 1992). Many different variables can be calculated using cross-sectional geometrics as a way to connect geometry, growth and biomechanical adaptation.
These data have well described methods for collection and standardization (Ruff and Hayes, 1983a,b; Bridges, 1989; Ruff, 1995, 2000a; Stock and Pfeiffer, 2004; I. Maggiano et al., 2008). For this project, similar calculations were run from scans bone cross-sections made on a desktop scanner. Scans were made face-down (proximal-down) so that the imaging perspective mimicked that of the image inversion encountered during microphotography on a standard optical microscope. ImageJ was used in conjunction with “MomentMacroJ,” an add-on for calculating various cross-sectional geometric variables (public access courtesy of C. Ruff). This software measured JPEG versions of the scans, thresholded to select only bone tissue, generating a binary mask (and in some cases trimmed to exclude non boney elements that could not be excluded by thresholding). In general the method was similar to that employed in previous cross-sectional studies on the material (I. Maggiano et al., 208). The current study requires only a subset of the total generated data, in that sole the point of interest is the relationship between ELP size, shape, and position; and the direction of the maximum second area moment, $I_{max}$. Second moments of area, such as $I_{max}$, correspond to the bending or torsional rigidity of a bone in a specific plane and can define the section modulus (a strength measurement important for biomechanical investigations) when raised to the power, 0.73 (Ruff, 1995, 2000). Other generated values are not utilized for the current study.

Additionally, the use raw of $I_{max}$ values as magnitudes cannot be easily pursued here (for example to compare ELP size and $I_{max}$) because a treatment like this necessitates
standardization by some estimate of body weight (Ruff, 2000b). The method outlined by Ruff (2000a,b), require femoral head diameter and reliable length measures, for example. These requirements overlain on those of the microstructural study, would yield a total sample size far too small to provide interesting results, particularly with the focus here on many non-fused bones of children who, due to preservation and recovery bias cannot often give body weight standardization measures.

Instead the current study focuses primarily on the angular direction of $I_{\text{max}}$ in its efforts to understand the ELP. This is because, as a proposed descriptor for mechanically adaptive response in bone, the orientation of $I_{\text{max}}$ should correspond with that of bone modeling drift as represented by the ELP. If bone modeling is the mechanism through which such cross-sectional adaptation must occur, and cross-sectional adaptation is measured by geometry, then they should tell the same story. If they do not, then a source of human variation has been uncovered. In order to facilitate these comparisons, angular data was collected from the image composites generated after hand-drawn analysis, as described in Section 5.4.5.4. The process of generating cross-sectional geometric images (scans with associated $I_{\text{max}}$ and $I_{\text{min}}$ axes and the total centroid) was important for the following point-count efforts in that the centroid was necessary to accurately set up the regions of interest for microscopic analysis.

5.4.4 Point-count technique
Point-count microscopy typically involves the use of a counting reticule which is a grid integrated into one of the oculars. The grid is superimposed on the field of view at a known scale and assists in counts of objects per area, length measurements, and area measures in general. The basic principle for area measurements using the grid is to count the number of intersections on the grid that are superimposed over the object of inquiry. These intersections are typically referred to as “hits” and can later be converted into an estimation of area. In some studies counters or clickers are used to tally a handful of variables simultaneously to speed the process. Typically the counts are entered onto lab sheets and transferred to a spreadsheet or database for storage and statistical analysis.

5.4.4.1 Starburst sampling method

The current study accomplished point-count measures using 100x magnification (10x objective, and 10x ocular) using a “Merz grid,” 36 intersections, “sine function” curved horizontal lines with tick marks for intersections and a dominant X:Y axis with an associated measurement scale. Less powerful or more powerful magnification was used for clarification on primary membrane of origin or fine detail cement-lines or other features, respectively. Parfitt and colleagues’ (1983, 1987) recommendations, terminology, and notation for the technique were used here along with terminology applied in a collection of other works (Enlow, 1962a; Frost, 1973; Robling and Stout,
2003, Maggiano, 2011) except for novel terms introduced below for differentiating variables and methods used in the current research.

Many different sampling schema have been employed in bone Histomorphometric studies to select which fields of view (FOV) to count and in what regions of interest (ROI), largely because of the time intensiveness of the point-count process (see Iwaniec, 1998 for a quick reference with schematics for several studies). The choice of sampling structure depends on the level of desired predictability and the time available for the analysis (Iwaniec, 1998). The time investment per sample increases dramatically as one raises the number of desired variables per field. The basic sampling effort in this study is similar to that employed by Robling and Stout (2003), except that where he counted two FOV tracks for each of four ROIs (anterior, posterior, medial, and lateral), the technique employed here samples four FOV tracks, one in each of 8 ROIs (anterior - A, anterio-lateral - AL, medial - M, posterio-medial - PM, posterio-lateral - PL, lateral - L, and anterio-lateral - AL) (Maggiano et al., 2009b).

Here, 8 analyzed ROIs are positioned as single tracks of FOVs along rays extending from the bone’s calculated centroid to the bone’s external surface (Figure 23).
Figure 21: Schematic representation of the starburst sampling pattern. Four axes correspond to eight regions of interest (ROIs). The abbreviations: A, AL, L, PL, P, PM, M, AM, correspond to anterior, anteriolateral, lateral, posterio-lateral, posterior, posterio-medial, medial, and anterio-medial ROIs. Counts were made from the outside field of view (FOV), inward. This array increases the likelihood of sampling directionality equally while keeping efficient total times of analysis.

In this way the bone is divided into eight regions along four equally represented bending axes. Each ROI contains a various number of FOVs from one or two in the smallest and youngest individuals to above 7 or 8 in the largest and oldest. FOV number always increased as the ROI was sampled interiorly; therefore FOV number 1 always targeted the most external position on that ray. This, of course, means that the last FOV in any ray was the FOV most likely to contain the fewest “on-bone” hits.

Several factors that necessitated this unique sampling pattern, referred to as the “starburst” pattern (Maggiano et al., 2009b). First, the total number of counted variables was considerably higher here than in most bone Histomorphometric studies (at 3 total FOV counts, and 12 hit-counted primary variables). Collecting as many variables as
possible was a major priority for future work targeting specific variance or even spatial relations between bone and void-space along geometric axes. Second, the major focus of the current work is primary tissue which can be best assessed by sampling the cross-section in tracks that represent the entire cross-section, while considering a larger number of total ROIs corresponding to major and minor anatomical axes. Even more important was choose ROI tracks that would provide the highest potential contact with ELP tissue, without using more subjective means to directly target it. In this way, differences between the regions housing the ELP and others manifest as a function of measured variables rather than the sampling technique. Third, greater directional representation was necessary for later comparisons with hand-drawn and cross-sectional geometric angular measures, permitting a rare simultaneous consideration of geometric and histomorphometric circumstance along similar axes, within the same individuals. Fourth, the FOV numbering system itself needed to be standardized such that it represented the position of each FOV: on the bone’s periphery or interior. Finally, the starburst pattern is standard and systematic in that it is the same for all individuals, again decreasing subjectivity that could bias the technique. In short, to look at the ELP it was necessary to detect it while considering the entire cross-section.

Considerations regarding potential limitations of the starburst sampling technique should also be addressed. It may seem as though the starburst pattern refuses Iwaniec’s advice (applied by Robling and Stout’s technique in 2003), that two columnar paths per region is most efficacious (1998). In actuality, the starburst pattern abides by her
recommendation strictly. Split into zones, anterior/posterior/medial/lateral, and sampled with two paths, is the same relative amount of sampled tissue as the starburst pattern: one length-wise half of the anterio-medial and anterio-lateral lanes would be counted as “anterior” using her zoning. So, the cross section is equally as well-represented here as it would be using Robling and Stout’s (2003) technique and is in accordance with Iwaniec’s advice (1998). In addition, if two lanes for describing 1/4th of the cortex is representative, then mathematically, so is one lane describing 1/8th. Iwaniec’s also notes that as much as 90% anterior representation can be achieved by as few as 5 equally separated FOVs in the femur. Four times this number of FOVs is common in femoral application of the starburst pattern. Finally, this study targets the humerus, and younger individuals are included. In both these cases the relative sampled area per region is much larger than in the femoral example since the bones are smaller but the FOV’s stay the same size.

The above reasoning does not wholly excuse the starburst pattern from some inherent bias in cross-sectional or regional representation. The size of the bone is taken into account through a basic standardization of area values by total cortical area (TCA). However, since the same number of FOVs is used per region, the inner tissue is always more representatively sampled than the outer tissue. Considering the inner region the a half or third of the TCA makes no difference, the bias would still apply and has applied for all known studies of bone histomorphometry by region. In addition, the FOV stays the same size regardless of TCA meaning that smaller individuals are relatively better
sampled than larger, a factor standardization does not mend. No study design has been yet invented that corrects for this issue. This is understandable as such an effort would be prohibitively time consuming and analytically challenging. Regardless, the likelihood that these combined errors threaten the integrity of any meaningful data is low, for this to be a true concern the potential for dramatic outliers in regional zones would need to be significantly high, which does not appear to be the case. For this reason and for time efficiency, these considerations are taken as unavoidable costs pursuant to data collection in the described context.

5.4.4.2 Starburst data collection

Cross-sections were prepared by scanning on a Lexmark® All-in-One, printer/scanner at 300 dpi, leaving the lid up for contrast and associating the scan with a horizontal transparent ruler for scale verification. These large images were inserted into Microsoft Powerpoint® where they were copied and cropped into images of individual slides saved as TIFF image format for preservation of integrity. All files were named by I.D. and converted into JPEG images for use in ImageJ® as a batch function in Irfanview®, both of these programs are available as freeware for image analysis and image viewing/batch converting. In ImageJ, the scan was run through its cross-sectional geometric evaluation and \( I_{\text{max}} \) and \( I_{\text{min}} \) were overlaid as axes on the image, defining the cross-sectional centroid on newly saved JPEGs.
Great care was taken to standardize all aspects of file saving and naming and to rename folders with the dates of modification to facilitate data backup efforts. This is not only for security of the information but to permit batch functioning in changes of image type, color mode, cropping, size, compression, and naming. If files are even slightly haphazardly named these batching options are unavailable and it can take hours to simply convert and rename the files for one of the many steps outlined in various sections of this method.

In order to transfer the centroid to the physical thin-ground slide, scans with axes were opened individually and sized to actual. The slide was placed on the computer screen used for super-position; this is most easily accomplished when, in addition to the cross-section itself, the I.D. tag is left in the image for a distant reference. Two reference points at distance increase the accuracy of overlaying the slide on its digital scan. At this point the centroid was marked on the coverslip using permanent marker with a very fine point. These same markers were then used to draw the ROI axes for point-count, using a template measured by protractor on graph paper placed under the slide, aligned to the slide’s anterio-posterior axis. Each ray was drawn with a straight edge taking great care not to draw the line over the tissue itself but only outside the external and inside the internal margins (Figure 24).
Figure 22: Marking starburst ROIs on the slide employed graph paper, protractor, ruler and fine point permanent marker. The axes seen here are on the paper under the sample. Lines were not drawn that would obscure the bone viewing but were instead kept outside the cortex. Arrow marks posterior (femoral section).

The lines were not permitted to overlap at the center, obscuring the centroid. When mistakes were made with the permanent marker, it was found the easiest means of removal was to whet a sharply broken popsicle stick slightly with saliva – the porous abrasion so provided erases permanent markings from glass even easier than using chemical removers and is much safer.

Counting was accomplished by placing the sample on top of four cleaned, large sized glass slides as a platform. This permits the 360° rotations that necessary for tracking along each ROI without the XY aperture confounding efforts at proper slide positioning. The bone was aligned on the anterior mark, and the first FOV was targeted within the bone’s periphery with the “uppermost” edge of the Merz grid as close to parallel with the bone surface as possible and containing all of the bone’s area. All
variables were recorded and the FOVs “lowermost” edge landmarks were memorized placed on the “uppermost” Mertz periphery to frame the next FOVs which progressed intracortically in this manner repetitively. When the last FOV was counted, the next ROI was targeted and the process, begun anew. The counting order was designed for efficiency and is not to be confused with the numerical order assigned to each ROI which needed to be sequential. ROIs were counted in the following order: A, P, M, L, the sample was rotated 45° and then AM, PL, PM, AL. The counting process is slow and user error in ROI selection or FOV placement was best avoided by strictly adhering to protocol.

Another tool used to reduce user error was a customized data entry program (Figure 25).
Figure 23: Custom programmed Microsoft Access® bone histomorphological data entry and spreadsheet generator. This program, called “µ-Count Histo” is suitable for 15 variables per Mertz grid intersection (each intersection is one field on the above matrix). Simple and rapid “Key 9” entry can be preformed without ever taking the eyes from the ocular. All individual ID and cross-sectional context is recorded and reported along with the spatial orientation of each counted FOV.

This piece of proprietary software, referred to as µ-Count Histo was coded in Microsoft Access® in order to preserve data interrelation, a security feature that neither lab sheet notation, nor spreadsheet software can offer. That being said, the end goal is of course a spreadsheet for statistical analysis rather than an inclusive database with a rigid data structure. Therefore, µ-Count Histo has three phases of user interface: data entry, data editing, and data export. Setup proceeded by completing the fields identifying the individual, the site of origin, bone, side, duplicate number, ROI and FOV. Info
identifying the individual needed only be entered once and the rest was nested, permitting at any time for a new bone or contralateral element to be added to the individual’s record.

Data entry was accomplished by first entering the three total FOV count variables for each FOV: osteon count, fragment count, and primary osteon count. Next the counting window entries were made. The counting window is a series of 36 (6x6) cells that are arranged mimicking the intersections on the Merz Grid. Key Nine variable associations permit each grid intersection to be labeled as fast as it is identified without the eyes ever leaving the microscope. Variables counted per intersection were: non-bone, secondary osteonal bone, drifted osteonal bone, periosteal lamellar, endosteal lamellar, non-identified, woven, trabecular, primary Haversian canal, resorption cavity, Volkmann’s canal, and trabecular void. At the end of the window, a “double enter” ticks the FOV up by one, unless a button for “New ROI” is clicked, in which case the ROI ticks up by one (to the next ROI) and the FOV count is reset to one for the new region. In this fashion and using tab or enter to progress between fields, point-count can accommodate a larger number of variables with very little effort on the part of the user. Each counting window can be recalled by selecting the ROI and FOV; it can be edited at any time in the Data entry window or in the Data editing mode which permits access to the database itself. Finally, the export function saves a comma-delimited file on the choice drive and portrays only the necessary data (does not include the contents of the counting window, only their sums per FOV) and restructures the data in the necessary format for statistical analysis. The use of µ-Count Histo was a vital component of a study.
such as this, greatly increasing the speed of multivariate counts, data entry, and manipulation. Reading errors were much easier to correct with the ability to simply switch data subsets into or out of position. In addition, the potential for transcription errors in entering data from lab sheets, or in reorganizing data by hand into different forms, is removed completely.

5.4.4.3 Parameters for histological variables

These variables represent the data relevant to the current study, taken from the larger dataset (Table 1). Primary data were collected per FOV (as described above) and were tabulated per ROI and per cross-section (in which case the prefix “r” or “t” is added to the variable abbreviation). Area measures were normally transformed to mm$^2$ and were standardized by total cortical area (rSA or tSA) before analyses.
Table 1: Major variables collected during this study’s point-count method. Note Values with no asterisk were collected for use during forthcoming research endeavors and are not referred to in the current text.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Used</th>
<th>Description of the Hit (mm²)</th>
<th>Variable Type</th>
<th>Prog. Designation Or Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>*</td>
<td>Periosteal lamellar area (point-count)</td>
<td>Reticle Sampled</td>
<td>PeriLamBone</td>
</tr>
<tr>
<td>EAp</td>
<td>*</td>
<td>Endosteal lamellar area (point-count)</td>
<td>Reticle Sampled</td>
<td>EndLamBone</td>
</tr>
<tr>
<td>NDSBA</td>
<td></td>
<td>Non-drifted Secondary Bone Area</td>
<td>Reticle Sampled</td>
<td>SecOstBone</td>
</tr>
<tr>
<td>WA</td>
<td></td>
<td>Woven bone</td>
<td>Reticle Sampled</td>
<td>WovenIntBone</td>
</tr>
<tr>
<td>TBA</td>
<td></td>
<td>Trabecular bone</td>
<td>Reticle Sampled</td>
<td>Trabeculae</td>
</tr>
<tr>
<td>DOA</td>
<td></td>
<td>Drifted osteonal area</td>
<td>Reticle Sampled</td>
<td>DriftedOsteon</td>
</tr>
<tr>
<td>NID</td>
<td></td>
<td>Non-identified bone</td>
<td>Reticle Sampled</td>
<td>NonIDIntBone</td>
</tr>
<tr>
<td>Ocan</td>
<td></td>
<td>Primary or secondary osteonal canal</td>
<td>Reticle Sampled</td>
<td>HavPrimCan</td>
</tr>
<tr>
<td>RC</td>
<td></td>
<td>Resorption bay</td>
<td>Reticle Sampled</td>
<td>ResopCav</td>
</tr>
<tr>
<td>Vcan</td>
<td></td>
<td>Primary or secondary Volkmann’s canal</td>
<td>Reticle Sampled</td>
<td>VolkCan</td>
</tr>
<tr>
<td>NBTBA</td>
<td></td>
<td>Off-bone within trabecular void</td>
<td>Reticle Sampled</td>
<td>NoBoneTrab</td>
</tr>
<tr>
<td>NB</td>
<td></td>
<td>All other off-bone</td>
<td>Reticle Sampled</td>
<td>NoBone</td>
</tr>
<tr>
<td>OC</td>
<td>*</td>
<td>Osteon Count</td>
<td>FOV Total Count</td>
<td>Osteon Count</td>
</tr>
<tr>
<td>FC</td>
<td>*</td>
<td>Fragment Count</td>
<td>FOV Total Count</td>
<td>Osteon FragCount</td>
</tr>
<tr>
<td>PC</td>
<td></td>
<td>Primary Osteon Count</td>
<td>FOV Total Count</td>
<td>Primary Osteon Count</td>
</tr>
<tr>
<td>tCA</td>
<td>*</td>
<td>Total Cortical Area</td>
<td>Scanned</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>*</td>
<td>Sampled Area</td>
<td>Calculated</td>
<td>NDSBA+DOA+PA+EA+NID+WA+Ocan+RC+Vcan</td>
</tr>
<tr>
<td>SBA</td>
<td>*</td>
<td>Secondary Bone Area</td>
<td>Calculated</td>
<td>NDSBA+DOA</td>
</tr>
<tr>
<td>OPD</td>
<td>*</td>
<td>Osteon Population Density</td>
<td>Calculated</td>
<td>OC+FC</td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td>Primary Lamellar Bone</td>
<td>Calculated</td>
<td>PA+EA</td>
</tr>
<tr>
<td>PB</td>
<td></td>
<td>Total Periosteal Bone</td>
<td>Calculated</td>
<td>PA+WA</td>
</tr>
<tr>
<td>VASC</td>
<td></td>
<td>Vascularization</td>
<td>Calculated</td>
<td>Ocan+Vcan</td>
</tr>
<tr>
<td>POR</td>
<td></td>
<td>Porosity</td>
<td>Calculated</td>
<td>Ocan+Vcan+RC</td>
</tr>
<tr>
<td>TRBZ</td>
<td></td>
<td>Trabecularization</td>
<td>Calculated</td>
<td>NBTBA+RC</td>
</tr>
<tr>
<td>PEI</td>
<td></td>
<td>Periosteal Endosteal Bone Index</td>
<td>Calculated</td>
<td>PA/EA</td>
</tr>
<tr>
<td>OPD/HAp</td>
<td>*</td>
<td>Remodeling Index</td>
<td>Calculated</td>
<td>OPD/HAp</td>
</tr>
</tbody>
</table>

1) **Sampled Area (SA):** Total bone area quantified in hits converted to mm².

SA was tabulated as all hits counted on all “on-bone variables” plus any void-space within the cortical margins, forgoing trabecular void-space. All regions combined are represented tSA, whereas per ROI is rSA.

2) **Periosteal Lamellar Area (PA) and Endosteal Lamellar Area (EA):**

Determined by the number of hits made on primary lamellae which, by location, lamellar orientation, and morphology, can be associated with periosteal or endosteal formation. The endosteal primary bone count also tallied bone that was not deposited strictly by the medullary surface when to...
call this bone BMU tissue would be contentious, as illustrated by the following example:

a. When large void-spaces (much larger than the largest osteons) deposited tissue that was directly adjacent and contributive to the ELP, it was always counted as endosteal primary bone and added to this variable. This is because on some occasions the ELP is constructed by prior vessel, or once-resorptive, cavities that find themselves functioning as per the endosteum – only separated by the main medullary endosteal activation by a thin trabecularized region and depositing phase after phase of bone in lock step with PEM deposition. In this case they contribute to the same PEM event but with separate activation foci (Figure 26). On occasion, but very rarely, even large drifting osteons comprised the ELP’s net deposit (Figure 27).
Figure 24: Large resorptive bay (two merged together) that is still active in formation phase on one of its surfaces (white solid arrows). This formation is similar in timing to the overall process of drift, which is evident by the drifting trabecularized bridge (top) encasing it, and the local lamellar stratigraphy at zones of adjacency between tissue deposited by the endosteum proper (top bone margin), and that deposited by the active surface of the bay (white lined arrow). Since the concerted effect is the formation of the ELP this tissue is counted as primary for the purposes of ELP analysis. Merged-micrograph 40x magnification. 530 nm red quartz compensated cross-polarized microphotograph.
3) **Secondary Bone Area (SBA):** Determined by the number of hits made on secondary bone at counting reticule intersections (all formations from Haversian systems including drifters). This count also tallied bone that was not Haversian in origin only when to call it PEM tissue would be contentious, as illustrated by the following example:

a. When large void-spaces (much larger than the largest osteons) deposited tissue that *was not* directly adjacent and contributive to the ELP, it was always counted as secondary bone and added to this variable. This includes drifting vessels remote from the ELP because it would be difficult to argue this is an endosteal event when it
transpires within the intercortex, rather than having been formed consecutively with endosteal deposition (Figure 28).

Figure 28: Vessel drift permitting its retention in the lagging cortex despite rapid cross-sectional linear drift. In this case the deposition is within the lagging intercortex, but is remote enough from the body of the ELP (bottom) to not have its drift included in the tissue area calculated for the ELP. A) Vascular void, B) pericortical surface, C) endocortical surface; Cross-polarized micrograph, scale bar indicates 500 µm

4) **Osteon count (OC)**: The total counted number per FOV of intact secondary osteons. These osteons must contain a Haversian canal that is at least 90% complete, and must contain ≥50% of their total area within the counting reticule. The following considerations were also made in the tally:
a. In keeping with Robling and Stout (2003), resorptive bays that were approximately the size of an osteon or smaller were counted as an osteon for these purposes. This was done because such a feature most often represents more than half of the process of generating an osteon and to avoid recognizing this underestimates remodeling processes in that area.

b. There are, especially within younger individuals, a preponderance of non-circular or even amorphous results of remodeling that cannot rightly be called an “osteon” by typical definition. These features are typically collections of osteons whose canals have joined to make a highly irregular void-space. These occurrences were always counted in terms of osteonal/fragment equivalency as follows:

   i. Two or more osteons diverging with evidence of cement-line between, while both contain a complete canal: ≥2 osteons

   ii. Two or more “osteons” diverging with evidence of cement-line between, while only the “newer” contain complete canals:

       complete canals = osteon; others = fragments

   iii. Two or more “osteons” mutually disrupted by an active resorption bay with evidence of cement-line between them, but neither canal is complete: ≥2 fragments
iv. Two or more canals diverging within one osteonal cement-line, no interposing reversal: 1 osteon

v. Two or more osteons in proximity, incomplete canals disrupted only due to an interconnecting secondary Volkmann’s canal: ≥2 osteons

vi. One slightly drifted osteon (more lamellae on one side than the other: 1 osteon

vii. One greatly drifted osteon (3x longer in total deposition than the original resorptive circumference – as suggested by the cement-line: 1 osteon

viii. One irregular void-space, with one or more associated partially closed osteons (lamellae congruent to void) with interposing cement-lines and little evidence of resorption (Howship’s lacunae): ≥1 osteon

ix. One irregular void-space, with one or more associated partially closed osteons (lamellae cross-cut by void) with interposing cement-lines and ample evidence of resorption: ≥1 fragments

x. One regular or irregular void-space larger than the largest osteons, with deposition not separated by interposing cement-lines, or with no deposition and ample resorptive evidence: not
counted (these are typically vessels or large resorptive bays in various states of resorption, formation, or drift)

c. A secondary deposition $\geq 3x$ larger than the original resorption bay (as suggested by the oldest cement-line drifter), but is now separated into one or more segments by interposing BMU events: $I_{frAGMENT}$ (even if separated into more than one segment).

d. Circumferentially drifting osteon larger than the FOV, or $\geq 50\%$ within the reticule: $I_{OSTEON}$

e. Circumferentially drifting osteon larger than the FOV, but is now separated into one or more segments by interposing BMU events: $I_{FRAGMENT}$

5) **Osteonal fragment count (FC):** The total counted number of fragmentary secondary osteons per FOV. Fragments exhibit only a fraction of the original lamellar area and more than 10\% removal of the Haversian canal via later remodeling events. There was no maximum or minimum size limit placed on fragments. The diagnostic difference between a fragment and a drifted osteon segment created by reversal in the direction of drift was cement line orientation and morphology. In the rare case that the drift was slight (less than one osteon’s diameter, and reversed exactly on its path to form a complete cement line separating it from its previous path, that path was named a fragment. This is because this circumstance is indistinguishable from a
fragment facing a similarly sized osteon. It is important to note that the number of cases where this “tie-breaker” was used was incredibly small due to the rarity of perfect osteonal drift reversal. Also see previous entry for handling special circumstances in osteon/fragment equivalency.

6) **Osteon population density (OPD):** The total sum of intact and fragmentary secondary osteons per mm$^2$: $OPD = (OC + OF)$, this can be measured per regional or total sampled area

7) **Primary Bone (PR):** The total sum of hits counted as periosteal or endosteal per mm$^2$: $PR = (PA + EA)$

8) **Periosteal/Endosteal Bone Index (PEI):** The ratio of periosteal bone to endosteal bone: $PEI = (PAp/EAp)$

9) **rOPD/rHAp Index:** The ratio of OPD per Secondary tissue area: a measure of regional remodeling that relates large areas of secondary tissue with how densely populated they are by Haversian systems. In this way regions with low secondary bone areas but high OPD can be singled out against those with low OPD but high secondary bone areas.

It is important to detail at this point how void-spaces were analyzed since treatment of trabecularized cortex is non-standardized and often vague. For this study, beginning from the intercortex, “resorption bays” were within the average cortical thickness of the cross-section (their deposition was secondary when distant from, and
primary when acting in concert with the endosteum); whereas, “trabecularized voids” were those separated from the medullary canal only by a thin trabecular strut and were not contained within the average cortical thickness of the cross-section (their deposition, if at all present, was considered primary when part of endosteal formative event).

5.4.5 Hand-drawn data collection

The point-count technique is less well suited for an analysis of shape, distribution, angles, or position. To measure these aspects of the ELP several requirements become obvious: 1) lower magnification is necessary to fit the entire ELP into view. Accomplishing this in a single FOV is not possible for the ELP, even at the lowest setting objective available (4x), necessitating the stitching of many images into a merged micrograph; 2) due to the need to source interstitial lamellar tissue to its membrane of origin and in order to differentiate tissue types at low magnification, contrast between tissue types becomes a high priority; 3) resultant merged micrographs require massive computational ability to accomplish digital stitching with no coordinate data, to generate final format composite images, and for rapid image manipulation and digital zooming.

Hand-drawn techniques were the most rapid and simple method for satisfying these requirements. Particularly, they are excellent for analyzing the distribution or geometry of tissue relative to the cross-section itself. These techniques make no effort to avoid the subjective perspective of the histologist, instead taking advantage of the trained
eye. In this case, the intention was to permit a fully detailed microscopic view of as much tissue as is necessary to outline the ELP with respect to orientation relative to the total cross section.

In order to accomplish these objectives, hand-drawn area measurements of merged micrographs were combined with all collected visual information, generating composite overlays of total transection scans, marked anatomical axes, cross-sectional geometric axes, hand-drawn zones of import, high-detail merged micrographs, hand-drawn ELP area masks, and the ELP centroid. The comprising images themselves and this composite offered a second dataset which, in combination with point-count data, provides a wide approach to sensing patterns of modeling drift within the population sample.

The protocol for hand-drawn ELP measurement is as follows: acquire raw images, stitch micrographs, outline the ELP, construction composite images, take angular measurements. These steps are each detailed below.

5.4.5.1 Image acquisition

Polarization microscopy augmented with false color compensation, as discussed previously (Section 5.4.2) was used in this study to generate much higher levels of contrast between tissue types and facilitate the reading of lamellar orientation, disruption, and patterns. The Maltese cross artifact was a limitation, twisted to advantage and
informs the most important aspect of primary image collection. This is true not only for the current work but is vital for any future efforts to automate ELP detection using advanced image analysis. To make this liability a benefit, the current project applies a novel microphotographic technique, 45° image acquisition, to the adult samples.

By taking images of ELP tissue (40x total magnification) only when lamellae are at 45° with respect to the artifact orientation (Figure 29), the photographer targets the orientation contributing the highest birefringence, and therefore the highest intensity.

![Figure 29: Schematic showing microphotographs capturing lamellar tissue only when at one 45° orientation (upper left) for the generation of merged images. This technique generates maximum birefringence and eliminating the Maltese cross artifact from primary tissue, while permitting it for contrast generation in Haversian tissues.](image)

In addition, the artifact is avoided in the primary tissue due to its larger circumference, relative to that of Haversian systems which at any angle of photography, contain the entire Maltese cross and therefore are dimmed in comparison to primary tissue, yielding a
much higher contrast differential between the two tissue types. In addition, the use of the red quartz compensating filter meant that as long as microphotography was standardized in orientation and targeted the tissue at one quadrant hue-zone, the ELP tissue would always appear bright blue since it was never photographed with its lamella at an opposing angle. In comparison, haversian systems couldn’t avoid the fact that their entire circumference must be photographed at these low magnifications. This means that, in addition to the interference artifact, each osteon displayed all the colors added by the compensator: in the images this manifests as blue in the upper left and lower right, and orange in the upper right and lower left quadrants (in reality it is the inverse since the microscope inverts the images upon condensation and magnification).

To accomplish this technique the upper right quadrant was chosen for ELP imaging, defining it in bright, blue light. The first image was taken, typically at the left-most insertion of the ELP (the area where ELP lamellae either about the medullary cavity or merge with the thin endosteal bone feature typically referred to as the internal “lamina” (for more information on how to distinguish the internal lamina from the ELP see Section 6.2.2.2). The priority order for imaging is to 1) maximize the blue birefringence by choosing an imaging angle that summarizes the orientation of lamellar tissue at as close to 45º as possible, 2) ensure that no tissue is left un-imaged, 3) force all images to contain enough between-image overlap and defining histomorphological features to facilitate the software’s stitching capabilities, 4) avoid any inclusion of interference zones in complex more acute primary lamellar topography by ensuring tissue
in the artifact is also imaged in blue at some point and that any orange-hue tissue is minimized in its area of frame inclusion as much as possible 45° while the contour of the bone’s inner (or outer) surface, if near the FOV was positioned such that it entered one, and exited the adjacent frame side. This ensured no loss of tissue in the image, particularly when two histological markers of reference were picked on the advancing edge of the image, one on the contour if one was present and one deeper in the image. These markers were moved from their lead position in image one to the trailing position of image two, using the X:Y armature. The image was rotated gently by hand to account for any change in lamellar direction, maintaining the 45°. At least 15-25% of the total FOV was required to be overlap with the prior image. Between each image, focus was checked. First the lamellae that were “central” for the image were placed as close to Resultant images must be saved as TIFF file format in order to avoid introduction of compression, or the potential copy degradation that is possible with other formats. This must be done every 75 images or the Spot Advanced® imaging software will risk hitting the memory limit of the associated desktop. Folders were labeled with identification along with the last image in the set (the rest kept their image names, Image1, Image2, Image3, etc.).

5.4.5.2 Image stitching
The 45° technique was accomplished using Kolor Autopano Giga® stitching software for all adult humeri in order to prepare them for automated image analysis at a later date; prior right-angle, non-compensated, Photoshop CS2® mergers were used to represent the two younger age-category subpopulations. This posed no problem for contrast concerns and reading accuracy because the younger individuals had much fewer obstructions (osteons) of the primary tissue, facilitating recognition and delineation of the ELP dramatically. The quantitative variation between mergers generated using these imaging techniques is extremely slight, as evidenced by the tight agreeance between not only Photoshop right-angle merged and Autopano 45° merged micrographs, but also between these micrographs and the tabletop scan and full sample mergers used as controls. Tests of merged micrographs generated using both techniques against these controls were well-within the total deviation of 50-150 µm that was found in some rare cases within only one or the other techniques sample. The mergers generated by both techniques were so accurate that the microstructure was preserved flawlessly between images, much more accurately than any “by-hand” technique that could have been employed.

The methodological duality was necessitated by uncontrollable factors of the research schedule. First, appropriate equipment for color image stitching was not available during the period of juvenile analysis. During that time the study was limited by computational strength and software suitability in that only Adobe Photoshop CS2® was available for image stitching. Although completely sufficient for these smaller bones (fewer total images in the merger), Photoshop was not capable of automatically stitching
these images if they were taken with the 45º technique, therefore right angle mergers were stitched instead. Imaging the adult tissue required not only more processing power but more efficient stitching software. Kolor Autopano Giga®, was used to meet these requirements. This software is specially designed for large computational demand image stitching, is wonderfully intuitive, and features more robust algorithms for pairwise image stitches, using hundreds of points per comparison when necessary and giving statistical data on the quality of fit used to generate a given merger (all mergers generated reported “excellent” fits – the highest rating). Unlike other software, Autopano easily stitches odd-angle images, like those generated by the 45º image acquisition technique. In addition this software offers extremely efficient batching options, speeding up the stitching and merging processes incredibly. All setup options and specifications used during the Autopano stitching process were recorded; the most important of which was to set the focal length to 1000 mm, select “multiple viewpoint.” permit color correction (to summarize the color shows the proper blue present in the overall merger and reduces “coronal” artifacts from off-sample), and choose “planar” image projection. This setup most approximates the effect generated by microscopic imaging where the camera is moved in perfect orientation to the photographed media and there is no need for projection compensation (fish eye, etc).

After the images are stitched together, the result is a very large image – so large that the preview scale needed to be set to 3% to view the bone merger. At this point the merger was cropped out to reset the image size appropriately (since this is not a
resolution change the scale is not affected). At this point the preview is rendered to complete the actual ELP merged microphotograph. The largest images (fully merged femora, for example) would run into a limitation in Autopano file size permissions (it is unknown if there is a workaround for this). Therefore, a standard rendering reduction in image resolution of 50% was used to ensure that all images were kept size standard and to a single scale, this change in scale was accounted for (photography at 0.630 pixels/µm meant mergers were 0.315 pixels/µm). Reducing the resolution output by 50% was necessary to save as a TIFF file format, but was not necessary if compression was used. For image analysis however, TIFF file format is a necessity for data integrity. This resolution decrease was found not to cause any real change in ability to identify primary tissue types or complete drawings under digital zoom. Resulting images were still extremely large and regularly demanded upwards of 70 gigabytes of storage for only the base images and mergers. Batch processing permitted by the Autopano software was also quite useful and time saving but required, along with storage, considerable computational strength.

The hardware used to efficientize image stitching, merger quantification, and composite generation was a custom portable workstation (very large laptop), providing portable power for all aspects of the analysis. This system was built by Eurocom®, a small company based in Canada that specializes in special needs portable computing. Their Panther II®, used here, includes specified high-end components necessary for image analysis: 6 core 3.3 GHz Intel processor, 64-bit Windows 7® Operating System, 12
GB RAM, and a 485M Nvidia GeForce GTX® video card. Of these, the multi-threading on the processor and the powerful video card are most helpful, particularly for image work. Additional equipment required by for the handling and storage of data included over 4 TB of memory for primary and backup files.

5.3.5.3 Composite image construction and analysis

Composites were necessary for two main necessities: 1) providing easy access to all levels of information for observation and comparisons, 2) relating microscopic, hand-drawn, and cross-sectional data for angular and positional measurements between them. The media used for construction of composites was Adobe Photoshop CS5®. This software was well-suited for overlaying image types, axes, and angular measures in that it permitted precise control of both adjustment and maintenance of component image positioning, size, and scale, and also facilitated image reordering (layer stack reorganization), file type transformations, layer transparency, and figure construction for publication and report.

Composites were generated by beginning with the original merged-micrograph of the ELP with no scale adjustment. It was at this time (and in this file) that a new layer was generated for the outlining of the ELP. The ELP was outlined at high digital zoom (larger than 40x magnification) of various degrees depending on the complexity of tissues in the region, when any doubt in tissue assignment was encountered that region could be
checked again in the microscope. The layer containing the ELP was then saved as a JPEG and imported into ImageJ®. From here it was converted into 8-Bit format and set to scale in pixels per micrometer. The thresholding function was used to select the ELP as a region, and the measurement window was set to analyze only this thresholded zone, reporting the total area (µm²) and the centroid (in x:y coordinates). The centroid was drawn on the ELP JPEG at the designated coordinates before it was added as a new layer to the composite in CS5. Here its centroid was made a separate layer.

For notation and observational study, another merged image was generated at right angles and using circularly polarized light (employing a hybrid 135nm and 530 nm setup found to be useful for low magnification viewing). This “notation merger” was added to the composite along with the original tabletop scan. A duplicate of the table top scan was useful for contrast enhancement. Digital zoom was used to ensure proper overlay of this and all other merger and ELP layers on top of the imported and resized tabletop scanner image layers. Other layers were made to permit viewing of anatomical axes and to facilitate overlaying images. In total, composite images contained 11 layers, including (in order of appearance from top down): ELP centroid, the ELP area, the Antero-posterior axis, a copy of the tabletop scan with heightened contrast for overlaying orientation, the actual tabletop scan with cross-sectional axes and centroid, any drawn notation on tissue types other than ELP, the notation right angle circular polarization merger, the original associated ELP area and ELP centroid, the 45° micromerger used to originally draw the ELP, a black background layer. All of these
were resized for file size limitations and agreement using scalar transformations equalizing their scales, except for the original table top scan whose scale was not needed for the composite portion of the project. It is important to note that all actual measurements, including cross-sectional calculations and axes were made using other images with their original scale and that angular measurements were centroid-based and did not depend on scalar information.

The final set of measurements could only be made at this point, from the completed composite images. These were the angular measurements, accounting for the position and axis of the ELP and the axis of $I_{\text{max}}$. It is important to note that the following method references the orientation for a right element; the left element would be measured in counter-clockwise fashion. This difference means that the angle always indicates degree of laterality as acute and obtuse angles and mediality as reflex angles (Figure 30).
Figure 26: Composite image file of a left humeral cross-section. Layers here are ordered to permit visualization of, A) the circular-polarized 530nm partial compensated merged-micrograph (used for notes), and B) the green mask identifying the ELP area, the two centroids (red = cross-sectional, orange = ELP centroid), and the angle between the posterior ROI and the ELP position (yellow line).

ELP and I_{max} position were measured by exporting JPEGs from the composites that showed only the cross-sectional scan and geometric axes, the ELP centroid, the anterio-
posterior axes, and the ELP area itself. In ImageJ, these files were analyzed using the software’s accompanying angular measurement tools. Primary angles of interest were defined with respect to the posterior ROI in order to collect the angle in a fashion that would avoid ELP angular measures around 0º which could confuse interpretation. ELP position was measured as the lateral angle (0-360º) between a line defined by the posterior ROI and another, by the cross-sectional and ELP centroids. Likewise, I_{max} was measured by noting the angle (0-180º) between the line defined by the cross-sectional centroid and posterior ROI, and the line defined by the nearest lateral arm of the I_{max} axis.

5.3.5.4 Parameters for hand-drawn and composite image variables

These variables (Table 2) represent the data collected from the ELP using hand drawn assessments of its area and digitally measured cross-sectional axes. These measured comparisons are only possible due to the construction of composite images discussed in detail in Section 5.4.5.3). Primary data were collected per cross-section (as described above) and were compared to projections of point-count data, used to inform a second set of angular estimates of drift via weighted averaging of endosteal or total primary bone hits per region. Area measures were normally transformed to mm² and were standardized by total cortical area (tCA) before analyses. Many of these measures had to be designed particularly for this application.
1) **Endosteal lamellar area, hand-drawn (EAh):** This measured area was calculated digitally as described above from hand-drawn areas outlined using digital zoom to view merged micrographs of the entire ELP. Tissue was considered as belonging to the ELP when demonstrated the vital characterizing features: Thickly deposited hemicircumferencial endosteal lamellae, demonstrating fewer osteons than other inner cortical areas, and vascularized by large, primary Volkmann’s Canals (for more information on ELP characteristics see Section 6.2.2). Not all endosteal deposition is included in the ELP. Most commonly included was the “internal lamina” deposited in a thin slip around the medullary cavity, particularly visible in younger individuals. The following guide facilitated discernment of when the ELP included or did not include endosteal lamellar tissue. The ELP was considered to “begin” in its middle, thickest region, and extend in two directions toward either aspect of the drifting medullary canal. In either

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Table 2: Major variables collected during this study’s hand-drawn method.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description (mm² for areas)</th>
<th>Variable Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAh</td>
<td>Endosteal lamellar area (hand-drawn)</td>
<td>Hand-Drawn</td>
</tr>
<tr>
<td>Ec</td>
<td>ELP centroid</td>
<td>EAh digital</td>
</tr>
<tr>
<td>EhAng</td>
<td>Lateral angle, from posterior ROI to ELP</td>
<td>EAh digital</td>
</tr>
<tr>
<td>EpAng</td>
<td>Endosteal point-count angle from weighted avg. endosteal hits</td>
<td>Calculated</td>
</tr>
<tr>
<td>PRpAng</td>
<td>Endosteal point-count angle from weighted avg. primary bone hits</td>
<td>Calculated</td>
</tr>
<tr>
<td>CSc</td>
<td>Cross-sectional centroid</td>
<td>Digital cross-sectional</td>
</tr>
<tr>
<td>ImAx</td>
<td>Nearest lateral angle, from posterior ROI to Imax axis</td>
<td>Digital cross-sectional</td>
</tr>
</tbody>
</table>
direction, if the ELP narrowed to less than the average osteon (200-300 µm) and did not thicken again into ELP typical depositions, then that point would be used as a cut-off. In outlining debatable tissue deposited by large voids either distant from or within the ELP depositional event, the same parameters listed for point-count technique inclusion were used. Disassociated interstitial endosteal lamellar tissue contributing to the ELP was only included in the area assessment if it was larger than the average osteon. All interposing secondary structures were avoided completely by the final hand-drawn mask of the meta-feature’s area.

2) **ELP centroid (Ec):** The x:y coordinates for this point were part of the Image J® output generated from the EAh mask.

3) **Endosteal hand-drawn angle (EhAng):** This angle was calculated as the angle between a line bisecting the Ec and CSc, and one along the posterior ROI. This angle could run a range of 0-360°.

4) **Endosteal point-count weighted vector angle (EpAng):** ELP point-count angle from weighted avg. endosteal hits. This description of ELP position and drift direction was generated using equations that weight each ROI by its comprising endosteal lamellar hit count. The result was a vector whose strength represented the statistical significance of the weighting, and whose position indicated the angle of the ELP when measured versus the posterior ROI. The equation used was:
5) **Endosteal and periosteal point-count weighted vector angle (EpAng):**

ELP point-count angle from weighted avg. primary bone hits. This description of ELP position and drift direction was generated using equations that weight each ROI by its comprising primary lamellar hit count. The result was a vector whose strength represented the statistical significance of the weighting, and whose position indicated the angle of the ELP when measured versus the posterior ROI. The equation used was exactly similar to that for EpAng except that PA and PE were summed to weight the vector.

6) **Cross-sectional centroid (CSc):** The x:y coordinates for this point were part of the Image J® output generated from the total cross-sectional scan during geometric analysis.

7) **Maximum second area moment angle (I_max):** The I_max axis was part of the Image J® output generated from the total cross-sectional scan during geometric analysis. This angle measure, however, was generated between nearest component of the I_max axis and the posterior ROI. In this way the axis was always “named” by nearest, rather than most distant component, in the same fashion that EhAx axis was measured. This angle could run a range of 0-180°.

5.4.6 Phase one methods: Qualitative histomorphological drift assessment
Qualitative assessment of relative tissue maturation and drift within the cortex was accomplished through drawings, notes, and microphotography documenting observations on several major aspects of change in cortical tissue structure and distribution. Dominant foci were patterns of primary formation, secondary remodeling, and resorption in each of the two bone types: periosteal and endosteal; with separate attention given to their composition/structure and distribution. Structural notation targeted the manipulation of phases of formation as evident in the lamellar stratigraphy recorded by BSUs, primary and secondary. Distributional notation targeted the anatomical regions, the relative cortical depth, and the linear drift frame of reference (leading and lagging cortices) where events transpired. Special or rare occurrences not directly related to tissue maturation and drift were not included for sake of efficiency.

5.4.7 Phase two through four: Histomorphometric statistical analyses

The statistical analyses for this project use SPSS where possible and are divided into three major histomorphometric phases, (these follow the first phase which was non-quantitative). Phase two compares point-count versus hand-drawn measures of a) ELP size and b) ELP position. In this way trends across the sample can be assessed using completely different types of data acquisition, augmenting verification of patterns observed. Phase three compares the hand-drawn ELP angle, and its generated axis with
that of $I_{\text{max}}$, to determine if the growth history of the bone as represented by endosteal
drift agrees with the axis of highest resistance to bending. Phase four explores the trends
for ELP presence, area, and position among subgroups defined population sample as well
as age and sex.

Two methodological circumstances are important for understanding the research
design outlined here. 1) The data was typically non-normally distributed. This could be
due to the sample bias, though this is unlikely since sexes and ages are fairly well
represented for most of the comparisons. It is also possible that development in bone
modeling could be more constrained than we would expect, with growth and adaptation
removing variance when possible, not to mention the effects of individual behaviors or
environs which are also far from randomized. This property would be even more
noticeable in angular point-count data – since the point-count technique was collected at
set angles numbering 8 rather than across a larger range of sampling. In order to account
for this attribute of the data, non-parametric methods were used for all elements of this
study. 2) Angular data provides its own challenges. Namely, zero and 360° are the same
location and any axis has two points of measure. This required the use of statistical
methods specially developed to address angular means. In addition, the comparison of
measures of different total ranges is discussed as a special consideration as well $E_{\text{Ang}}$
(range 0-360°) with $I_{\text{max}}$ (range 0-180°). Unless noted, all statements regarding statistical
significance were marked against an $\alpha$: 0.05, or 95% confidence interval.
5.4.7.1 Phase two methods: Point-count and hand-drawn data comparisons

The two methods, point-count and hand-drawn, will yield differing absolute measurements of areas and angles relating to drift. This is especially true since one of these measures, hand-drawn, discerns between total endosteal area and that of the ELP by omitting the internal “lamina” (for more on this see Section 6.2.2.2. However, the intention of this section is to compare the patterns each method indicates rather than only their absolute values. Especially if both are sufficient for quantification of primary tissue patterns in bone, these patterns should be similar. If one is dramatically different or more highly variable then comparisons will aid in future methods development in the larger study. To facilitate general visualization of the data, they were plotted on line graphs. Analyses was also aided by the use of a profile analysis in SPSS comparing area and angle values generated by the two techniques (despite the violation in this case of normality since it permits visual comparison with interesting results).

5.4.7.1.1 Comparing area methods

Comparisons of endosteal area between the techniques, EAp and EAh was accomplished as follows. Comparisons of EAp (in endosteal hits/cross-section standardized by rSA) and EAh in mm² standardized by tCA) were first made using descriptive statistics which showed very strong similarities in the pattern of endosteal
deposition across the total pooled sample (in some cases \( N = 61 \) (rather than 76), since individuals with no ELP in the hand-drawn dataset are excluded in pairwise comparisons). It is important to note that differences were expected due to the fact that the methods collect different representations of the data and because the point-count technique a) has lower representation of periosteal compared to endosteal FOVs, and b) EAp includes the thin endosteal circumferential lamellaer slip that EAh ignored. Regardless comparisons should yield similar patterns even if the magnitude of measures are different. All sub-samples were pooled for this level of investigation to facilitate a broad perspective on the two techniques.

Accurate assessment of the relationship between two methods of area data collection required the application of the Wilcoxon ranked-sum test for paired data with non-normal distributions). In order to use this test three assumptions were confirmed: 1) each observation pair was from a random sample and were independent from other pairs, 2) the sample size was relatively large (much greater than suggested minimum of 16 pairs), and 3) the distribution of differences scores was continuous and symmetrical (Green and Salkind, 2008).

5.4.7.1.2 Comparing angle methods

Comparing angles between the two tests employed techniques developed particularly for generating and comparing angular means (Zar, 2010), and addressed the
total pooled sample. First it was necessary to generate a single angle, or vector, (0-360°) from point count data collected only every 45°. This was accomplished by weighting each measured ROI by its hit frequency for the given tissue and accounting for the circularity of the distribution. The obtained weighted average can be visualized best as a vector whose angle averages the orientation of the weighted mean ROI, but whose length indicates the statistical significance of that direction. A shorter vector would arise form a lower calculated r value (dispersion measure between 0 and 1, where 0 = no vector exists summarizing the distribution, and 1= the vector contains all angles in the distribution – it is not related to “r” terms in linear statistics). This r value has the potential to be too low to significantly represent the net angular effect. For example, a distribution of angular data that are in groups exactly oriented across from one another on a circle will cancel each other out (r = 0) in exactly the same way that angular data distributed widely around the circle will cancel one another out. This problem is significant due to the fact that the ELP is hemicircumferential and internal, striking several ROIs even when relatively small, necessitating corrective efforts. In addition, many of the most internal FOVs have at least some endosteal hits (fewer than 6 normally), and this competition with the ELP signal could conceivably cause trouble.

Another goal of this phase of study is to assess whether considerations of endosteal tissues alone are sufficient for drift summary or if the addition of endosteal and periosteal point-count consideration provides a more useful summary. For this reason, two different methods of determining a point-count drift summary were used by changing
the hit frequency used to weight ROIS: 1) rEAP weighted EpAng and, 2) rPRp (rEAp + rPAp) weighted PRpAng (both with ranges 0-360º).

Having constructed the summary vectors for use, the two weighted point-count vector sets (EpAng distribution and PRpAng distribution) can be compared to the angle defined during the hand-drawn data collection (EhAng) (range 0-360º). EpAng was compared to EhAng using the non-parametric paired-sample test for circular means (Zar, 2010). This test forms a single sample of the paired differences between the two drift angles for each individual were examined, non-parametrically, by Moore’s test for paired data on a circular scale of measurement (since Hotelling’s test requires normality and these distributions were not normal).

5.4.7.2 Phase three methods: Comparing drift and I\textsubscript{max}

The ELP centroid-defined EhAng was used to represent ELP drift direction by defining its axis. EhAng was used rather than EpAng/PRpAng, due to the aforementioned potential for point-count measures to generate insignificant vectors, and because EhAng is a more visual variable that can be readily seen in the composite images, confirming its positional relevance to drift; where point-count angle measures cannot be visually assessed.

In this phase, EhAng and I\textsubscript{max} were directly compared in degrees. EhAng, like I\textsubscript{max}, was reported as the angle between the posterior ROI and the nearest lateral angle at
which EhAng or the nearest arm of the $I_{\text{max}}$ axis is encountered. However, these measures have different ranges because one is an axis (0-180º) and the other is an angle (0-360º), a factor that could disrupt their comparison. Fortunately, pilot studies showed that $I_{\text{max}}$ was extremely low in variance and predictable in position, such that multiple occurrences of confounding $I_{\text{max}}$ orientation relative to EhAng was completely avoided in all but three cases. In this fashion, the comparison is still relevant and useful, even if statistical significance is threatened by these outliers.

5.4.7.3 Phase four methods: comparing ELP presence area and position among regions and subpopulations

5.4.7.3.1 ELP presence

ELP presence has been assessed in a pilot study reporting on adult femora (I. Maggiano et al., 2011a). The intention of analysis in this section is to provide similar data for the Humerus. Basic presence will be reported as a total percentage of the pooled data, and was reported in the context of each population sample, males and females, and the three age groups.

5.4.7.3.2 Comparisons of area
In this phase, cross-sectional area variables were analyzed first from the pooled population sample in order to address large scale patterns in the data. These measures include EAh, tEAp, tPHAp, tHAp, and tOPD, where each is standardized accordingly by tSA. Values for tCA and tOPD/tHAp, the remodeling index, were also reported here. All of these variables were compared between the total sample and a subsample of only individuals bearing an identifiable ELP.

The next step was to break down each tissue type by region to illuminate the distribution of tissues across all axes. Whenever possible both raw and standardized data was included in this manuscript. Color notation was used to emphasize several important elements of the analysis: 1) the origin of bone tissue in question (green = periosteal, blue = endosteal, orange = secondary, and red = osteon population density), 2) to identify maximum and minimum means, important for discussion, and 3) to call attention to statistical treatment if applicable (using “*” to denote significance at a 95% confidence interval). In addition, regional visualization of tissue distribution was aided by the construction of radar graphs that have each of the ROIs as coordinates. Reports and discussion of data from these tables and figures was often made using relative secondary variables relating to the difference between mean values expressed in percentages (i.e., “males had X% more of Y variable than females on average”).

More detailed investigations were made regarding sexual dimorphism across the pooled population sample dataset. Likewise, the population samples, Xcambó and Xoclan were compared using the total pooled sex dataset age matched to the third age
category (20-65). These relationships were tested by the application of Mann-Whitney tests for comparing two non-parametric distributions.

After pooled analyses, each site was examined individually for relationships at the cross-sectional and at the regional level between the sexes and age categories. Since three age categories only exist in the Xcambó sample this style of analysis was not performed on the Xoclan sample. It is important to remember that for age comparisons differences between age groups were treated as such, although the heuristic false chronological perspective is also useful for understanding the data and at times in results or discussions areas will be referred to as “increasing” with age in a given ROI, knowing full-well that longitudinal comparisons are not possible from deceased remains.

Identification of trends in age distribution of tissues was facilitated by the use of line graphs plotting areas against ages (sometimes pooled). Other than this, the structure used for these analyses was the same as described above, using color-coded tables and radar graphs and appropriate statistical techniques: Mann-Whitney for two-sample, and Kruskal Wallis for multi-sample comparisons.

5.4.7.3.3 ELP position

The ELP centroid-defined EhAng was used to represent ELP position by defining its relative angle. EhAng was used rather than EpAng/PRpAng, due to the aforementioned potential for point-count measures to generate insignificant vectors, and
because EhAng is a more visual variable that can be readily seen in the composite images, confirming its positional relevance to drift; where point-count angle measures aren’t visually measured, but calculated instead. Kruskal Wallis for multi-sample comparisons.

5.5 Summary

The current research introduces two methods of data collection in order to compare the patterned results of each. This has two major benefits: 1) it functions as an internal comparative control, verifying the trends observed via two different datasets, 2) it permits comparisons in efficiency and recorded variability between the two techniques which can be used for justifying protocols for future research. The point-count technique is well-suited for count data and statistical treatments on various tissue distributions. It also has the added benefit of permitting collection of other histomorphometric data of interest, such as osteon population density and tracking drifting osteons. However, it is difficult to compare the shapes rather than merely the magnitudes of tissue distributions. The hand-drawn line method is better suited for shape analysis and could be much more rapid for limited analyses of one tissue type (the ELP for example) but does not provide count data on osteon population density. Several new methods were introduced to fit these techniques to the current research. These include: 1) the standardized starburst sampling pattern used to define regions of analysis on the cross section; 2) the
application of a data entry and databasing program designed specifically for histomorphometric analysis in bone; 3) the use of angular statistics to generate vectors from point-count distributions that indicate drift directions; 4) the generation of the 45° image acquisition technique which permits cross-polarized microphotography, increasing contrast between primary and secondary tissue types; and 5) new software applications for micromerging large numbers of high-resolution, color images.
CHAPTER 6: RESULTS

6.1 Introduction

This chapter reports the results of the research project, focusing separately on histomorphological observation and microscopic point-count or hand-drawn data. First, macroscopic measures are addressed because long bone length, humeral torsion, and other measures are important for interpreting modeling drift and the ELP as its indicator. Also, these measures maintain a connection to perspectives used more commonly in bioarchaeology like stature, body proportion, robusticity, locomotion, or biomechanics. Second, qualitative assessment of humeral primary and secondary histology is provided in order to facilitate the reading of bone microstructures related to modeling drift. Third, data analysis is provided, comparing basic ELP size and location as reported by the two datasets: point-count and hand-drawn. Then these measures are used to calculate the axis suggested as the major modeling growth vector to explore its relationship to cross-sectional geometric reports regarding axes most resistant to bending. Finally, this section includes the results of tests and exploratory analyses that track variation in ELP traits (presence, position, and size) across subgroups based on age, sex and population membership.
6.2 Phase One Results: Qualitative Histomorphometric Drift Assessment

It is very important here to detail qualitative histological observations for several reasons: 1) observational results are difficult to address elsewhere and a dissertation provides the perfect forum for their solidification, 2) observational results are the foundation for new hypothesis driven testing, 3) basic descriptions of human primary bone histomorphology have been lacking in the last several decades (Maggiano, 2011). Therefore, here special attention is given to areas that seem underrepresented in the literature including: 1) important structural differentiation between periosteal and endosteal, “interstitial tissue.” notorious for being labeled generally rather than with respect to the formative membrane; 2) distinction between primary and secondary osteons, types of osteonal drift, laminar and pseudo-laminar bone, and traditional Volkmann’s canals and those present in the ELP; and 3) commentary on the arrangement of histological features on a cross-sectional scale as they relate to modeling drift.
6.2.1 Periosteal bone

6.2.1.1 Pericortical primary microstructure

Early periosteal deposition in children is somewhat similar to what has been described as “laminar bone” in non-humans (Maggiano, 2011) in that it occurs in relatively quickly growing sets (175-250 µm thick) (Figure 31). True woven bone was seen rarely within these age categories, but in some occasions the youngest individuals still demonstrated this tissue type.

Figure 27: Images from the youngest age category, demonstrating, A) periosteal apposition in pseudo lamina (white bracket) enclosing a sheet of longitudinally oriented primary vessels in its interior, and B) the continuum within one FOV from woven tissue (white solid arrow) to lamellar tissue (white lined arrow). Cross-polarized
It is, however, nothing near as regular as it is in most other mammals and so here has been called pseudo-laminar in appearance. When completely formed, appositional, laminar-like sheets have a barely perceivable disturbance to the lamellae adjacent to their boundaries, much like a slight arrest line (notoriously difficult to spot in primary tissue). Much more easily visible, are the vessel voids within each pseudo-lamina (Maggiano, 2011).

Unlike those in most mammals, the vessels trapped by periosteal deposition in humans are nearly always longitudinal and are positioned roughly 500 µm apart from one another along the midline of the pseudo-lamina. On occasion and particularly later in life, the bone does not manifest a laminar tendency at all (being comprised of tight and clearly organized lamellae) and the row-like organization of these longitudinal vessels is absent or disguised by variation in their timing of entombment or secondary remodeling (according to lamellar stratigraphy). A typical field of view in this study might include between 14-20 primary longitudinal vascular voids, traditionally called “primary osteons.” In most cases these primary osteons are the same size as secondary Haversian canals, but in other cases are much smaller (approaching 25 µm rather than the 50 µm or more common in secondary osteons). Regardless of size, they provide vascularity for the immediate vicinity but also serve as the major sites for subsequent remodeling activation events.

In immature primary periosteal bone, different tissues could be observed that were clearly tissue-age-at-formation dependent and highly ordered due to the process of
periosteal apposition. That is, primary osteonal seams (as in pseudo-lamina) along the bone’s peri-cortex would be least likely to have associated “first generation” secondary osteonal remodeling compared to their neighbors in “older tissue” toward the intracortex (Figure 32).

Figure 28: The bracket indicates a deeper pseudo-lamina with its longitudinal primary vessel seam under the process of remodeling at the time of death. The more external (top) seam has no secondary osteons. Primary vascular inclusions in the youngest tissue are even capable of their own slight drifts (insert), also note the greater effect of the vessels presence on the periosteal side of the original primary void location (top left of insert). Resorption bays during this early phase of maturation often include several vessels either along a seam or between them (white solid arrows). The results are Haversian morphologies that are dominated by osteonal drift of various magnitudes.
Pure primary osteonal lamellae (or even pseudo-lamina) mark the youngest tissue in the periosteal deposition. Tissue with one generation of secondary osteons is typically intracortical in position in the youngest individuals, marking it as older. Once the bone is more mature, its intracortical region is populated by second and third generation secondary osteons, and only the outer-most peri-cortical deposition possesses the occasional un-remodeled primary canal.

Primary bone was deposited in sheets that are roughly congruent with the circumference of the cross-section, particularly in areas of more recent deposition. Deeper tissue, older primary tissue, demonstrates lamellae sometimes angled toward the endosteum. Prime examples of this phenomenon exist on either side of the ELP. Interestingly these layers are contiguous with those on the inner cortex of the bones leading drift aspect, and matches the external contour there, despite being out of alignment with the nearest cortical surface (which is roughly “drift-neutral.” or lateral to the drift vector) (Figure 33). Changing orientations in periosteal depositional phases is otherwise uncommon, yielding arrest or partial reversal lines on only infrequent occasion.
Figure 29: Merged-micrograph including a portion of the ELP (white solid arrow) demonstrating periosteal tissue orientation which changes (white lined arrows) particularly outside an ELP with significant evidence of rotational drift, as in this case. Also note the presence of a postmortem micro fracture which takes advantage of the course suggested by circumferentially drifting osteons common in this region.

6.2.1.2 Pericortical secondary microstructure
In the case of standard first generation secondary osteons, activation creates a resorptive cavity adjacent to the primary vessel which refills in the formation of a typical secondary osteon; sometimes in the exact position of the prior primary vessel, other times slightly off-position. It is unclear how often these events transpire, a) during, b) after, or c) without the formation or continuance of a new vessel. In the literature, the formation of a new vessel along with the A-R-F sequence (activation-resorption-formation), is typically assumed. These observations suggest that may not be the case. The area of a fragmentary canal, having been partly resorbed during the formation of the new osteon in that region, shows evidence under high magnification of being part of the formative event leading the new osteon. On no occasion was a canal cut and the void-space left unincorporated into formation generating the newer adjacent secondary osteon. Often this event manifests as a “comma shaped” osteon emerging from the fragment, micro-structurally distinct from the “figure eight) osteon that is associated with Haversian branching events (Figure 34). The fate of prior vessels and other Haversian canal contents in this case is unclear.
Figure 30: This scene contains many occurrences of fragments which by position have apparently passed their vessels forward into the most recent secondary osteon which now is not only oddly shaped but has a void space that could be inhabited by at least two vessel systems. Several other similar occurrences take place in this FOV.

Osteonal stratigraphy (overlapping cementlines) also permitted identification of first, second, third, and sometimes even fourth “generation” secondary osteons. In this setting two factors were seen to be particularly informative of local processes of turnover 1) the total area of secondary tissue, 2) the total number of complete and fragmentary osteons (OPD) (also regions with high OPD after a point tend to have the highest number
of visible osteonal “generations”), and 3) the interaction of these two. On many occasions the most central or intracortical FOVs had reached the OPD asymptote whereas periosteal origin tissue positioned more interiorly had not. In these cases, primary periosteal tissue reappeared even adjacent to the medullary margin (obviously this is likely to only transpire on the leading modeling side of the bone). Occasions like this were especially clear when these primary regions still contained primary osteons and other markers of primary periosteal deposition, despite their position in a surprisingly interior local. Also, immature peri-cortical tissue seemed to have often “haversianized” before it “fragmented” due to new osteonal generations. Therefore FOVs entirely secondary tissue but few fragments were associated, younger, more recently deposited tissue. The phenomenon responsible for this circumstance is often the drifted osteon.

So “stable” secondary osteons do not construct the only maturation pattern for periosteal tissue. Drifted osteons seem to be the odd tissue out, covering large territories with a single activation (Robling and Stout, 1999). In some immature regions for example, a single seam of first generation secondary osteons drift impressive distances toward the intercortex, sometimes accounting for large percentages of the total cortex in that region. Especially when this transpires, OPD can be unexpectedly low, compared to the high number of hits for Haversian area. In other cases drifted osteons seem to have less patterned arrangement; in one case even literally drifting 360°, in another cartwheeling around one another while borrowing the same voidspace (Figure 35).
Figure 31: Highly irregular drifters are actually quite common in younger individuals in the Humerus and seem on occasion highly patterned in orientation. Here however, it seems a conflict of necessary remodeling despite inability to achieve multiple activations for some reason and is not ordered, manifesting a circularly drifted osteon in the main image, and two drifters spiraling around the same shared void in the insert. Also note that even in the highly Haversianized tissue in the main image, identification of the primary interstitial tissue is quite easy thanks to the presence of a primary vascular canal (white lined arrow).

The current quantitative study only considered secondary tissue “drifted” when osteons cleanly formed bone on one longitudinal side of their bay and resorbed on the other “leading” edge, resulting in an osteon 3x or more long than its original resorptive area (as suggested by its original cement-line). However, this may have been only a partial consideration of the phenomenon of osteonal drift.
Qualitative histomorphometric observation turned up a completely different type of drifted osteon, not well described in the literature, and very common in the humerus. That they are true secondary osteons, in the remodeling sense, is evidenced by their clear and completely encompassing reversal lines. They seem to form at the site of prior primary canals in immature periosteal tissue. Instead of refilling as a standard secondary osteon, or drifting, however circuitously in the axes with the centroid as most drifters do, these are completely locked within the pseudo-lamina of their origin and travel, sometimes for 1-2 mm, always within the circumference suggested by the current or one-time peri-cortex (Figure 36).
Figure 32: Circumferential drifting osteons. This form of drifted osteons seems directly connected to large expanses of periosteal primary bone deposited in the vicinity of the ELP, particularly when significant evidence of rotational drift exists in the orientation of endosteal lamellar phases. On occasion the drift is “typical” in that the longitudinal void is the only comprising void-structure, whereas in other circumstances (particularly over larger distances) several longitudinal voids may be connected during the drift (white lined arrows) and a transverse void is left bridging them.

Each of these circumferential drifters demonstrates one or both of the following structural patterns. A) One or several longitudinal vessel voids are positioned along their central line, an area marked sometimes by an uncommonly acute lamellar bend (possessed of seeming higher lacunar accumulation) that “opens” towards the next canal. (B) Other times they contain voids similar to Volkman’s canals in appearance, except that they
exhibit their own associated lamellae, do not deviate from the osteon’s midline and so do not penetrate a reversal sheath, and often do not connect to longitudinal voids within the space provided by the plane of section.

The three dimensional structure of these osteons is unknown, but their voids are at least 20-50 µm thick and they have associated lamellar deposition on either side of well-over 100 µm. Therefore, their length completely rejects concerns regarding plane of sectioning artifacts (a 1 mm long, >150 µm thick remodeling event cannot transpire within a depth of 50-70 µm no matter how you slice it). They also in no way resemble longitudinally sectioned osteons, whose void space is continuous rather than broken and perforated who do not typically have two ends closing with no cutting cone. In addition, the chance that the plane of section would just happen to luckily slice them down their longitudinal axis in large numbers (sometimes 10 within a field of view) is extremely low. Longitudinal sections made during this study demonstrated clearly different perspective on typical osteonal morphology not seen in the circumferential osteon (Figure 37).
Figure 33: Single longitudinal section of the ELP (proximal is top left, distal is bottom right). To the left side of the section images (intercortical regions), the appearance of the tissue is highly disorganized. This is what Enlow (1962a) referred to as coarse cancellous bone resulting from the infilling and haphazard remodeling of tissues that were once trabecular in this region earlier in development. In addition, osteons are hard to discern due to the simultaneous viewing of variable thicknesses of their sections. Note the ELP is easily visible to the right (medullary surface) and continues for the entire length of the section with no
sign of diminishment. Also of interest is a particularly strange formative process (left image, midway down) on the medullary surface. This node is highly suspicious as an indicator of a slow growing, proliferative pathological process of some kind and not at all like typical endosteal deposition. Combined image is ~2 cm long.

Also of interest regarding drifted osteons is their distribution. In general, drifters associated with peri-cortical region outside the ELP, for example can have some of the “longest” transectional axes. As the bone is drifting away some osteons seem to drift to keep up, sometimes a whole lens of them. This phenomenon also affects large (1-2 mm diameter) vessels or the nutrient canal as well (see Figure 28) One of their surfaces slips into resorptive phase while the lagging edge deposits tissue, effectively burying the void within the bone as the bone drifts away. The distribution of circumferential drifters is of interest as well. Close inspection of notes and diagrams identifies their most likely position at either side of the ELP in the intercortex or associated indirectly with a position of muscular attachment (Figure 38).
Figure 38: Circularly polarized partially compensated full humeral merged micrograph from juvenile notes files. Circumferential drifters are present at locations marked by white solid arrows and wrap deep into the inner region of the posterior cortex which is periosteal in origin (lined white arrow). ELP position is in the upper left (anterio-lateral) and bears evidence of rotational drift in that its most medial and oldest deposits
(black solid arrow) suggest a more anterior drift whereas the youngest regions to the anterio-lateral region face posterio-medially (black lined arrow).

6.2.1.3 Pericortical resorption

Another aspect of periosteal histomorphology that should be addressed is the distribution resorptive structures within periosteal primary tissue - or in the case of the humeri observed here, the lack of resorptive structures. Very little evidence of large resorption bays was present in the age categories analyzed here. Most resorptive bays were not much larger than a large osteon and could easily have been filed in by a standard secondary osteon in most occasions and by a drifter in the cases where the bay was slightly larger. This seemed to hold true for the leading drift face of the cross-section as well as the lagging. In some cases, both in younger and older individuals, “irregular spaces” could be seen that had no evidence of active resorption. Instead they had 2 or more (sometimes up to five) loci of osteon-fragment-like deposition and/or a thin slip of lamellar bone around their inner perimeter.

6.2.2 Endosteal bone

6.2.2.1 Differentiating periosteal and endosteal origin tissue
Endosteal primary bone was easily differentiated from primary periosteal tissue, even when it occupied “peri-cortical” regions, or when the periosteal tissue was adjacent to medullary cavity. The discerning histomorphology can be difficult to assign only when the adjacent tissue context is completely haversianized and the larger pattern of lamellar orientation in the opposing type is roughly circumferential. Yet even in this case, differentiation can often be made with careful attention. Qualitative assessment highlights differences between the two tissues, at local and global levels (Table 3).

Table 3: Means of primary tissue differentiation

<table>
<thead>
<tr>
<th>LOCAL:</th>
<th>GLOBAL:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary osteonal presence</td>
<td>Lamellar orientation</td>
</tr>
<tr>
<td>Pseudo-lamina</td>
<td>Drift reversal line</td>
</tr>
<tr>
<td>Minute Lamellar disruption</td>
<td>Drift landscape</td>
</tr>
<tr>
<td>ELP primary Volkman's canals</td>
<td>Anatomical region</td>
</tr>
<tr>
<td>Terminal lamellae</td>
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As previously reported, primary osteons and pseudo-laminar organization is unique to periosteal deposition. Endosteal lamellae, however, are deposited in phases that more often include arrest and resorptive evidence along their interstitial margins. In particularly large endosteal depositions, such as at that often constructing thickest region of the ELP, its formation seems closer to continuous (or hides arrest well enough not to permit observation, even under high magnification), and lacks even partial evidence of
resorptive foci. This continuity is disrupted with greater frequency when the net drift direction is noted to contain a rotational, rather than simple linear, vector. Periosteal lamellae also give the impression of being less tightly associated. This is due to very small wavering seen in the lamella themselves and is augmented by their tendency to veer around common primary vascular voids. Even areas of primary periosteal bone rather distant from a primary osteon (upwards of 100 µm) can be affected by its presence, particularly when on the “downstream.” or intracortical, side of the vessel (Figure 39).

Figure 39: Primary vascular canals incorporated into periosteal deposition seem to affect lamellae positioned intracortically to them up to a depth of several 2-300 µm. This is the cause for the “wavy” appearance of primary periosteal lamella.

Likewise, endosteal tissue seems more “tightly packed.” but this is not the case. Measured lamellar thickness, the distance between one bright birefringent layer and
another is similar between the tissue types at an average of around 6 µm. This would indicate that the average individual layer visible in polarized light cross-sections is around 3 µm, which is 0.5 µm larger the actual measured average across 10 sets of 20 lamellar pairs each (the discrepancy favors the average measure since individual lamellae have quite amorphous borders). The apparent greater “density” of endosteal bone might result from an impression lent by the apparent lack of the small ripple present in periosteal tissue.

Endosteal lamellae are not affected by minute wavering but are disrupted by arrest and resorption evidence, and by primary Volkmann’s canals. These canals are referred to here as “primary” to distinguish them from typical Volkmann’s canals which have no lamellae, have a more random association in the cross-section, and form via penetrance of the osteonal reversal sheath.

Primary Volkmann’s canals that characterize the ELP (Figure 40) are not found elsewhere.
Figure 34: Dense primary endosteal lamellae, comprising nearly a fourth of the total cortical area, have failed to entrap a single longitudinal vessel from the endosteal surface. Instead, lamellae formed under endosteal vasculature, pushing it away, filling the spaces between transverse vessels (Volkmann’s canals denoted by black solid arrows). These vessels extended to maintain connectivity to the intercortical Haversian network (out of view to the left). A) As bone is laid between transverse vessels its lamellae “pillow” slightly, perhaps due to slower rates of calcification adjacent to the pulsating vessels. B) This effect can be easily seen in lamellae merely adjacent to transverse vessels, even when these vessels are not within the plane of sectioning. Micrograph taken with red quartz cross-polarized filter (hilfsobject). Scale bar indicates 200 µm. Image courtesy of I. Maggiano (Maggiano, 2011)

They appear in quite high numbers in the ELP (large ELP’s will have upwards of 10-20 of them in the transection, despite resting in only 70 µm of tissue. In fact, when observed in highly haversianized regions, remnants of these structures nearly always occur in the current or previous position of the ELP (hinting that the ELP is largely obscured by remodeling). ELP canals are much larger than typical Volkmann’s canals that connect
longitudinal osteonal systems, on the order of 2-5 times wider and many times longer (some of these canals run nearly the entire cortical thickness on the ELP side of the cross-section). They are the primary source for remodeling activity within endosteal tissue, serving as the for many first generation osteons at a time period where the rest of the bone tissue is less likely with increasing age to have remaining first generation osteon. Primary Volkmann’s lamellar stratigraphy indicates that canals originate at the endosteal surface, extended by active bone deposition under the endosteal membrane between them. As the deposition advances, so too must the canal elongate in the direction of drift. This is because the advancing endosteal bone layers push the vessel’s insertion origin at the endosteum further from its branching penetrants, “locked” deep in the intercortex. Primary Volkmann’s canals have a regular distribution and introduce a gentle pillowing in endosteal lamellae. This results from changes in bone apposition due in some way to vessel adjacency. Affected lamellae bend into the canal to parallel its inner walls, leaving no reversal line despite the sometimes near-perpendicularity of these layers as evidenced by alternate colors (warm versus cool) in the quartz compensated polarized light. The effect gives the impression that associated lamellae “belong” to the Volkmann’s canal when in actuality they were formed by the endosteum (Figure 41).
The lamellae themselves also hold clues that help to identify endosteal tissue. Endosteal deposition was nearly always mature lamellar bone. In one occurrence, individual xc-252a-LH, woven bone was seen inside the entire ELP endosteal surface. In this case, the lamellae marking the ELP’s most recent layer were completely unreturbed, however, a seam, 200-300 µm thick rested against it as a sharp margin. No evidence of fracture or hairline fracture in the region was evident.

In all other cases endosteal phase deposition pattern itself varied uniquely from periosteal. Since endosteal layers are apposed on the bone’s medullary interior, they are the only layers capable of intersecting their formative surface (see Figures 32 and 38). This happenstance is normally part of the ELP structure (either end can abruptly
terminate at the medullary margin). In order for periosteal lamellae to abut their formative surface they would have to somehow be deposited with in a concavity facing the outside of the bone. Although this is quite possible near muscular attachments or more complex topography near epiphyses or in flat bones, it is not common at the diaphysis which is, by definition columnar or cylindrical.

Layers of bone in endosteal tissue are often not in agreement with the circumference suggested by nearby periosteal lamellae or with the external bone’s circumference at all. In most cases, in fact, the ELP lamellae dive away from the curvature of periosteal tissue quite abruptly. Though they can also attack the medullary margin at a near-right angle, even then their hemi-circumferential trend agrees with the previous contour of the old medullary cavity (see Figures 32 and 38).

In addition, the total tissue distribution of the cross-section and the predictable position of the ELP aid in its location and differentiation. Particularly, circumferential drifters occupy the region outside the ELP’s terminal ends and the more scarcely remodeled side of the pericortex denoting the leading drift edge of the diaphysis. The most difficult region of PEM differentiation was the lagging edge of the ELP.

In the youngest individuals, discernment was not necessary. Endosteal deposition accounted for the entire cortex on that side on a number of occasions. In slightly older individuals and on rare occasion, a drift reversal line could be seen – sometimes even followed for many millimeters around the lag side of the diaphysis (Figure 42).
Figure 36: Low magnification image of the lagging drift cortex. The ELP is positioned on the left of the image, and the white bracket indicates the extent of periosteal deposition. The reversal line under this phase of periosteal growth is evidenced by partially resorbed osteons (A), and resorptive activity targeting the region (B). The reversal line runs nearly the entire lagging half of the bones intercortex, expanding as it gains distance from the associated ELP. Solid white arrows indicate endosteal bone lamellar orientation and lined white arrows indicate periosteal lamellar orientation. Note that near B, orientations of the two bone types are more easily distinguished than near A.
This reversal line separates endosteal and periosteal deposits with no mediation in some areas and is potentially the most interesting feature of modeling drift. It could not have been found in a study using fewer individuals or in one addressing only adult structure.

The circumstance permitting its visibility is apparently fleeting, yet its stratigraphy is unmistakable. The drift reversal line is generated by early childhood growth that constructs the majority of the lag side cortex. This phase is accompanied by surface resorption at that edge of the bone, presumably by cells under the periosteum itself. At some point this process reverses and periosteal tissue is added in this region. Sometimes this thin slip is all the periosteal tissue on that cortex even in peri-adolescents or early adults. The feature records the prior resorption in a whole series of intracortical osteons cross cut by a perfect line of resorption congruent with what is now the periosteal surface. Primary periosteal is bone built on top of this line of damaged osteons in wide formative events (general circumferential growth). Few samples still have a drift reversal line that is visible over enough distance to confirm it undoubtedly within the age ranges examined in the current study. It is important to admit that this is not truly a feature of “endosteal bone.” since both the resorption and formation that cause it are controlled by the periosteum, however, discussing it anywhere but here would seem out of context since it is literally a feature distinguishing the two tissue types even when they have, at that location, their most similar orientation and curvature. Just a few osteons here is enough to make discernment of the boarder more of a chore in individuals that still have
primary bone on the periosteal margin of the lagging drift side. Other occasions have no obscuring osteons dividing the two PEM bone deposits, which meet instead, gently and subtlety as potentially the longest reversal, or cement line possible, in the element.

6.2.2.2 Endocortical primary microstructure

Differentiating ELP from endosteal circumferential lamellae (ECL) was made arbitrarily due to the often unclear boundary between the ELP as a drift remnant and the ECL. The ECL has no radially oriented primary vasculature, its deposit is typically small (100-400 µm thick). It can be separated into waves, particularly along irregularly contoured medullary margins and rarely is formed by more than two consecutive formation phases. This endosteal tissue also sometimes abuts cut Haversian systems in a reversal line that can be quite long, indicating that previously the entire local surface was in resorptive retreat and is now completely in deposition/arrest. The location for this particular feature was interestingly opposite the ELP. This is also the location where, on occasion a smaller “second” ELP was visible. In a handful of cases this minor ELP was nearly as large as the major or was even connected by a bridge of endosteal deposition only barely thicker than the ECL. In some cases, enough specificity in lamellar orientation exists to propose that resorption, had transpired faster than formative processes could maintain, such that upon termination of major drift processes, too much bone had been resorbed. Touching up the endocortical surface is then necessary, in
addition, on occasion to lamellar compaction of any large resorptive bays present in the leading drift cortex. On occasion this “touch-up” is significant enough to leave evidence similar to that seen in the ELP, on other occasions it represents as a thin slip of ECL. Later ages remove the ECL completely.

Another pattern in phase deposition seen in the endocortical microstructure is phase staggering. Pristine ELPs with few osteons invading the region leave enough undisturbed lamellar tissue for visible differences in formation phase structure between the terminal ends of the ELP and its thicker central region. The oldest deposition seems to have been most intracortical and progresses nearly continuously but with regular rhythm on occasion introduces activation phases of formation on either side as it generates concavity there. This leads to staggering of phases in arrest or even partial resorption on the sides, flanking the most central ELP’s more evenly paced phases. Unfortunately phase structure is nearly always not easily countable because arrest on this bone surface is extremely subtle without major simultaneous switches in the direction or morphology of the subsequent formation phase.

The final and perhaps most interesting qualitative result of the histomorphology of the ELP was its frequent curvilinear drift. This rotational aspect to the humeral ELP could be readily observed in some individuals as having affected the distribution of all tissues through ought the cortex, equating to over 90º of internally twisted cortex (Figure 43).
Figure 37: Rotational drift in a juvenile left humerus (posterior is toward the bottom). The ELP is rotating in the anterio-medial to posterio-medial fashion that is common among many of these samples, but in this individual is uncommonly strong (white arrow). All other tissue types agree with this summary of rotational drift. The periosteal tissue (black arrows) contributes differing degrees of rotation depending on the location and regions where osteonal obscurment are low, contain the least information on rotational drift due to the dominance of linear trends on the leading drift surface (posterior). The total ovality of
Haversian systems aligns with agreement to the primary lamellar orientation in both PEM tissues (gray arrows).

More commonly, especially in adults, this rotation was more slight and visible as the medial induction of medullary expansion while the anterior endosteal surface phases are in deposition/arrest and are stacked, step-wise around the anterior to medial aspects of the medullary margin. This rotation was nearly always in lateral to medial twist, clockwise in the left element and counter-clockwise in the right.

6.2.2.3 Endocortical secondary structure

Endosteal secondary osteonal remodeling was clearly age dependent. Younger ELPs for example had clear intracortical borders and were in some cases completely clear of osteons whereas the periosteal tissue just adjacent to the ELP, especially in the regions outside each of its flanks, contained prolific osteons and fragments. The flanks of the ELP were interesting for another reason too: secondary bone there had the greatest likelihood to have a background constructed of the circumferential drifters, as discussed previously. Drifters were not common, however within the ELP itself, except for occurrences where the ELP constructed the entire cortex on one side (in these cases some of the biggest drifters were seen). First generation osteons within the ELP spring from the radially oriented Volkmann’s canals that are the only voids in the thick endosteal
deposit. In general, osteon density increased toward the ELPs intracortical boarder. Observations suggested the depth of first generation secondary osteons in periosteal bone is shallower than in the ELP, and that they are less numerous in the endosteal tissue. This is true despite that region between the ELP and primary periosteal tissue seems to be even more osteon prolific than some similar regions on other cortical regions. Finally in the oldest individuals included in the study, the ELP and endosteal tissue in general was smaller, even likely to be missing – including any intracortical remnants of the Volkmann’s canals.

6.2.2.4 Endocortical resorption

The reason older individuals were more likely to have smaller or no ELPs present is that the surface of their medullary cavity displayed ample evidence of dramatic resorption. The bone had been removed by two major means. One was through surface resorption at the medullary cavities margin. The other was through “trabecularization” of the compact tissue through proliferation and expansion of resorptive bays to sizes 10-50x larger than any osteon.

The beginning of these processes could be seen in much younger individuals from the adult sample in regions to either side of the ELP, but particularly on the side of the ELP aligned with the anterior axis. In these individuals it was common to find a large (1-3 mm) “resorption bay” in this area (Figure 44).
Figure 38: Note the void space at the anterior inner cortex (top). In some individuals this feature is smooth and round, similar to a large vessel void, in others it is irregular and more similar to a resorption bay.

Sometimes its shape was smooth and circular, hinting that it could house a larger vessel, in other cases it was more irregular. In some individuals this void was highly osteogenic, meaning its bone-side surface was as active in endosteal bone formation as the medullary cavity was on that side of the bone, augmenting the ELP tissue in two large formative
events that would have had to have formed over many years. This characteristic (typically single) large void at the anterior aspect of the midshaft also seemed to be “leading” the curvilinear aspect of drift in individuals with significant endocortical rotation. In other cases, (typically older individuals where the bone surfaces were often in deposition/arrest phases) this void-space slip of ECL bone around its internal surface – or no lamellar bone whatsoever and evidence of active medullary expansion through resorption in the form of Howships lacunae.

Resorption via trabecularization was clearly a separate phenomenon, more common starting at the posterior aspect of the humeral medullary cavity younger individuals (including young adults). This would be the lagging drift side of the endosteal surface. In older individuals, resorption that could be called trabecularization seemed to target the ELP first. However, in the oldest individuals, other areas became involved in endocortical resorption. The degree of resorption was not equal in all regions. In fact, across all ages endosteal resorption in the form of intra cortical voids and trabecularization was much more common in the AL-PM, or A-P axes than elsewhere (Figure 45).
Figure 39: Left humerus of an older adult, demonstrating the typical pattern and locations of trabecularization of compact tissue. Caution in interpretation is necessary though in that medullary surfaces with no trabeculae have actually undergone more resorption than the trabecularized axis here, anterio-lateral to posterio-medial.

Cortical thickness was relatively uniform in most axes in the oldest individuals, and two different types of resorptive bay destruction were noted: 1) proliferation of smaller bays within the average thickness of the cortex, and 2) the remnants of vast swaths of prior cortex now fully trabecularized by extremely large “resorptive bays” (which at this time could be referred to as trabecular voids). In the latter case the trabecular tissue is identifiable as thin spans and arches or sheets, oriented inside the average cortical thickness and typically marked by thin slips or even completely reconstituted by primary modeling deposition of the endosteum or internal
compartmental BLC layer (in some cases arguably by hemi-osteonal deposition although for reasons discussed in Section 3.2.1.4 the distinction is unclear).

6.3 Phases Two through Four: Histomorphometric Statistical Results

6.3.1 Phase two results: Point-count and hand-drawn data comparisons

Comparisons of point-count and hand-drawn angles (N = 61) found that, in general, the two methods return similarly patterned angular distributions (Figure 46).

Figure 40: Relatively tight agreement between hand-drawn and point-count methods in indicating drift angle.
This generality was developed in more detail using a profile analysis (Figure 47), because the graphical representation is still useful for interpretation despite the non-normality of the data.

![Profile analysis chart](image)

Figure 41: Profile analysis of hand-drawn and point-count methods of ELP area and angle assessment

It is worth noting, that the profile analysis test of between –subjects effects indicates that there is not a significant effect of the measurement tech on these reported results ($p = 0.563$). This indicates that both techniques report similar results according to the profile analysis. Other testable interpretations from profile analysis are not applicable.
here due to either 1) the presence of only 2 variables, or 2) the fact that the variables are not trials or any other type of tied data changes.

To further examine potential similarities between these methods of area assessment, a Wilcoxon test was performed, supporting that, indeed, EAp and EAh were not significantly different (p = 0.000). For more information on trends in each of these area measures across subgroups and ages refer to total cross-sectional tables that are in the following sections. Similarly, Moore’s non-parametric test for paired data on circular scales of measurement shows that mean EpAng and EhAng are not statistically different (R’ = 0.230932, which is well above the lowest confidence limit of R’ = 1.007).

The next step was to test whether this relationship was improved by the use of an angular weight factor that considers both periosteal and endosteal tissue hit frequencies. Combined primary bone weight vector, PRpAng was compared to EhAng and was also found to be statistically similar at the same confidence level (R’ = 0.2612193). The two weighted vectors, however, have completely different variances in that EpAng is nearly 2x more tightly constrained than EhAng (Figure 48).
In addition, the previously mentioned issue with weak vector significance was explored further. In order to compare the representative strength of the PRpAng vector to that provided by EpAng, Rayleigh’s test for circular uniformity was applied. The Rayleigh’s z-statistic showed that both tests generated potentially weak z-statistics for their vectors, but that EpAng was roughly 20x more likely to generate vectors that pass Rayleigh’s test due to their lack of circularity in distribution. For more discussion on Rayleigh’s test and weighted measures of angle from point-count data see Section 7.3.

6.3.2 Phase three results: Comparing drift and $I_{max}$
In this phase, the axis of drift, EhAng, was compared to that of $I_{\text{max}}$, or $I_{\text{max}}$ (Figure 49). EhAng was used rather than EpAng or PApang due to the latter inability to be visually cross-checked on the composite image to verify summarization of drift direction (and the inability to generate statistically significant vectors from frequency weighted regions.

The drift axis, represented by EhAng had higher variance than $I_{\text{max}}$, which was tightly constrained around 150-155° in nearly all humeri in the pooled sample, regardless of pooled sample age. In addition, the average value of EhAng or was lower than that of

Figure 42: ELP location and $I_{\text{max}}$ compared across pooled sample age
\(I_{\text{max}}\), indicating that drift direction was not only more variable, but was slightly more laterally oriented, particularly in younger samples. That EhAng and \(I_{\text{max}}\) are statistically different (\(R' = 2.142\), confidence interval critical value of 1.007 indicates reject null hypothesis) in their distributions was verified by a pair-wise nonparametric test on the circular means (Zar, 2010). However, three individuals here represent potential outliers that may have affected the data. They are represented by the highest peaks and lowest troughs and occur due to difficulties associated with conditional comparisons of a unimodal distribution (EpAng) with a bimodal distribution of \(I_{\text{max}}\) since each \(I_{\text{max}}\) can be described by two angular comparisons to EpAng.

6.2.3 Phase four results: Comparing ELP presence, area, and position among regions and subpopulations

6.3.3.1 ELP presence

In total, 76 individuals were examined during this portion of the project (all but 18 were represented by left humeri), 28 males and 31 females (Table 4).
Fifteen individuals in the study did not display an ELP that was easily observable, leading to a total prevalence of ELPs in the humeral sample of 80%. Of those individuals with no evidence of an ELP, all 15 were from age category 3 and were of known or estimated ages above 49 on average (only 4 individuals missing ELPs were below 50). The Xcambó sample contained 59 individuals (16 males and 26 females), whereas Xoclan contained 17 individuals (12 males and 5 females). Males and females were equally likely to demonstrate visible ELPs. The left humerus was more likely to be missing an ELP than the right humerus, though this is only true in the pooled sample due to the fact that all right humeri come from Xcambó and nearly all are from the juvenile sample, which never lacked ELPs.

6.3.3.2 Pooled comparisons of area and position

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6.3.3.2.1 Pooled sample cross-sectional and regional area results

In the pooled sample (Table 5), mean cortical area itself was 137 mm$^2$ and the ELP mean area (EAh) was traced 10.2 mm$^2$, standardized by tCA at 0.1021.

| Table 5: Descriptive Statistics ELP size and Endosteal, Periosteal and Secondary Bone Hits (both samples pooled) |
|---------------------------------------------------|---------------------------------------------------|
| **All individuals** | **Individuals with ELP** |
| | N | Minimum | Maximum | Mean | Std. Dev. | N | Minimum | Maximum | Mean | Std. Dev. |
| **Cortical Area**$^1$ | 76 | 38.78 | 224.47 | 132.2872 | 53.8252 | 61 | 38.78 | 224.47 | 136.7885 | 53.4597 |
| **EAh**$^1$ | 61 | .94 | 22.54 | 10.2178 | 6.1389 | 61 | .94 | 22.54 | 10.2178 | 6.1389 |
| **EAh** standardized | 61 | .01 | .49 | .1021 | .01069 | 61 | .01 | .49 | .1021 | .01069 |
| tEAp hits | 76 | 3 | 300 | 113.26 | 79.5776 | 61 | 21 | 300 | 136.82 | 70.8737 |
| **tEAp**$^4$ | 76 | .00 | .52 | .1248 | .1238 | 61 | .02 | .52 | .1508 | .1250 |
| tPAp hits | 76 | 6.00 | 344.00 | 99.5658 | 72.9885 | 61 | 14.00 | 344.00 | 114.2623 | 73.4082 |
| **tPAp**$^4$ | 76 | .01 | .31 | .0986 | .0700 | 61 | .02 | .31 | .1114 | .0711 |
| tHAp hits | 76 | 80.00 | 1303.00 | 732.2500 | 281.3444 | 61 | 80.00 | 1303.00 | 732.9672 | 299.3104 |
| **tHAp**$^4$ | 76 | .19 | .90 | .4534 | .1570 | 61 | .19 | .85 | .6818 | .1604 |
| tOPD$^2$ | 76.00 | 4.49 | 36.1953 | 19.002992 | 7.365536 | 61 | 4.485 | 28.5513 | 17.034601 | 6.40446 |

1 in mm$^2$, 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone

This value, ~10% of tCA compares to the total endosteal point-count value of 137 mm$^2$, standardized by tSA at .1508, or 15% tSA. Individuals with ELPs have 3% more primary endosteal tissue than those without, but only 1% more primary periosteal tissue; the roughly 3% less tCA in those lacking ELPs results from the lack of this endosteal tissue. Those lacking ELPs have 3% higher OPD, and 3% more tHAp.

In the pooled analysis of regional periosteal tissues (as measured by rPAp) (Table 6), periosteal tissue is much higher in the P ROI at 26% of the total periosteal bone,
followed by PM at 14%, than it is in the rest of the cross-section, particularly the lowest rPAp regions: A, AL, and L, where it only achieves a mean of around 7%.

Table 6: Periosteal bone hits per ROI all individuals pooled (n=76)

<table>
<thead>
<tr>
<th>ROI</th>
<th>Minimu</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>81.00</td>
<td>3.20</td>
<td>9.92</td>
<td>0.00</td>
<td>0.49</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>AL</td>
<td>0</td>
<td>66.00</td>
<td>3.50</td>
<td>9.56</td>
<td>0.00</td>
<td>0.48</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>L</td>
<td>0</td>
<td>25.00</td>
<td>4.43</td>
<td>5.20</td>
<td>0.00</td>
<td>0.23</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>PL</td>
<td>0</td>
<td>76.00</td>
<td>10.36</td>
<td>12.47</td>
<td>0.00</td>
<td>0.68</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>120.00</td>
<td>33.26</td>
<td>26.98</td>
<td>0.00</td>
<td>0.77</td>
<td>0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>PM</td>
<td>0</td>
<td>116.00</td>
<td>18.49</td>
<td>22.41</td>
<td>0.00</td>
<td>0.64</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>71.00</td>
<td>12.84</td>
<td>14.76</td>
<td>0.00</td>
<td>0.81</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>AM</td>
<td>0</td>
<td>83.00</td>
<td>13.49</td>
<td>15.20</td>
<td>0.00</td>
<td>0.79</td>
<td>0.12</td>
<td>0.16</td>
</tr>
</tbody>
</table>

1 in mm², 2 by cortical area, 3 by Number of Hits per ROI, 4 by Number of Hits in the whole bone; red background = high values, orange background = low values

In stark contrast, pooled analysis of regional endosteal tissues (as measured by rEAp) (Table 7), endosteal tissue is much higher in the AL ROI at 24% of the total endosteal bone, followed by L at 21%, than it is in the rest of the cross-section, particularly the lowest rEAp regions; P, PM, and M, where it only achieves a mean of around 7%. These results are most easily visualized by a radar graph (Figure 50).
Table 7: Endosteal bone hits per ROI all individuals pooled (n=76)

<table>
<thead>
<tr>
<th>ROI</th>
<th>rEAp hits</th>
<th>rEAp³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>56.00</td>
</tr>
<tr>
<td>AL</td>
<td>0</td>
<td>104.00</td>
</tr>
<tr>
<td>L</td>
<td>0</td>
<td>76.00</td>
</tr>
<tr>
<td>PL</td>
<td>0</td>
<td>67.00</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>59.00</td>
</tr>
<tr>
<td>PM</td>
<td>0</td>
<td>49.00</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>56.00</td>
</tr>
<tr>
<td>AM</td>
<td>0</td>
<td>50.00</td>
</tr>
</tbody>
</table>

¹ in mm², ² by cortical area, ³ by Number of Hits per ROI, 4 by Number of Hits in the whole bone; red background = high values, orange background = low values

Figure 43: Radar graph of standardized periosteal and endosteal bone hits per ROI (all individuals pooled, n=76)
In the pooled analysis of regional secondary tissues (as measured by rHAp) (Table 8), secondary tissue is slightly higher in the M ROI at 73% in that region compared to an average of 70% in the rest of the cross-section. The M ROI is even higher still, in comparison with the lowest rHAp regions: P and AL, where it only achieves a mean of around 63%.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>191.00</td>
<td>114.46</td>
<td>41.86</td>
<td>0.00</td>
<td>0.92</td>
<td>0.72</td>
<td>0.14</td>
</tr>
<tr>
<td>AL</td>
<td>0</td>
<td>191.00</td>
<td>94.66</td>
<td>40.90</td>
<td>0.00</td>
<td>0.91</td>
<td>0.65</td>
<td>0.21</td>
</tr>
<tr>
<td>L</td>
<td>10</td>
<td>153.00</td>
<td>81.53</td>
<td>34.03</td>
<td>0.13</td>
<td>0.96</td>
<td>0.69</td>
<td>0.20</td>
</tr>
<tr>
<td>PL</td>
<td>0</td>
<td>161.00</td>
<td>84.93</td>
<td>34.00</td>
<td>0.00</td>
<td>0.94</td>
<td>0.72</td>
<td>0.19</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>162.00</td>
<td>77.79</td>
<td>37.33</td>
<td>0.00</td>
<td>0.90</td>
<td>0.59</td>
<td>0.20</td>
</tr>
<tr>
<td>PM</td>
<td>13</td>
<td>201.00</td>
<td>95.50</td>
<td>42.14</td>
<td>0.23</td>
<td>0.97</td>
<td>0.68</td>
<td>0.18</td>
</tr>
<tr>
<td>M</td>
<td>6</td>
<td>176.00</td>
<td>93.96</td>
<td>40.47</td>
<td>0.07</td>
<td>0.95</td>
<td>0.73</td>
<td>0.20</td>
</tr>
<tr>
<td>AM</td>
<td>6</td>
<td>158.00</td>
<td>89.42</td>
<td>36.69</td>
<td>0.06</td>
<td>0.97</td>
<td>0.71</td>
<td>0.19</td>
</tr>
</tbody>
</table>

1in mm², 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values

In comparison, pooled analysis of OPD (Table 9) indicates a differing pattern where the AM ROI is highest at 21, and closely similar at an Anterior zone defined by A, AL, AM, L, and M which average at 20.34. For comparison a smaller posterior zone defined by PL, P, and PM demonstrate much lower values (15.19 at P, and averaged at 16.77 across that zone).
These results are more easily compared by including a secondary variable that increases if rHA is low and OPD is high, indicating more relative remodeling per unit area: as an index, OPD/HA. The distribution of the secondary tissue index is meaningfully different from either other measure of secondary tissue in that AL demonstrates the highest index value at 31.72 against a mean of 27.78, with a minimum index value of 24.45 at the PL ROI. Visualization of these results is facilitated by a radar graph (Figure 5).
6.3.3.2.2 Pooled population sample, comparing males and females

There were no statistically significant differences (Mann-Whitney, p < 0.05) in the sexes when both sites were pooled (Table 10). See below for a comparison of sexes within each population sample separately, at both cross-sectional and regional levels. Basic trends seen there are also reflected here in the pooled data, despite statistical insignificance here. Namely, males had higher tCA and higher standardized values for tHAp and tOPD, as well as tOPD, whereas females had higher values for primary tissues.
6.3.3.2.3 Pooled sexes, comparison of population samples

There were no statistically significant differences (Mann-Whitney, p < 0.05) in the pooled sexes analysis of Xcambó adults compared to those in Xoclan (Table 11). However, the hand-drawn assessment of the ELP in this case seems to be detecting a slight, if insignificant, decrease in the modern sample’s endosteal area compared to the archaeological sample.
Table 11: Descriptive statistics, ELP size and Endosteal, Periosteal and Secondary Bone Hits (both sexes pooled), split by age-matched (age category 3) population sample for comparison

<table>
<thead>
<tr>
<th></th>
<th>Xcambo</th>
<th></th>
<th>Xoclan</th>
<th></th>
<th>Mann-Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>Std.Dev.</td>
</tr>
<tr>
<td>Cortical Area</td>
<td>34.00</td>
<td>39.64</td>
<td>224.47</td>
<td>135.2909</td>
<td>50.63</td>
</tr>
<tr>
<td></td>
<td>23.00</td>
<td>1.16</td>
<td>22.54</td>
<td>9.5796</td>
<td>6.42</td>
</tr>
<tr>
<td>EAH</td>
<td>23.00</td>
<td>0.01</td>
<td>0.31</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>EAH standardized</td>
<td>34.00</td>
<td>3.00</td>
<td>240.00</td>
<td>84.41</td>
<td>67.64</td>
</tr>
<tr>
<td>EAH standardized</td>
<td>34.00</td>
<td>0.00</td>
<td>0.20</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>tEAp hits</td>
<td>34.00</td>
<td>6.00</td>
<td>218.00</td>
<td>78.4412</td>
<td>58.17</td>
</tr>
<tr>
<td>tPAp hits</td>
<td>34.00</td>
<td>0.01</td>
<td>0.19</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>tHAp hits</td>
<td>34.00</td>
<td>54.79</td>
<td>1303.00</td>
<td>823.8824</td>
<td>184.30</td>
</tr>
<tr>
<td>tOPD</td>
<td>34.00</td>
<td>0.59</td>
<td>0.86</td>
<td>0.75</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>34.00</td>
<td>9.68</td>
<td>36.20</td>
<td>22.8280</td>
<td>5.93</td>
</tr>
</tbody>
</table>

1 in mm2, 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

6.3.3.3 Sexual dimorphism in Xcambó

Within Xcambó Males had larger tCA and bigger ELPs, as well as more rHAp and rOPD – but less tPAp than females. These measures, however, were not significant, indicating that there was little effect of sexual dimorphism on any measure of primary or secondary bone distribution within the total cross-section (Table 12).
Table 12: Xcambó descriptive statistics, ELP size, OPD and endosteal, periosteal, and secondary bone hits, split by estimated sex for comparison

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Mann-Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Cortical Area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>63.53</td>
<td>224.47</td>
</tr>
<tr>
<td>EAh</td>
<td>14</td>
<td>1.16</td>
<td>22.54</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>.01</td>
<td>.31</td>
</tr>
<tr>
<td>EAh standardized</td>
<td>14</td>
<td>.01</td>
<td>.31</td>
</tr>
<tr>
<td>fewer hits</td>
<td>16</td>
<td>3</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>.00</td>
<td>.16</td>
</tr>
<tr>
<td>OPD</td>
<td>16</td>
<td>20.00</td>
<td>218.00</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>.02</td>
<td>.18</td>
</tr>
<tr>
<td>OPD hits</td>
<td>16</td>
<td>627.00</td>
<td>1303.00</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>.61</td>
<td>.84</td>
</tr>
<tr>
<td>OPD standardized</td>
<td>16</td>
<td>.61</td>
<td>.84</td>
</tr>
</tbody>
</table>
| 1 in mm², 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Regionally, females seemed to have a stronger AL, L drift trend than males who demonstrated more of an A-P trend (Table 13). That trend in difference was statistically significant at the AM ROI where males had an average of 9% and females 4% endosteal hits out of the total. Variance in regional distributions between the two sexes are high (standard deviation equals mean value) but are roughly equal to one another. The M and PM aspects had the lowest recorded areas overall. A radar graph aids visualization (Figure 52).
Table 13: Endosteal bone hits per ROI, Xcambó males compared to females

<table>
<thead>
<tr>
<th>ROI</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev</th>
<th>P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>0.24</td>
<td>0.09</td>
<td>0.07</td>
<td>0.28</td>
<td>0.07</td>
<td>0.08</td>
<td>0.238</td>
<td>0.238</td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>0.01</td>
<td>0.40</td>
<td>0.14</td>
<td>0.12</td>
<td>0.66</td>
<td>0.20</td>
<td>0.17</td>
<td>0.300</td>
<td>0.213</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.00</td>
<td>0.27</td>
<td>0.10</td>
<td>0.08</td>
<td>0.53</td>
<td>0.17</td>
<td>0.16</td>
<td>0.745</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.00</td>
<td>0.38</td>
<td>0.07</td>
<td>0.10</td>
<td>0.23</td>
<td>0.06</td>
<td>0.06</td>
<td>0.237</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.00</td>
<td>0.50</td>
<td>0.07</td>
<td>0.12</td>
<td>0.06</td>
<td>0.02</td>
<td>0.02</td>
<td>0.305</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>0.00</td>
<td>0.15</td>
<td>0.04</td>
<td>0.04</td>
<td>0.11</td>
<td>0.02</td>
<td>0.03</td>
<td>0.305</td>
<td>0.03</td>
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</tr>
<tr>
<td>M</td>
<td>0.00</td>
<td>0.16</td>
<td>0.06</td>
<td>0.05</td>
<td>0.45</td>
<td>0.05</td>
<td>0.09</td>
<td>0.237</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td>AM*</td>
<td>0.00</td>
<td>0.26</td>
<td>0.09</td>
<td>0.06</td>
<td>0.11</td>
<td>0.04</td>
<td>0.03</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 in mm², 2 by cortical area, 3 by Number of Hits per ROI, 4 by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Figure 45: Radar graph comparing endosteal bone hits per ROI, between Xcambó males and females

Periosteal tissue distribution in Xcambó was oriented strongly toward the posterior in both males and females (Table 14). Between the sexes, despite the general occurrence of
higher rPAp values in females (with a peak of 6% difference at the P ROI), there was no significant difference (Mann-Whitney, p < 0.05). Trends in periosteal tissue between Xcambó males and females are most easily observed in a radar graph (Figure 53).

### Table 14: Periosteal bone hits per ROI, Xcambó males compared to females

<table>
<thead>
<tr>
<th>ROI</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Mann-Whitney P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>0.49</td>
<td>0.04</td>
<td>0.12</td>
<td>0.00</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
<td>0.763</td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>0.00</td>
<td>0.48</td>
<td>0.06</td>
<td>0.13</td>
<td>0.00</td>
<td>0.08</td>
<td>0.02</td>
<td>0.02</td>
<td>0.625</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.00</td>
<td>0.18</td>
<td>0.05</td>
<td>0.05</td>
<td>0.00</td>
<td>0.23</td>
<td>0.04</td>
<td>0.05</td>
<td>0.273</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.00</td>
<td>0.22</td>
<td>0.06</td>
<td>0.06</td>
<td>0.00</td>
<td>0.24</td>
<td>0.08</td>
<td>0.06</td>
<td>0.392</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.00</td>
<td>0.54</td>
<td>0.18</td>
<td>0.14</td>
<td>0.01</td>
<td>0.62</td>
<td>0.24</td>
<td>0.16</td>
<td>0.244</td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>0.00</td>
<td>0.42</td>
<td>0.10</td>
<td>0.11</td>
<td>0.00</td>
<td>0.44</td>
<td>0.12</td>
<td>0.13</td>
<td>0.876</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.00</td>
<td>0.30</td>
<td>0.07</td>
<td>0.09</td>
<td>0.00</td>
<td>0.26</td>
<td>0.08</td>
<td>0.07</td>
<td>0.476</td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>0.00</td>
<td>0.24</td>
<td>0.08</td>
<td>0.07</td>
<td>0.00</td>
<td>0.37</td>
<td>0.10</td>
<td>0.10</td>
<td>0.613</td>
<td></td>
</tr>
</tbody>
</table>

1 in mm², ²by cortical area, ³by Number of Hits per ROI, ⁴by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05
The difference between Xcambó men and women were even less in the case of secondary tissue. For example, tHAp in males was nearly exactly the same in value, distribution and variance between males and females, aside from a slightly higher standard deviation in females. In both sexes the M aspect of the cross-section contained the peak secondary area, 80%, and the P, the lowest at around 65.5% in both sexes. A similar pattern was seen in rOPD (AM 24 in both sexes, P, 17.5%). None of these differences were statistically significant (Mann-Whitney, p < 0.05). This data can be easily visualized in the summary provided by the rOPD/rHAp index’ radar graph (Figure 54).
6.3.3.4 Sexual dimorphism in Xoclan

In comparison, the Xoclan sample demonstrated a significant age effect (Table 15) in all tissues measured except for EAh (Mann-Whitney, p < 0.05). Despite significantly higher tEAp in females who had 5% more endosteal tissue than males, a similar trend in EAh (2% higher in females) was not significant. Females in Xoclan also had 3% more tPA. Correspondingly, females had significantly lower amounts of tHAp, 8% lower, and insignificantly lower tOPD despite being 16.6% lower than in males (due to much higher variance in tOPD). Considering totals of primary versus secondary tissues, Xoclan males had 8% less primary tissue and 8% more secondary tissue, but had 16.6% higher average tOPD.
From a regional perspective, Xoclan endosteal tissue was distributed differently in males and females, in that females had more endosteal tissue than males in all regions except PL and P where values were equally low in both sexes (Table 16). Most notably, females had significantly higher rEAp in their A and AM ROIs than males (8% and 14% more, respectively) (Mann-Whitney, p < 0.05). The M ROI also was higher in females, by 5%, but due to variation this difference did not achieve significance. The P and PM aspects had the lowest recorded area. A radar graph assists visualization (Figure 55).
Table 16: Endosteal bone hits per ROI, Xoclan males compared to females

<table>
<thead>
<tr>
<th>ROI</th>
<th>Males (n=12)</th>
<th>Females (n=5)</th>
<th>Mann-Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rEAp³</td>
<td>rEAp³</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
</tr>
<tr>
<td>A*</td>
<td>0.00</td>
<td>0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>AL</td>
<td>0.00</td>
<td>0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>L</td>
<td>0.00</td>
<td>0.35</td>
<td>0.08</td>
</tr>
<tr>
<td>PL</td>
<td>0.00</td>
<td>0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>P</td>
<td>0.00</td>
<td>0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>PM</td>
<td>0.00</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>M</td>
<td>0.00</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>AM*</td>
<td>0.00</td>
<td>0.15</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 in mm², ²by cortical area, ³by Number of Hits per ROI, ⁴by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Figure 48: Radar graph comparing endosteal bone hits per ROI, between Xoclan males and females

Periosteal tissue distribution between the sexes at Xoclan (Table 17) was very similar in comparison to the endosteal tissue. In general, female periosteal area was...
slightly higher than males in most ROIs, even approaching (though not satisfying) significance at the P region with 14% more rPAp in females than males there (Mann-Whitney, p < 0.05). The radar graph still shows the expected trend for more primary periosteal tissue in females (Figure56).

Table 17: Periosteal bone hits per ROI, Xoclan males compared to females

<table>
<thead>
<tr>
<th>ROI</th>
<th>Males (n=12)</th>
<th>Females (n=5)</th>
<th>Mann-Whitney</th>
<th>p Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>Std.Dev.</td>
<td>Minimum</td>
</tr>
<tr>
<td>A</td>
<td>0.00</td>
<td>0.22</td>
<td>0.03</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>AL</td>
<td>0.00</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>L</td>
<td>0.00</td>
<td>0.06</td>
<td>0.03</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>PL</td>
<td>0.00</td>
<td>0.21</td>
<td>0.08</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td>0.01</td>
<td>0.45</td>
<td>0.18</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>PM</td>
<td>0.00</td>
<td>0.27</td>
<td>0.12</td>
<td>0.08</td>
<td>0.00</td>
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<tr>
<td>M</td>
<td>0.00</td>
<td>0.14</td>
<td>0.04</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>AM</td>
<td>0.00</td>
<td>0.13</td>
<td>0.05</td>
<td>0.05</td>
<td>0.00</td>
</tr>
</tbody>
</table>

1 in mm², ²by cortical area, ³by Number of Hits per ROI, ⁴by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05
In both sexes the P, PM, M zone had lower remodeling as measured by rHAp, rOPD (Table 18), and the rOPD/rHAp index (FIG). Values for secondary tissue, as measured by rHAp were higher in Xoclan males in all occasions except for PL and L where the values were more similar between the sexes. These relationships passed statistical significance at the AL, M, and AM ROIs, where the difference in rHAp was 9%, 13%, and 16%, respectively (Mann-Whitney, p < 0.05). Again the P aspect had the lowest recorded area.
Table 18: Secondary bone hits per ROI, Xoclan males compared to females

<table>
<thead>
<tr>
<th>ROI</th>
<th>Males Minimum</th>
<th>Males Maximum</th>
<th>Males Mean</th>
<th>Males Std.Dev.</th>
<th>Females Minimum</th>
<th>Females Maximum</th>
<th>Females Mean</th>
<th>Females Std.Dev.</th>
<th>Mann-Whitney p</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.65</td>
<td>0.92</td>
<td>0.80</td>
<td>0.07</td>
<td>0.60</td>
<td>0.83</td>
<td>0.71</td>
<td>0.08</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>AL*</td>
<td>0.67</td>
<td>0.91</td>
<td>0.79</td>
<td>0.08</td>
<td>0.61</td>
<td>0.74</td>
<td>0.69</td>
<td>0.05</td>
<td>0.035 *</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.59</td>
<td>0.94</td>
<td>0.80</td>
<td>0.10</td>
<td>0.70</td>
<td>0.85</td>
<td>0.79</td>
<td>0.06</td>
<td>0.752</td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.64</td>
<td>0.90</td>
<td>0.79</td>
<td>0.09</td>
<td>0.72</td>
<td>0.87</td>
<td>0.81</td>
<td>0.07</td>
<td>0.598</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.48</td>
<td>0.90</td>
<td>0.71</td>
<td>0.14</td>
<td>0.52</td>
<td>0.70</td>
<td>0.59</td>
<td>0.07</td>
<td>0.114</td>
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</tr>
<tr>
<td>PM</td>
<td>0.66</td>
<td>0.93</td>
<td>0.76</td>
<td>0.09</td>
<td>0.56</td>
<td>0.77</td>
<td>0.69</td>
<td>0.09</td>
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<td></td>
</tr>
<tr>
<td>M*</td>
<td>0.65</td>
<td>0.93</td>
<td>0.85</td>
<td>0.07</td>
<td>0.56</td>
<td>0.82</td>
<td>0.72</td>
<td>0.10</td>
<td>0.006 *</td>
<td></td>
</tr>
<tr>
<td>AM*</td>
<td>0.77</td>
<td>0.90</td>
<td>0.84</td>
<td>0.04</td>
<td>0.48</td>
<td>0.81</td>
<td>0.68</td>
<td>0.13</td>
<td>0.006 *</td>
<td></td>
</tr>
</tbody>
</table>

1 in mm², 2 by cortical area, 3 by Number of Hits per ROI, 4 by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

In the case of rOPD, only the A ROI had significantly higher rOPD in Xoclan males (7.25% more) than females, though the trend was consistent across all regions (Table 19), particularly also at the AM aspect which narrowly missed achieving significance at 6.42% more rOPD than in females (Mann-Whitney, p < 0.05). The OPD/HA index (Figure 57) reveals a trend toward more remodeling per unit area secondary tissue at the AL and L zone for females, whereas remodeling is most concentrated in the wider range of AM through anterior to PL zone in males.
Table 19: OPD per ROI, Xoclan males compared to females

<table>
<thead>
<tr>
<th>ROI</th>
<th>Males (n=12)</th>
<th>Females (n=5)</th>
<th>Mann-Whitney p</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>Std.Dev.</td>
</tr>
<tr>
<td>A*</td>
<td>17.79</td>
<td>39.05</td>
<td>25.87</td>
<td>5.93</td>
</tr>
<tr>
<td>AL</td>
<td>20.00</td>
<td>28.25</td>
<td>23.02</td>
<td>2.56</td>
</tr>
<tr>
<td>L</td>
<td>18.88</td>
<td>36.00</td>
<td>26.00</td>
<td>4.92</td>
</tr>
<tr>
<td>PL</td>
<td>9.64</td>
<td>27.74</td>
<td>19.34</td>
<td>5.73</td>
</tr>
<tr>
<td>P</td>
<td>8.84</td>
<td>30.41</td>
<td>17.71</td>
<td>6.17</td>
</tr>
<tr>
<td>PM</td>
<td>11.88</td>
<td>25.00</td>
<td>18.04</td>
<td>4.84</td>
</tr>
<tr>
<td>M</td>
<td>12.24</td>
<td>37.43</td>
<td>22.06</td>
<td>6.85</td>
</tr>
<tr>
<td>AM</td>
<td>17.02</td>
<td>36.85</td>
<td>25.93</td>
<td>5.98</td>
</tr>
</tbody>
</table>

1 in mm², 2 by cortical area, 3 by Number of Hits per ROI, 4 by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Figure 50: Radar graph comparing rOPD/rHAp index per ROI, between Xoclan males and females

6.3.3.5 Age categories in Xcambó

Within Xcambó, where all age categories were represented (Table 20), there was a strong, statistically significant age effect on all tissues at the total cross-sectional level.
and for nearly all variables within each region at the local level. Age was negatively correlated with EAh and tEAp, with averages at around 30% of the youngest age category reducing to roughly 12% by the second, and 7% by the third. Values for tEAp were higher on average than those for EAh, but particularly so in the two juvenile age categories. Values for tAPp, however were actually highest in the second age category, where the first and third categories were both roughly 5% lower on average. Changes in tHAp were accomplished during younger ages, starting at 38% in the first age category and ending at 76% in the third; whereas tOPD changes were more spread over age categories, doubling between each group.

Table 20: Xcambó descriptive statistics, ELP size and endosteal, periosteal and secondary bone hits across all age categories: 1) 4.5-12; 2) 12-20; 3) 20-65

<table>
<thead>
<tr>
<th>Agegroup 1</th>
<th>Agegroup 2</th>
<th>Agegroup 3</th>
<th>Kruskal-Wallis P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Area</td>
<td>38.78</td>
<td>81.34</td>
<td>56.2450</td>
<td>16.9747</td>
</tr>
<tr>
<td>tEAp hits</td>
<td>108</td>
<td>300</td>
<td>61.4094</td>
<td>220.25</td>
</tr>
<tr>
<td>tAPp hits</td>
<td>14.00</td>
<td>217.00</td>
<td>102.0000</td>
<td>64.7653</td>
</tr>
<tr>
<td>tHAp hits</td>
<td>80.00</td>
<td>389.00</td>
<td>242.8333</td>
<td>88.3432</td>
</tr>
<tr>
<td>tOPD</td>
<td>4.49</td>
<td>12.48</td>
<td>7.815348</td>
<td>2.330619</td>
</tr>
</tbody>
</table>

1 in mm²; 2 by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Representation of the point-count data against all pooled age information eases interpretation (Figure 58). Across ages rEAp values decline sharply until roughly age 10,
where they begin to decline more gently and even flatten out. In comparison, rPAp values increase dramatically within the first 16 or so years of life, but also decline thereafter, gently approaching a flat distribution in adult years. Values for tHAp remain low or even decrease during periods of initial periosteal deposition and substantial endosteal tissue presence, but increase sharply from roughly age 8 to age 28 before flattening. Variation in bone type areas across ages seemed to decrease. In comparison, tOPD (Figure 59) was seemingly more variable in later years. Values for tOPD, similar to those for tHAp remain low before age 8 or so, but increase for a longer period of time, until around age 37. Increases in secondary tissue as measured by both variables seems to be linear during its increasing phase despite variability.
Figure 58: Each major bone type: endosteal, periosteal, and secondary bone (rEAp, rPAp, and rHAp) across all ages pooled

Figure 59: Total cross-sectional standardized osteon population density (tOPD) across all ages pooled
Significant differences in all area variables existed at each region across age categories – except for rAPp at the L ROI (TABLE). In addition, regions were significantly different from one another within each age grouping for all variables (bottom row in following tables, Kruskal-Wallis, p < 0.05).

In general, rEAp values decreased with age (Table 21). Endosteal tissue was concentrated dependably across all ages in the AL and L ROIs. Only the youngest age category demonstrated considerable rEAp in some M, PM, and AM ROIs, rendering this age category the most variable in rEAP values and positioning, despite its strong AL and L bias. This data is recognizably similar in its distribution across ROIs regardless of the age, as is seen easily in the radar graph (FIG). In addition, the most dramatic change in endosteal tissue area between the youngest and oldest categories took place in the AL, L, PL zone which averaged at 37.5; over 1/3rd more difference in area was contained within this zone than across the rest of the cross-section (AL, L, and PL demonstrate higher “rates” of change over ages if the data is viewed from a false chronological perspective). A radar graph aids visualization (Figure 60)
Table 21: Endosteal bone hits per ROI, Xcambó age categories compared

<table>
<thead>
<tr>
<th>ROI</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>0.00</td>
<td>0.92</td>
<td>0.31</td>
<td>0.24</td>
<td>0.05</td>
<td>0.28</td>
<td>0.14</td>
<td>0.08</td>
<td>0.00</td>
<td>0.24</td>
<td>0.07</td>
<td>0.07</td>
<td>0.000</td>
<td>*</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AL*</td>
<td>0.29</td>
<td>0.97</td>
<td>0.32</td>
<td>0.21</td>
<td>0.04</td>
<td>0.64</td>
<td>0.38</td>
<td>0.21</td>
<td>0.00</td>
<td>0.66</td>
<td>0.17</td>
<td>0.16</td>
<td>0.000</td>
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</tr>
<tr>
<td>L*</td>
<td>0.28</td>
<td>0.81</td>
<td>0.57</td>
<td>0.20</td>
<td>0.06</td>
<td>0.41</td>
<td>0.28</td>
<td>0.13</td>
<td>0.00</td>
<td>0.53</td>
<td>0.14</td>
<td>0.15</td>
<td>0.000</td>
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</tr>
<tr>
<td>PL*</td>
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<td>0.82</td>
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<td>0.20</td>
<td>0.03</td>
<td>0.38</td>
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<td>0.12</td>
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<td>0.23</td>
<td>0.05</td>
<td>0.06</td>
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<tr>
<td>P*</td>
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<td>0.00</td>
<td>0.50</td>
<td>0.09</td>
<td>0.18</td>
<td>0.00</td>
<td>0.16</td>
<td>0.03</td>
<td>0.03</td>
<td>0.000</td>
<td>*</td>
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<tr>
<td>PM*</td>
<td>0.00</td>
<td>0.67</td>
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<td>0.23</td>
<td>0.00</td>
<td>0.15</td>
<td>0.04</td>
<td>0.05</td>
<td>0.00</td>
<td>0.11</td>
<td>0.02</td>
<td>0.03</td>
<td>0.003</td>
<td>*</td>
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<td></td>
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<tr>
<td>M*</td>
<td>0.04</td>
<td>0.64</td>
<td>0.27</td>
<td>0.22</td>
<td>0.03</td>
<td>0.08</td>
<td>0.04</td>
<td>0.02</td>
<td>0.00</td>
<td>0.13</td>
<td>0.04</td>
<td>0.04</td>
<td>0.000</td>
<td>*</td>
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<tr>
<td>AM*</td>
<td>0.00</td>
<td>0.70</td>
<td>0.25</td>
<td>0.24</td>
<td>0.04</td>
<td>0.09</td>
<td>0.07</td>
<td>0.02</td>
<td>0.00</td>
<td>0.26</td>
<td>0.06</td>
<td>0.06</td>
<td>0.003</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kruskal-Wallis: there is a significant difference between the ROIs

1 in mm2, 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Figure 51: Radar graph comparing endosteal bone hits per ROI, among Xcambó age categories

Periosteal tissue also saw the most distributed deposition across regions within the first age group which included more of a medial component than the other two age groups (Table 22). Its general distribution across all age categories, however, was strongly posterior, accounting for significant differences in regional rPAp distributions within each age group (Kruskal-Wallis, p < 0.05). Across categories of age, significant
differences existed in rPAp in all regions except for L ad PM, where trends were
maintained despite lack of significance. The difference between regionally distributed
rPAp in the youngest and oldest categories was far larger at the zone defined by PM
through L, but not including P, each of these 3 regions contained an average of 22% of
the total difference, compared to the rest of the cross section which contained an average
of 1% of that difference (PM, PL, and L demonstrate higher “rates” of change over ages
if the data is viewed from a false chronological perspective). A pattern also existed
across the zone, A and AM, in that these ROIs were the only two to have significantly
more periosteal tissue in the youngest age category, decreasing into adulthood; the rest of
the cross-section (the anterior zone and lateral zone) demonstrated significantly higher
rPAp values in the second age category than they did in the younger or older category
(Figure 61).

Table 22: Periosteal bone hits per ROI, Xcambó age categories compared

<table>
<thead>
<tr>
<th>ROI</th>
<th>Age Groups 1 (n=12)</th>
<th>Age Group 2 (n=7)</th>
<th>Age Groups 3 (n=34)</th>
<th>Kruskal-Wallis p Value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rPAp^3</td>
<td>Min</td>
<td>Max</td>
<td>Mean</td>
<td>Std.Dev.</td>
</tr>
<tr>
<td>A*</td>
<td>0.00</td>
<td>0.17</td>
<td>0.02</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>AL*</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>L</td>
<td>0.00</td>
<td>0.21</td>
<td>0.04</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>PL*</td>
<td>0.00</td>
<td>0.68</td>
<td>0.11</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>P*</td>
<td>0.00</td>
<td>0.77</td>
<td>0.32</td>
<td>0.27</td>
<td>0.00</td>
</tr>
<tr>
<td>PM</td>
<td>0.00</td>
<td>0.61</td>
<td>0.13</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>M*</td>
<td>0.03</td>
<td>0.81</td>
<td>0.32</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>AM*</td>
<td>0.03</td>
<td>0.79</td>
<td>0.31</td>
<td>0.27</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Kruskal-Wallis: there is a significant difference between the ROIs.

1 in mm^2, 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05
Secondary bone area (Table 23) clearly increased across all age categories for all regions of interest, achieving high statistical significance in all cases (Kruskal-Wallis, p < 0.05).

This pattern of increasing rHAp was most different between youngest and oldest individuals at the M ROI, though in comparison to primary tissues within Xcambó age group comparisons, the total differences across age groups were far more similar (each region saw relatively similar “rates” of change over ages if the data is viewed from a false chronological perspective). Within each age group, cross-sections were also significantly different from one another in their distribution of rHAp (Kruskal-Wallis, p < 0.05). For example the A ROI yielded nearly double the rHAp than posterior in the first two age categories, while the difference between these two regios had largely disappeared in the oldest age category.
The distribution of OPD was highly similar to that of rHAp (Table 24) Within each age group, cross-sections were also significantly different from one another in their distribution of rHAp (Kruskal-Wallis, p < 0.05) with more anterior regions yielding higher rOPD values than posterior regions (particularly P, itself). Across age categories every region also significantly increased in rOPD. This difference was greatest between youngest and oldest categories at the M ROI and lowest at the PM and A. However, analysis of the rOPD/rHAp index (Figure 62) clearly shows a trend towards a remodeling focus in the AL quarter of the cross-section across the age groups.
Table 24: rOPD, regional osteon pulaitoin per ROI, Xcambó age categories compared

<table>
<thead>
<tr>
<th>ROI</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std.Dev.</th>
<th>P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>0.00</td>
<td>19.61</td>
<td>10.67</td>
<td>4.91</td>
<td>9.40</td>
<td>19.33</td>
<td>15.19</td>
<td>3.26</td>
<td>10.44</td>
<td>35.56</td>
<td>21.25</td>
<td>5.67</td>
<td>0.000 *</td>
<td></td>
</tr>
<tr>
<td>AL*</td>
<td>0.00</td>
<td>15.50</td>
<td>6.33</td>
<td>4.43</td>
<td>5.13</td>
<td>18.54</td>
<td>14.37</td>
<td>9.38</td>
<td>7.40</td>
<td>63.85</td>
<td>25.44</td>
<td>10.08</td>
<td>0.000 *</td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>2.67</td>
<td>19.24</td>
<td>7.21</td>
<td>4.62</td>
<td>10.59</td>
<td>15.55</td>
<td>14.66</td>
<td>2.64</td>
<td>10.44</td>
<td>50.71</td>
<td>24.26</td>
<td>7.95</td>
<td>0.000 *</td>
<td></td>
</tr>
<tr>
<td>PL*</td>
<td>0.00</td>
<td>14.47</td>
<td>5.99</td>
<td>4.47</td>
<td>6.46</td>
<td>16.43</td>
<td>11.12</td>
<td>3.24</td>
<td>10.49</td>
<td>40.82</td>
<td>21.35</td>
<td>7.20</td>
<td>0.000 *</td>
<td></td>
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<tr>
<td>P*</td>
<td>1.50</td>
<td>10.48</td>
<td>5.59</td>
<td>3.19</td>
<td>6.05</td>
<td>9.90</td>
<td>8.41</td>
<td>3.11</td>
<td>5.31</td>
<td>37.87</td>
<td>18.66</td>
<td>7.14</td>
<td>0.000 *</td>
<td></td>
</tr>
<tr>
<td>PM*</td>
<td>4.46</td>
<td>16.04</td>
<td>10.31</td>
<td>3.56</td>
<td>7.70</td>
<td>16.85</td>
<td>12.04</td>
<td>3.62</td>
<td>5.94</td>
<td>40.23</td>
<td>20.88</td>
<td>6.99</td>
<td>0.000 *</td>
<td></td>
</tr>
<tr>
<td>M*</td>
<td>2.00</td>
<td>16.02</td>
<td>7.22</td>
<td>3.87</td>
<td>10.99</td>
<td>15.53</td>
<td>12.74</td>
<td>1.58</td>
<td>10.24</td>
<td>47.00</td>
<td>23.34</td>
<td>7.84</td>
<td>0.000 *</td>
<td></td>
</tr>
<tr>
<td>AM*</td>
<td>1.12</td>
<td>16.50</td>
<td>7.09</td>
<td>3.95</td>
<td>9.69</td>
<td>17.08</td>
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<td>44.55</td>
<td>25.44</td>
<td>6.72</td>
<td>0.000 *</td>
<td></td>
</tr>
</tbody>
</table>

Kruskal-Wallis: there is a significant difference between the ROIs

1 in mm², 2by cortical area, 3by Number of Hits per ROI, 4by Number of Hits in the whole bone; red background = high values, orange background = low values; * Denotes Significance at P < 0.05

Figure 53: Radar graph comparing rOPD/rHAp index per ROI, among Xcambó age categories

6.3.3.5 Age categories in Xoclan

Since Xoclan only had one individual outside age category three, Section 6.3.3.4 also includes all meaningful data on this age category and regional treatment is not necessary.
6.3.3.6 Comparisons of drift summaries among subpopulations

Section 6.3.2 addressed the similarity between the axis of drift suggested by the ELP and $I_{\text{max}}$ axis. It is important to remember that ELP position in general was found to be between the antero-lateral and anterior ROIs. This corresponds to roughly 150-160° from the posterior aspect which would indicate a net linear drift of roughly +180° with respect to ELP position or posterio-medially. An ELP angle closer to 180° means the trend is more anteriorly oriented and closer to 90° means the trend is more lateral. Here the variation in drift direction is measured and compared between the sexes and among age categories from each population sample (Table 25).

Table 25: Variation in drift direction and $I_{\text{max}}$ as measured by EpAng and EhAng

<table>
<thead>
<tr>
<th></th>
<th>Pooled</th>
<th>Xcambo</th>
<th>Xoclan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=14)</td>
<td>Females (n=18)</td>
<td>Test Sex Diff.</td>
</tr>
<tr>
<td>EpAng</td>
<td>141.9</td>
<td>156.0</td>
<td>127.9</td>
</tr>
<tr>
<td>EhAng</td>
<td>139.9</td>
<td>159.6</td>
<td>127.1</td>
</tr>
<tr>
<td>ImAx</td>
<td>156.5</td>
<td>161.2</td>
<td>155.6</td>
</tr>
</tbody>
</table>

Wheeler-Whatson Test Critical Values: 5.991

Blue background = sex, purple = age groups; red = high values, orange = low values; * Denote Significance at P < 0.05, when n > 17 on $x^2$ distribution, and when n < 17

In the pooled data on ELP indicated drift, EpAng and EhAng means were similar at around 140° but were both more lateral in position than the more anterior alignment of $I_{\text{max}}$ which averaged 157°. When the sexes were split for each population sample, there was a significant difference between males and females, particularly at Xcambó, in that...
males (averaging 158° between the two methods) had a more anterior trend to their position than females (127°), whose ELP position was more lateral (Wheeler-Whatson, p < 0.05). This was true for both EpAng and EhAng methods. Sexual dimorphism in ELP position was greater than that of Imax. Interestingly, these trends were completely absent from the data from Xoclan. In Xoclan males and females were more similar in their average ELP position and they both seemed even more anteriorly positioned than Imax, demonstrating more variance and a mean around 169°.

As for comparisons between the two population samples (all age matched within age category 3), Xoclan individuals demonstrated ELP positions indicating drift along more of an anterio-posterior trend than did those from Xcambó. This trend though apparent by a comparison of means was not statistically significant (Wheeler-Whatson, p < 0.05). Within Xcambó and across ages, the position of the ELP was significantly different (multi-sample Wheeler-Whatson, p < 0.05) in EpAng which was more anterior in the middle age category than it was in either those younger or, older. This trend was also picked up by EpHang but was not significant. Another factor contributing to age variation in Xcambó was that with increasing age category, the average angle (as measured by both methods) was more anterior and at its third category maximum was around 138.5°. This anterior trend with age is also statistically significant in Imax.
6.4 Summary

Histomorphological patterns in primary tissue indicated structural differences between lamellar bone deposited by periosteal and endosteal membranes. Although the thickness of lamellae were similar between the two tissue types, periosteal tissue contained longitudinal primary vasculature entombed in seams that are reminiscent of laminar bone in other animals, but less regular and less clearly defined. The result, however, is that deeper (older) tissue is more likely to contain first generation secondary osteons emanating from these primary voids. These regions often contain osteons with highly irregular perimeters that are likely to drift at least slightly (but sometimes over impressive distances – particularly on the lagging side of the drifting cortex). On either side of the ELP, deeper within the cortex this periosteal origin tissue showed circumferentially drifting osteons in younger ages but was also the area containing the first second and third generation osteons, indicating that these regions are likely to hit the asymptote for osteon population density earlier in development than in other regions of the bone due to their older tissue age.

Likewise, endosteal primary tissue contained unique bone structural patterns involving deposition around radially oriented primary Volkmann’s canals in phases of mixed resorption and formation to either side of the ELP and continuous deposition within the axis of drift. Phases of deposition here were often stepwise oriented in a fashion that generated a rotational aspect to drift which was always from lateral towards anterior (medial rotational drift). The total effect is shape change in the cross-sectional
perimeter and net linear posterio-medial drift in the humeral mid-shaft that transpires simultaneous to overall diametric growth, accompanied especially later in adolescence or young adulthood by expansion of the medullary cavity that continues in late adulthood, thinning the cortex.

The two quantitative techniques applied to drifting tissues in the study both showed statistically similar patterns in data and corroborated histomorphological descriptions of drift (with significantly more endosteal tissue in the anterio-lateral and lateral regions, but more periosteal tissue in the posterior and posterio-medial regions). Endosteal lamellar pockets had an 80% prevalence in the pooled sample and nearly all individuals lacking an ELP were above 50 years of actual or estimated age. The average ELP made up 10% of the total cortical area and was much larger in individuals that were in the pre-adolescent age category. Although $I_{\text{max}}$ was in the same general region as the ELP, there was a statistically significant difference in their positions, caused by a generally more lateral position for the ELP than the $I_{\text{max}}$ at the time of death.

There was not a significant difference in between the archaeological and modern sample in the distribution of tissues by area, however, ELPs in the archaeological sample had a non-statistically significant tendency to occupy a more lateral position than those in the modern sample. Females had significantly more primary tissue in general, especially endosteal tissue, but had less secondary tissue and lower osteon population density. Sexual dimorphism between primary and secondary tissue, however, did not co-occur in
the same regions. Xcambó females also had more lateral ELPs than males, although this trend was not significant in the Xoclan sample.
CHAPTER 7: DISCUSSION

7.1 Introduction

Nearly all investigations of human skeletal biology in some way or another measure or draw inference from bone size, shape, and position. Efforts to quantify how these relative characteristics are formed have been limited. In experimental and clinical work, the scope of inquiry is focused on methods that require non-invasive procedures and on pathology and treatment. For human research, they are often left with indirect measures of internal bone tissues (µCT, densiometry, radiography) and typically don’t focus on developmental or populational perspectives due to a lack of attention paid to younger individuals (sampling bias) (see Garn, 1970 as an important exception). In bioarchaeology, a different limitation is encountered. The story told by bone remains becomes all the richer when life processes can be drawn from their study, but the connection between osteological techniques and the physiological processes that underlie our observations is challenged by our post-mortem perspective on the lives of those we study.

In these circumstance there is a growing need to provide new perspectives on how boney features form; to uncover hidden variation in developmental process; to find its
remnants, visible even in the adult remains; to deconstruct the view of a bone as “a thing” and instead differentiate activities of comprising tissues constructing the element; and to promote the view that considering different tissue-ages of formation could be a great benefit to histological research. With each effort to accomplish these goals, bone science steps closer to understanding how our skeletons inform our health and record important aspects of our life-ways, and bioarchaeology gains greater understanding of patterned variation informing the connection between bone and behavior.

Human bone growth lends itself easily to analysis via non-invasive techniques and so has been studied readily. But modeling drift, the pattern of PEM tissue activity during growth and adaptation has been largely uninvestigated in humans since pioneering efforts of a select few (e.g., Foote, 1916; Amprino and Bairati, 1936; Enlow, 1962a; Frost, 1973). Within recent years, a small resurgence of interest has been seen in tissue level activities of bone, using techniques that do not require experimental bone labeling and that are therefore more applicable to bioarchaeological applications. Some of these focus on non-humans and pay particular attention to remodeling processes due to their continued activity in the adult (Paine and Godfrey, 1997, Skedros et al., 2004), others, target modeling (Skedros, 2001; McFarlin et al., 2008). Still fewer have to push forward, to investigating human long bones from cadavers (Goldman et al., 2003, 2005, 2009) and from archaeological and cemetery sources (Wanner et al 2007, C. Maggiano, 2011; Maggiano et al., 2008, 2009b, 2011; I. Maggiano et al., 2008, 2011a,b).
Prior research has shown, the cross-sectional distribution of tissues is highly variable in bone (Goldman et al., 2003, 2005), but some of these same authors also aim at finding patterns in that variation (Goldman et al., 2009). Many such efforts arbitrarily separate the cross-section into thirds (outer, middle, and inner) that could introduce overestimations of variability confounding comparisons of drift among individuals and subpopulations (Goldman et al., 2005; McFarlin et al. 2008). None of these studies addressed direct measures of remodeling such as OPD, instead relying on hand-drawn area measurements to address only Haversian area. In addition, typically all cortices are described as equal informers on drift (representing conflation of drift and modeling). This dilutes our ability to see patterns and disrupts any attempt to compare individuals or subgroups – a contributing factor to the fact that no previous comparative measurements of modeling drift have been made in humans.

The current investigation of humeral primary tissue compliments this body of research, addressing the issue of tissue complexity in long bones from the perspective of both point-count and hand-drawn analyses and comparing these, quantitatively, to important measures of cross-sectional geometrics. The problem of complexity and hidden variation is removed by the consideration of the ELP as an indicator of net linear drift. Rather than attempting to measure all cortices simultaneously, the ELP is used as a summary for all bone modeling processes contributing to drift. It does not describe the important cross-sectional geometry of the diaphysis, however. This is why the current investigation considers both histomorphometric drift and cross-sectional geometrics. By
combining these analyses, a true tissue-level perspective can be achieved, explaining variation rather than noting it and permitting its measurement and comparison among individuals. As such, research implications are significant, within both experimental and bioarchaeological efforts to understand and apply bone biology.

7.2 Phase one: Qualitative histomorphometric drift assessment

7.2.1 Periosteal bone

Histomorphological observation of the periosteal tissues in this study yielded several important results. Of primary interest to this investigation was differentiating the activity at periosteal versus endosteal cortices. In general, the total area deposited under the periosteum was highly variable. On occasion only a thin margin of deposition was present while another side of the bone contained only primary periosteal tissue. Though the same was true for endosteal tissue to a degree, the circumstance was much more complex in periosteal tissue. This is actually to be expected since the periosteum has mixed demands on its activity: 1) local limitations of formation (muscle bellies and insertions or vessel pulsatile disruptions), 2) the general need to add or remove tissue in response to both mechanical demands of both local and general muscular activity, and 3) the general demand for a net diaphyseal growth necessitating increases in periosteal deposition in nearly every direction. In contrast the endosteeum seems to simply recensters
itself in response to all these periosteal changes, as a mechanically adaptive process removing bone that is less useful on the interior than the new bone placed on the exterior, and depositing tissue accordingly so that the cavity is consistently positioned over the cross-sectional centroid. It would be interesting to test the perfection of this process as a function of time, since theoretically, if the medullary cavity responds this way with accuracy, then the centroid of the medullary cavity should be in the exact same position as the centroid of the total cross-section – the neutral torsional axis. Deviations from this theoretical occurrences could be interesting to document.

Perhaps most interestingly though was the evidence found during periosteal histomorphological observation (and very likely present in point-count data yet to be analyzed), periosteal origin tissues housed many longitudinal primary canals. The true difference between these canals and those termed “primary osteons” can be debated of course. The important thing to notice in these age categories (from 5 onwards), is that primary vascular voids have no (or very few) lamella of their own and so represent a process in which periosteal deposition is all that is necessary, rather than subsequent infilling of void-space. These findings are similar to those reported by other authors as well (Bright and Elmore 1968; Chamay 1970; Currey 2002). This is perhaps the single greatest indicator of periosteal versus endosteal tissue, other than lamellar orientation, because endosteal tissue completely lacks these longitudinal vessels. In the periosteum they are very regularly spaced and nearly every field of view has the exact same number of them, regardless of the individual’s age. Future analysis of collected point-count data
should bear this out statistically once FOVs are assigned to types based on membrane origin. These efforts should also connect this high regularity to the idea that there is a minimum distance from the vascular supply of nutrients at which bone can survive. Tests could easily be derived to measure this possibility using average distances between intercortical surfaces (from one to the other canal or the external bone surface). For now though the use of primary longitudinal canals as an indicator of periosteal origin tissue is easily recommended, especially for regions far from their membrane of origin or for areas where the circumferential origin of both tissue types is seemingly congruous (in the lagging path of net drift).

Other interesting observations important to address here are not limited strictly to periosteal lamellar remodeling but are easier to see there due to the primary vessel field supplying layers of sequential tissue ages, each with varying degrees of secondary osteonal proliferation. One possibility deserves special attention: vessel repathering. More work needs to be done, preferably on tissue with vessels in each void so that counts can confirm suspicions voiced here. That being said, significant circumstantial evidence points to the possibility that, particularly for certain tissue (or chronological) ages, the goal of BMU activation, resorption and formation seems to not include angiogenesis, or vessel branching. Instead the goal is to send the BMU down the vessel, potentially repositioning it slightly while doing so, such that a micro-fracture or other disruption to the LCS is attended to without necessitating a new vessel. It stands to reason that this must be the case when one considers that new vessels in tissues are not age independent,
or cheaply formed, or formed without the primary need being more vascularization. A bone turnover system that necessitates angiogenesis at each remodeling event should crowd the tissue with far too many vessels. Since these vessels cannot be destroyed by osteoclasts (doing so would cause hemorrhaging and pain), extra vessels encountered by resorption cones or bays, are likely simply incorporated in whatever way they can, into subsequent newer secondary structures. Images shown in the results section seem to show evidence of this very occurrence: fragments from one time osteons passing forward their vessels into new osteons that become fragments when it is there turn to be repathed. This is one important possibility that could have ramifications for age estimation techniques and affects even our basic understanding of the many modes of operation possible for bone turnover. For example future research could find that repathing is far more common with advancing age since the tissue is already well-vascularized – and this would be exactly what we should expect. Such a process could also be linked to demands for freeing minerals in the bone reservoir. Only continued testing with “wet” bone will answer these questions directly however.

The other unique observation made here with regard to secondary tissue of the periosteum, is that there seems to be a second “type” of drifter, referred to here as circumferential drifters. These osteons do not share any structural similarity to longitudinally cut osteons, run exactly within the plane of section, and continue for even millimeters of tissue constructing entire fields of view with only a handful of them. They are particularly a nightmare for counting osteons because they result in fully secondary
tissue FOVs with an OPD of 6 or so – no frags. This confusing circumstance is highly likely on either side of the ELP and seems connected particularly to cross-sections with significant evidence for large rotational aspects to their drift vector. The exact “meaning” of these circumferential drifters is unknown, but like other drifters (also common in younger tissue areas attempting to account for drift, they seem to be what bone does when turnover is necessary far from the original vessel locations, but vessel branching is not an option for some reason. Circumferential drifters are interesting because they seem capable of connecting many primary longitudinal vessel canals along the pseudo laminar seam, but a complete understanding of this phenomenon awaits future examinations.

7.2.2 Endosteal bone

Endosteal deposition, particularly in the ELP demonstrates the complexity of formation phase modeling activity. This can be seen most readily in the occasional formation of partial arrest, and partial resorptive zones in endosteal modeling formation phases. These common, but largely unreported occurrences results in inconsistent “circumferential lamellae.” due to complex modeling phase interactions. Partially interrupted phases are more difficult to interpret due to the reduced visibility of the transition between consecutive uninterrupted apposition and lamellar arrest lines (Figure 63), compared with more obvious arrest lines in osteons.
Figure 54: Endosteal lamellae demonstrating complex phase activity. The white solid arrow marks continued uninterrupted formation through the entire field of view. Conversely, the black solid arrow denotes a modeling arrest line. Though subtle in appearance, the effect is significant, marking a pause in phase formation in the upper right region while formation continues for hundreds of micrometers (past the lower field of view boundary). Thin-ground, undecalcified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt. Micrograph taken with red quartz compensator. Scale bar indicates 100 µm.

There are also forensic or bioarchaeological implications for understanding the histomorphology of primary modeling arrest lines compared to uninterrupted lamellar apposition, particularly if it were found that they were diagnostic for certain mechanical or pathological circumstances. For example, the complex phase arrangement within the ELP could derive from local mechanical necessity, but it could also be affected by pauses in growth instigated by biological stressors such as malnutrition or disease.
Variation in lamellar orientation holds valuable stratigraphic information regarding growth and adaptation processes or stimuli. For example, if the newest lamellae (adjacent to the bone surface) are concentric with a membrane of origin, that membrane was either A) currently forming lamellar bone at the time of death or extraction, or B) it had paused after formation with little or no subsequent membrane activity. Alternately, lamellar phases can be cross-cut by those more newly formed. If a PEM phase is currently resorbing, lamellae can bear tell-tale evidence of osteoclastic activity (such as Howship’s lacunae) and can appear cross-cut at some oblique angle. Microscopic evidence of this process can persist long after its occurrence, especially when subsequent formation phases entomb cross-cut lamellae, typically marked by the presence of a reversal line (Figure 64).
Figure 55: Intracortical Haversian systems adjacent to endosteal primary lamella of the endocortex. These particular lamellae are laid in several formative phases. The white arrow marks consecutive, uninterrupted formation of a large phase, while the black arrow marks a cement line cutting across previous lamellae, splitting this region into two separate phases of formation. This presents stratigraphic evidence of a single phase of formation continuing in one region while at another reversal occurs, resorbing previously placed tissue and resuming formation, thusly directing drift in a specific net direction. Subsequent phases to the bottom right of the image also bear evidence of targeted modeling resorption in the form of cross-cutting cement lines. Also note the presence of an arrest line in a secondary osteon (black lined arrow) for comparison with cement lines with resorptive evidence. Thin-ground, undecalcified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt. Micrograph taken with red quartz compensation. Scale bar indicates 100 µm.

A reversal line is a region characterized by a localized disruption of typical lamellar microstructure, sometimes preserving Howship lacunae under high magnification, and similar to the margin at the periphery of a secondary osteon or hemi-osteon (also called a cement line). Some lines are smoother, with no resorptive evidence and are called arrest lines. Presumably modeling cement lines would demonstrate some of the same interesting attributes as their remodeling cousins, including increased mineral composition, unique
mechanical properties, and biomechanical importance as described by Skedros and colleagues (2005).

Interpretation of some lamellar structures is aided by remembering that at one time they were not solid, they existed as puddles of osteoid. For example, radially oriented vessels often introduce a pillowing of primary lamellae between them. This effect, when severe and along the periosteal surface has been implicated in advanced treponemal and leperous periostitis; the structures themselves being referred to as “polsters”, meaning literally “cushions” in German (Schultz 2001). Manifesting in a much less severe fashion, it can also be seen in typical endosteal primary lamellar regions, as in FIG. It has long been well-known that static and pulsatile strains can inhibit bone formation, or even induce resorption of bone tissue (Du Boulay 1956, Feik et al., 1987). Therefore, one potential cause for transverse lamellar orientation effects near vessels is the pressure applied by the vessel, or even through direct pulsation of the vessel which could hypothetically slow calcification through osteoid perturbation. This later possibility remains intuitive but unexplored, particularly in modern histomorphometry. Further experimentation on these issues could also weigh in on other debates such as how digital and vessel impressions are left on bone surfaces and why secondary osteon infilling slows as it approaches completion. The decline in formation rate during osteon closure has been further verified experimentally (Marshall et al., 1959; Manson and Waters, 1965) and supported theoretically by Martin (2000a). For a more detailed treatment of quasi-static and pulsatile effects on bone formation and resorption see
Carpenter and Carter (2008). For a summary of other hypotheses and mathematical models explaining rate of infilling during remodeling, see Martin and coauthors (1998) and Martin (2000a), respectively.

Interpreting modeling histology in regions like this also requires conceptualizing a changing surface area of apposition on the PEM. Theoretically a given phase focus is comprised of initially activated osteoblasts on the PEM bone surface. Changes in the osteogenic signal responsible for this activation could cause the active region (and therefore the associated phase of resorption or formation) to expand or contract. This occurrence should be visible in the bone’s primary microstructure. For example, increasing surface areas of apposition should result in unique lamellar organization relative to those that are decreasing (FIG). In addition, there exists the potential for “migrating” surface areas of apposition in which lamellar sheets are apposed in a stepwise fashion, changing the foci for the phase with each new team of osteoblasts. The transverse section of a migrating formation surface could be confused for changing surface areas of apposition if the lamellar migration was strong and its direction was perpendicular to the plane of sectioning. In practical consideration, however, conditions responsible for changing the surface area of an active formation phase seem far more likely than those necessitating its migration. Changes in a phase’s surface area of apposition are likely related to changes in the formation signal and should become more or less likely depending on the context of the phase’s formation, including the scale and topography of the original area of formation. It is also important to remember that
Howship’s lacunae could provide evidence of changing resorption surfaces as well, though without accompanying formation this phenomenon is not viewable stratigraphically. Although, not a modeling event, consider the migrating resorption accomplished by a drifting osteon as described by Robling and Stout (1999) as a conceptual example of how complex localized resorption of bone can be.

To further illustrate some interesting occurrences achievable by multiple foci of PEM activity, imagine an endocortical region in which a remnant of trabecular structure separates two membrane regions where formation is necessary. Two separate formation phases could occur on either side of the interposing structure (Figure 65).

Figure 56: The black solid arrows mark two different sets of modeling foci separated by an irregular trabecular spicule (potentially a remnant from an earlier growth period). If formation continued past this interruption these two foci could merge if timed correctly or the slower of the two would abut the faster. Thin-ground, undecalified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt.
Merged cross-polarized micrograph. Comprising images taken when primary lamellae were at 45° to normal x, y stage orientation. Scale bar indicates 300 µm.

Subsequent phases, not only pack on more and more endosteal lamellae, but also ensure that intracortical remodeling could eventually both obscure the circumstance originally separating these phases, and with continued significant formation, rejoin them. In other circumstances, formations are small and numerous, with evidence for prior resorption (Figure 6). Especially at these smaller scales (< 300 µm), distinguishing between modeling events and hemi-osteons, well-described by Parfitt (2003), could prove challenging (Jee et al., 2007). In order to understand the role of the hemi-osteon, we typically focus on trabecular tissue, the investigation of endosteal modeling phases during growth could contribute to our understanding of the complex circumstances created by spongy tissue.
Figure 57: A series of endocortical modeling formation phases present as adjustments to a much larger phase (past the lower field of view boundary). The white solid arrow marks the most recent phase, whereas the white lined arrows mark overlain fragments, indicating prior resorption took place before the final phase was formed. These phases are small enough to permit confusion with hemi-osteons, although the latter are typically only reported in trabecular bone. Black solid arrow denotes medullary surface. Thin-ground, undecalcified transection of archaeological, humeral mid-diaphysis, Dakhleh Oasis, Egypt. Micrograph taken with red quartz cross-polarized filter (hilfsobject). Scale bar indicates 50 µm.

Full consideration of results here shows surface areas under formation can increase in size as they progress or decrease. When they do so, they leave lamellar stratigraphic evidence containing tell-tale structure, important for consideration in all lamellar tissue (Figure 67).
Figure 58: Two theoretically distinct lamellar phase microstructures depending on changing surface areas of apposition over time, represented schematically. A) A convex surface, viewed in transverse cross-section, is filled in. Each successive layer is larger in surface area than the last, requiring the activation of more and more previously dormant osteogenic cells from the membrane (top left). On a concave surface the same event produces a different lamellar architecture (top right). B) A convex surface, in which each successive layer’s area is, instead, smaller than the last, where fewer and fewer cells are actively forming bone (bottom left). On a concave surface the same event produces a different lamellar architecture (bottom right). These changes in surface area accompany meaningful changes in some aspect of the formation signal that initiates or directs modeling activity. The size of the formation phase or its local microtopography could also be a factor influencing surface areas of formation. The prevalence or meaning of this type of microstructural variation is unreported (Maggiano, 2011)

This simple realization becomes important in interpreting the lamellar phase structure of the ELP. It seems that the point of greatest concavity during drift of the medullary cavity would be the focal point for activation of formation instigating its drift. That phase could continue uninterrupted theoretically, throughout the years in which drift transpires. However, the results of this study suggest otherwise.
That first theoretical phase of activation seems not to reach around the entire lagging half of the medullary canal, instead it accounts only for the apex of the curve and its deposition creates other centers of activation (Figure 68).

Figure 68: A schematic representation of activation phase structure in the ELP showing that each primary phase deposition (black dot) triggers secondary flanking depositions (gray dots). Theoretically if the formation signal is strong enough at the start, then something like what is shown in Figure 62B is possible. More often, however this staggering of phases in several locations is what constructs net linear medullary drift.

These are typically on either side of the first phase where an acute concavity has been formed by the first phase. Each of these locations enters into formative process but their progression creates an acute concavity in the lagging line of drift close to the epicenter of the first phase. The process continues.

Phase structure gets even more complex during rotational drift (Figure 69). This is because it can transpire via at least two major mechanisms: 1) staggering of primary, to secondary, to tertiary activation sites around the circumference of the concavity, or 2) pivoting on itself necessitating resorption at some regions of the phase.
Figure 69: A schematic representation of activation phase structure in the ELP showing that each primary phase deposition (black dot) triggers secondary flanking depositions (gray dots). In this case, however growth or mechanical demands necessitate rotational drift which asymmetrically triggers activation around the concavity and/or initiates partial phase resorption in order to pivot the constructed tissue. Note that at the regions of the black dots, deposition is roughly consecutive.

Together, these microstructural circumstances explain why a typical volume of “circumferential” lamellae can be constructed of non-circumferential phases of apposition interacting in a complex context that reveals the history of growth and adaptation in the region. More research is necessary to differentiate these processes clearly and determine their bioarchaeological implications. For example, reversal lines could indicate meaningful changes in adaptive response, and significant arrests in phase formation could indicate the severity of a malnutrition or disease event, interrupting growth. The tendency for drift to contain a non-linear component also requires further investigation, although measuring rotation of the cross section could pose interesting challenges.
7.3 Phase two: Point-count and hand-drawn data comparisons

Results of this study demonstrate that hand-drawn and point-count techniques provide similar data regarding drift direction and total area of endosteal tissue. This is true despite that point-count area measurement did not differentiate between the thin internal circumferential “lamina” and the ELP as a meta-feature. The difference between the two endosteal measurement parameters was intentional, meant to uncover whether or not the ELP (considered separately from total endosteal tissue deposits) was necessary to form a simple summary of drift direction and magnitude (relative to remodeling rates). The data suggest that in actuality the inclusion of existing internal circumferential lamellae is insignificant statistically throughout the investigation, since both measures of drift direction and endosteal area provide similar results. The only test where advantage seemed in favor of one or the other technique and therefore parameter of endosteal drift, was in comparisons of the age trend for increasing ELP anteriority with age, where EhAng failed to detect the trend as significant (albeit by an extremely slim margin of only 0.45 on the test statistic).

That the two methods produce similar results for areas and angles of orientation could be argued to render the consideration of the ELP meta-feature as unnecessary, opting instead to treat, discuss, compare, and measure total endosteal tissue instead. This could especially be true since each technique has differing amounts of time investment.
When the investigator intends to document variation in all tissue types and desires to include consideration of OPD, porosity, or the distribution of other histological features of interest such as osteon morphotypes or drifted osteons, they could conceivably gain much more efficiency of analysis by pursuing the starburst sampling pattern via point-counting. This is especially true with the application of the custom data collection program, “µ Count Histo.” which greatly speeds the collection of multiple variables per grid hit. In comparison, high magnification (necessary to reduce subjectivity and increase accuracy) hand-drawn differentiation between all these features or tissue types would be highly prohibitive with respects to time investment.

On the other hand, when a researcher intends only to summarize drift direction as indicated by a simplified vector of endosteal deposition, the hand-drawn technique has vastly superior efficiency. Ideally, the researcher could visually scan the section, identify the ELP, disregarding endosteal tissue not clearly indicative of drift and achieve highly similar results to the above point-count efforts regarding ELP area and size, as evidenced by the current dataset comparisons.

Others might argue that regardless of either applied technique, the use of meta-feature terminology is not necessary, opting instead to refer only to the tissue type. This conclusion is not without some support according to the current study. The troubles with this continued compositional treatment rather than the use of a meta-feature for drift are worth considering before such action is advised.
The current description of bone as either extreme: homogenous or highly variable, has been a road block, preventing comparative studies seeking patterns of variation. On one side tissue level comparisons and relations to larger total cross-sectional observations are ignored in favor of a more “micro” perspective. In the other, the total complexity of activity on all cortices is invoked; conflating growth, \( I_{\text{max}} \), and net drift and dampening meaningful biological signals. This is because most efforts to understand modeling consider it generally, rather than differentiating modeling drift from other aspects of diaphyseal growth and morphology. The effect, unfortunately is the overestimation of variation, the conflation of processes, and the destruction of an ability to measure governing factors with statistical strength. Indeed, this is one reason that modeling studies in humans have been comparatively rare until now.

Remodeling studies, conversely, have benefited from terms like “osteon” despite their notorious ambiguity (“primary,” “secondary,” “mega-,” “drifted,” “coupled or decoupled resorptive bays”), not to mention their relative inaccuracy in describing histological reality: these structures are only microscopic in one axis. In three-dimensions, Haversian systems are wonderfully complex and dynamic simultaneously accounting for static, targeted, metabolic, and adaptive bone turnover while ensuring proper bone matrix vascularization.

In much the same way that it is conceptually valuable to consider only one two-dimensional segment of a Haversian system as a “thing.” it is conceptually useful to permit modeling drift to be represented by a similar “thing”: incompletely but sufficiently
descriptive of meaningful, patterned variation, offering new variables for examining ancient bone and potential improvements on investigations in skeletal biology.

In summary: when considering all tissue types and other histological features and relatively large sample sizes, point-count techniques are recommended for ELP analysis. When considering smaller sample sizes and/or a focus only on endosteal tissue distribution for the purpose of investigating net drift direction, hand-drawn techniques will be more efficient. In any cases, the theoretical and conceptual value of the ELP as a meta-feature indicating an important summary of modeling drift is confirmed during this investigation and helpful for those planned in the future.

Several other studies, interested in tissue level dynamics at the mid-shaft of long bones have developed hand-drawn techniques for comparing tissue types (McFarlin et al., 2008, Goldman et al., 2009). These authors often employ the technique, avoiding point-count methods, though the results of the current study suggest that in fact the point-count technique might be more appropriate when considering the three major tissue types. It is also suspected that point-count may provide decreased subjectivity through random sampling and increased time efficiency, as discussed previously.

The current study also raised important issues for statistical treatments of modeling drift directions in general. Namely, each calculated vector has a statistical strength by which it represents the distribution of indicating tissue. For point-count data, this statistical strength was assessed here briefly through the application of Rayleigh’s test for circular distributions. Due to variability in ELP position and due to necessity of
considering a limited number of point-count ROIs (eight in this case), significance of generated vectors was challenged. Interestingly, despite the majority of vectors being insignificant, similarity was still apparent between both ELP position detection methods. Other mathematical limitations could also be at play, disrupting Rayleigh’s tests from detecting the significance of vectors within each individual. For example, Rayleigh’s test can be bumped in significance by applying a cut-off value to each ROI, removing those with non-contributory amounts of endosteal tissue (similar to the way hand-drawn ELP variables omit regions with only slight endosteal tissue present). However doing so in a method that contains only eight ROIs is a liability causing the loss of individuals available for potential statistical investigation, since Rayleigh’s requires at least 5 angles for consideration of the vector. A cut-off value would be a particular problem for its disproportionate tendency to remove older individuals from comparison since they would be more likely to have > 3 ROIs with insignificant amounts of endosteal tissue. In addition, Rayleigh’s test requires the circular equivalent of normality (von Mises distribution) (Zar, 2010), and the development of transformations to achieve this distribution or to circumnavigate the previously mentioned limitation is beyond the scope of current investigation. Other methods might avoid the conflict completely by focusing on weighted median angles rather than means, a technique that is friendlier to statistical comparisons where variation is relatively high. These possibilities require further testing.

Also an intended area for future examination, is the relative statistical significance of ELP positions indicated by hand-drawn techniques. Theoretically, the distance of the
ELP centroid from that of the cross-section is its vector strength. Methods capable of comparing centroid vector significance with those generated from Rayleigh’s test for the point-count data are the subject of continued work comparing the two major methodologies used in the current study. It is important to remember though that even without these more advanced analytical techniques, significant and biologically meaningful patterns are evident in the current data and corroborate the results of many different types of related research. This indicates that the techniques currently employed were successfully used to address endosteal remnants of modeling drift, in both area and position.

7.4 Phase three: Comparing drift and $I_{\text{max}}$

The current work compares histologically assessed drift with the direction of maximum resistance to bending, $I_{\text{max}}$. It also measures ELP position using several different methods and compares different subpopulations statistically. For efficiency in the discussion therefore, the connection between $I_{\text{max}}$ and ELP position will be addressed along with important age, sex, and populational variation regarding the angle of drift.

During this study, tests compared hand-drawn ELP position with the axis of $I_{\text{max}}$ and found that although $I_{\text{max}}$ is much less variable across individuals, the ELP’s position is most often generally in agreement with $I_{\text{max}}$. Particularly when combining the linear
and rotational observations made on drift in this study there is room to consider that the current position of $I_{\text{max}}$ could be different from the general position of the ELP because the ELP summarizes all efforts to achieve $I_{\text{max}}$ within the visible tissue-ages represented microstructurally. That being said, statistical evaluation across all individuals indicates that there are significant and potentially important differences between indicators of drift and those used to indicate adaptive bony response, like $I_{\text{max}}$. Namely, both ELP position and $I_{\text{max}}$ were statistically significantly different across age groups at Xcambó, but variation in ELP seemed to indicate an increasing anterio-posterior tendency with age that was not as visible a trend in $I_{\text{max}}$, despite $I_{\text{max}}$’s achievement of higher confidence in this comparison. This result could very well have been due to the difference in variance between the two variables, indicating that continued investigation of the ELP’s age-effect is necessary before sound conclusions can be made regarding the comparative relationship between drift and adaptive cross-sectional geometric measures. Limitations here are largely superfluous anyway as they test only the significance of the single vector summarizing the ELP. When the total distribution of ELP tissue is viewed in any of the radar graphs presented here, it becomes abundantly clear that, in all comparisons of means at any age bracket, the AL and L ROIs were much more likely to contain large deposits of endosteal tissue than other ROIs, especially P-PM. Against that backdrop and considering the general ontological changes present in the humerus, it is highly probable that the age trend noted here is indicative of increasing muscular development of the upper body with age and particularly the difference between development of muscles
with antero-posterior affects versus medial-lateral. This creates room for future exploration of drift as a response in early ages to deltoïd dominance and in lager ages to that of biceps, triceps, and brachial muscular action.

Information gathered here also corroborate Goldman and colleagues (2009) observations in the human femur, suggesting drift is roughly in accordance with reported ranges of the \( I_{\text{max}} \) axis, and that the lagging cortex during linear drift houses significant pockets of endosteal tissue (also in accordance with other observations from Enlow in 1962a and Streeter and Stout in 2003). That particular study, however, provides no statistical verification of observations and relies on surface depositional/resorption activity in order to identify drifting cortices, rather than actual measurements of angles of drift or quantitative consideration of internal distributions of bone tissues from differing origin types.

Interestingly, the observance of highly predictable AL-L to PM-M linear drift in human humeri from Xoclan and Xcambó also corroborates basic observational statements made in McFarlin and colleagues investigation of non-human primate humeral mid-shafts (though quantitative evaluation there is limited to percent Haversian Tissue and does not address standardized endosteal distribution quantitatively). This could be evidence worth considering in discussions on whether specific modeling drifts are ontogenetically required and how much they can be steered by variable biomechanical circumstances.

Also, important to note is that there was a trend in both sexes for higher ELP angles in Xoclan, even though significance was not achieved by a slim margin (0.420 less
than the critical value of 5.99). That trend was not seen in $I_{\text{max}}$ which was much more similar between the subpopulations. Several considerations come from these observations. The first is the need for continued study on the differences between archaeological and modern drift. The results here are still preliminary but could be explained by differences in physical activity patterns between sexes: particularly the dominance of the deltoid is likely to cause an increase in the medio-lateral drift trend in males. The fact that $I_{\text{max}}$ seems less affected and has less variation across all comparisons also begs further study. $I_{\text{max}}$ trends in the femur are known to be relatively well constrained to one dominant axis (Pauwels, 1980;), but its variation has been important as an indicator of mobility (Ruff, 1987). Similar investigations could be possible using drift assessment to uncover even more patterned variation, complimenting observations of cross-sectional geometrics. The other consideration is more general, but important for continued research: the fact that age trends observed in angular data narrowly missed significance (in one method, across ages; in another, across subpopulation) indicates that for the variation seen in angles, the population sizes explored here approach the minimum that could be used for future subpopulational studies.
7.5 Phase four results: Comparing total and regional ELP presence and area among subpopulations

7.5.1 ELP presence

ELP presence in the femur at Xcambó was tested in a pilot study evaluating whether or not the meta-feature’s consistency warranted its targeted quantitative and statistical evaluation (I. Maggiano et al., 2011a). The results of the current work suggest that the ELP has a prevalence rate in the humerus of 80% in the pooled sample and corroborate various elements of that brief communication. First, there was no apparent sex difference in ELP prevalence. Second, most individuals lacking an ELP were among the oldest individuals present in the sample. Although increased age-associated endocortical resorption is the likely cause for this, it shouldn’t be forgotten that a few individuals with no ELP had died before their 4th decade. Future work will be necessary to understand this occurrence which could be linked to lifestyle, diet, or health, but is beyond the scope of the current effort. Since femoral prevalence was 84% in the femoral pilot study, it might seem the prevalence is slightly lower in the humerus, but this conclusion would be premature. The two compared studies use different levels of analysis, that, observational, this, quantitative. In addition, the current study includes several individuals above 55 years of estimated or known age, whereas the pilot study did not. Forthcoming full statistical treatment of Xcambó femora may also alter the total prevalence in the femur slightly. For all these reasons a complete treatment on ELP
prevalence in the humerus compared to the femur awaits continued analyses.

7.5.2 ELP area

In general, current results using point-count assessment of standardized mean distributions of periosteal, endosteal, secondary tissues and OPD revealed trends that were readily compared and regularly demonstrated statistically significant differences among regions and subpopulations. This has not been the experience of work on other forms of tissue level variance (Goldman et al., 2003, 2005) and is difficult to compare with point-count studies that do not address the full cross-sectional distribution of variables. The reason for detection of patterns in various tissue types using the current methods could have to do with using the sampling technique: starburst patterned point-count, rather than a comparison of cortical thirds in regions (outer, middle, and inner). Comparisons of cortical thirds are typically made because of the general observation that older tissue is in the inner aspect of the cortex and is more remodeled than the outer zones (Enlow, 1962b; Skedros et al., 2001; Currey, 2002). This is true despite that Enlow (1962a) notes that adjacent periosteal tissue and endosteal tissue could be formed at completely different time periods of development. Interestingly, however McFarlin and colleagues (2008) research in non-human long bones used a similar technique to Goldman’s but found significant trends, particularly when interpreted from the perspective of the drifting cortex.
The general statement that deeper tissue is older and more remodeled is not necessarily false. However, results of the current study indicate that for at least one of eight regions of endosteal deposition (and more likely several), that trend is reversed; a circumstance rendering the most inner margin youngest and more external margin oldest, particularly in pre-pubescent age categories or individuals with large linear modeling drifts. What’s more important is that this phenomenon is not only detectable in young individuals but persists as a trend through all ages studied here, as evidenced by the quantification of statistically significant distributions of endosteal tissue appearing in the AL and L regions of the humerus and the presence of endosteal lamellar orientation (hemicircumferential) indicating the net drift of the bone in the opposite direction.

It could be expected then that Haversian area, or OPD, or some mixed index of the two, might have detected lower total remodeling in these areas with young endosteal bone. The fact that it is the opposite is at first confusing but makes sense in light of the greater drift perspective. Regions associated with the ELP, indeed, have significantly more Haversian area and OPD than adjacent regions… even higher than any adjacent ROIs, with the least remodeling on the leading drift ROI in the younger two categories. This is potentially the confounding factor inhibiting statistical strength in some histomorphometric regional analyses that use comparisons that do not account for the age of tissues under comparison, potentially dampening what otherwise should be stronger signals for things like targeted remodeling or regional distributions of osteon morphotypes or drifters.
The current results achieve significance potentially because they compare ROIs that do not as often split up cortices; they compare based on major and minor anatomical axes. This technique permits registry of the signal for differential tissue age because the two cortices with the youngest ages and the strongest age-at-formation signal are those at the leading and lagging drift ROIs, in this case AL and PM, for example. If the current methodological construction were successful in targeting drift signals and comparing tissues of similar regional tissue age, then we would expect not only to have the highest amounts of primary bone in the drift axis (AL – PM, for example), but also to have the highest amount of remodeling in the lagging cortex. This is because general diametric growth at the periosteum entombs the oldest layers of endosteal tissue either after drift stops/slow or periosteal deposition speeds up (most conceivably during the adolescent growth spurt). Thus, the oldest layers of endosteal deposition are repositioned by drift to the middle or even external cortical 3rd, depending on the age of the individual. Here it might undergo remodeling to obscure the drift reversal line. This reversal line is also reported by McFarlin and colleagues (2008) though no mention is made of its prevalence. Regardless, remodeling, both static and targeted, should be more dense here (not necessarily due to higher activation frequency, but this is also possible) for three potential reasons (particularly around the time of adolescence): 1) it is older tissue than many other areas of the bone, 2) it is not on the inner cortex and so experiences higher loads transmitted by the outer cortex (Biewener, 1992), and/or 3) the presence of the drift reversal line could be a structural liability for microfracture propagation. Only the latter
two options have the potential to result in more osteons because of an increased rate of local activation). The leading drift side of the bone should have less evidence of remodeling than the lagging side, since it is typically entirely periosteal in origin and the inner cortex (oldest, most remodeled) is continually resorbed, both by surface resorption in younger ages and by surface resorption and trabecularization of the compact tissue in older ages. Further research is necessary to differentiate between dense regions of secondary structures that are due to tissue-age-at-formation and those that might indicate variation in activation frequencies.

This is the pattern evidenced by the current results. The regions with the highest standardized primary bone areas are in the linear drift axis, the region of oldest bone in the individual is in the lagging drift intercortex, therefore both OPD and Haversian area were higher there. Expectations for detection of linear drift were also confirmed in the cortices at either right angle to the lagging drift cortex (AM and PM for example in the humerus). These ROIs have middling distributions of primary and secondary tissue, since their inner and outer surface’s age distributions is “slid sideways” and sandwiched the oldest tissue in the middle third (lending the true typical cortical pattern where internal and external primary tissue are actually very likely to be similarly aged but the space between them is older and more remodeled. Finally, age distributions of secondary tissue confirm that the adult age category quickly equalizes remodeling on all cortices (measured by Haversian area or OPD), indicating the potential for quicker remodeling rates in newer bone ROIs, despite higher absolute remodeling in the older, endosteal
bone-rich ROIs. To even more clearly quantify the significance of these tissue-level changes, field of view based comparisons for each region in this study are forthcoming in later efforts.

The importance of considering drift is mentioned over and over, yet rarely accounted for in application. In 1982, Newell-Morris and Siriani investigated neonatal macaques and found that distribution of tissue types was dominated by dynamic changes associated with modeling drift. This same conclusion is raised in the results of McFarlain and colleagues (2008) study as well, but not supported there wholly since they invoke many other potential reasons for deviations from their expectations during their zonal thirds analysis of catarrhine femoral and humeral mid-shafts. Although particularly this latter study seemingly collected hand-drawn data regarding primary bone distributions and used this information for the interpretation of results, its focus on percent Haversian tissue distribution meant that quantifiable or statistical treatment on the complete perspective of drift were not the focus. Not until the investigations by Goldman and colleagues (2009) and Maggiano and colleagues (Wanner et al 2007, C. Maggiano, 2011; Maggiano et al., 2008, 2009b, 2011; I. Maggiano et al., 2008, 2011a,b) has the focus been fully shifted away from intracortical remodeling to primary tissue distributions and descriptions of drift. This perspective has the potential to influence many aspects of bone biology and bioarchaeology.
7.5.3 Sexual dimorphism and Drift

Ample theoretical and experimental evidence suggests some sexual differences should be seen in the distribution of primary and secondary tissue across the cross-section, including the differential effect of estrogen on boney formation and resorption (Martin 2003). Also, Garn (1970) argues from longitudinal analysis of cross-sectional widths and perimeters in radiographs that endosteal surfaces were characterized largely by resorption throughout growth except for a period of net deposition during puberty, especially in girls.

The current research partially confirms these expectations in that individuals from both sites showed evidence that females had more primary tissue, particularly endosteal primary tissue. This trend was not significant in Xcambó, but it was significant in Xoclan where marked sexual dimorphism was found. Actually, augmenting Garn’s description of cortical behavior during growth, the current study found sexual differences in standardized mean areas of all three tissue types (endosteal primary, periosteal primary, and Haversian). The pattern was clear in that females had nearly double as much endosteal tissue as males did and 1/3rd more periosteal tissue. Of course the presence of all that primary tissue meant that females had lower total Haversian area, but not as much as would be expected given this amount of primary tissue difference between the sexes. These results statistically verify an extension of Garn’s observations through analysis of the histomorpholometric distributions of tissues in the female. Interestingly a combination of results shown here and Garn’s constructs a solid argument stating that the
larger ELPs in females than males, and in young adults rather than old, is not due solely to differences in remodeling, but is actually related to the magnitude of modeling drift itself.

However, the fact that OPD was not significantly different between Xcambó males and females is especially interesting in this light. This potentially indicates that OPD is more tightly constrained than the distribution of total Haversian area. One important consideration is that Haversian area can be increased without increases in OPD, namely through the action of drifted osteons, whereas the reverse statement might be harder to support. There is room for the testable hypothesis that OPD is connected primarily to age, whereas Haversian area is connected to some parameter sensitive to sex, such as drift direction and magnitude, potentially from developmental and adaptive differences in muscle strength and activity patterns. It is also supportive of this theory that there was a sample population difference in sexual dimorphism of tissue distributions. Since both samples are relatively similar genetically (i.e., both from the Yucatán Penninsula), it could be expected that the differences are potentially due to activity patterns, particularly increased sedentary lifestyles in the modern population. For now though, interpretations of this circumstance are somewhat conjectural and will require further investigation.

Interestingly, the ROIs responsible for the higher prevalence of endosteal tissues in females were not the same as those containing the most endosteal tissue for males, accounting for a statistically significant difference in endosteal distributions between the
sexes. Female endosteal deposition was more A and AM, whereas male, was more AL and L. This area based quantification corresponds to, indeed constructs, the sex difference discussed previously in which the average ELP position is more medial (~120°) in females than males (~158°) in hand-drawn and point-count determined drift direction as well.

A close examination of the data here provides evidence for why this might be the case. Two competing reasons exist for increased relative standardized primary tissue in females: 1) there is more drift in females which turns over “turned-over” (intracortically remodeled) tissue more quickly than in males leading to higher primary counts, and/or 2) there is simply more intracortical remodeling in males. These options are in no way mutually exclusive. Evidence exists here to support at least the first option in that increased primary area in females is accompanied by decreased Haversian tissue area in some of the same regions where males experience significantly higher Haversian areas and OPD than women (even where not statistically significant, the trend is clear). As for the second option, this is much harder to verify even with the breadth of data collected in the current study. The reason for this difficulty is that two equally sized bone cross-sections where one drifts 10x further posterior-medially than the other, might look extremely similar by tissue distribution to one another. This is because unless the low-drift cross-section drifted less than the distance of half a cortex it will also demonstrate the same relative regional tissue age distribution as the 10x cortical thickness drifted section.
The most useful way to consider drift magnitude then would be to completely describe the effect of net linear drift, differentiating two potential drift-effects: 1) curvature achievement which wouldn’t necessitate a change in the circularity of the cross-section, and 2) changes in circularity to promote an $I_{\text{max}}$ that is adapted to local and/or element-based biomechanical demands. In the case of the humerus, results here indicate that only the second option is likely, since the humerus has no real diaphyseal curvature to speak of but does undergo a process of increasing ovality across ages according to Sumner and Andriacchi (1996). Drift measured in this study lends histological evidence supporting the method of achievement of AL – PM ovality, namely via net PM linear drift rather than periosteal growth in this axis. This is because results here indicate periosteal deposition is comparatively absent in AL ROIs and endosteal deposition is comparatively absent in PM, rendering achievement of $I_{\text{max}}$ via net periosteal expansion an impossibility. The interference of local mechanical demands such as muscle bellies should be considered, but the most likely cause is direct muscular interaction with the bone surface itself in the form of insertions. Evidence can be seen in all ages that the point of muscle attachment at the linea aspera of the femur, for example, produces little to no periosteal appositional lamellar tissue. Although this could be due to the insertion and disruption created by sharpies fibers and biomechanical perturbation more research needs to be done to discern the exact nature of this disruption.

Because an area is “subendosteal” in no way means the bone there was endosteal in origin (likewise for subperiosteal tissue), therefore it could be necessary to reprioritize
explanatory options for tissue distributions. In this sense, histomorphological efforts too often compare tissues of similar origins but different ages, or tissues of different origins but similar ages; these attempts assume homogeneity of bone, despite knowing for the last century (at least the last half-century) that bone is not homogeneous—in structure or tissue age at formation.

In this light, for example, the arguments that remodeling rates are higher or lower in outer or inner regions due to lower mechanostat set-points, collagen reorganization, vessel reorganization (Ascenzi and Bonucci, 1967; Boyd and Riggs, 1990; Bromage, 1992; Skedros et al., 2001; McFarlin et al., 2008); all take on an entirely new dimension since the answer is: both, depending on the drift circumstance. It is also important to consider that not always is “rate” the variable collected, too often “comparative OPD” is taken as differences in rates of remodeling without first accounting for differences in tissue age. In short, older local tissue will give the impression that remodeling transpired faster there, when this isn’t necessarily the case at all. An example of how new interesting perspectives from the current work apply to a working hypothesis could be that we might expect periosteally formed tissue on the inner cortex to have higher rates of remodeling than endosteally formed tissue. Confirming this hypothesis could employ detailed analysis of the field of view level data collected in this study which is planned for future efforts.

Many other bone studies could be impacted by an analysis and complete characterization of bone drift for each skeletal element of interest. Both Enlow (1962a)
and Frost (1973) implicated bone modeling drift in curvature achievement. For investigations into modern human variation, whether in clinical or archaeological contexts, this generality is not sufficient to address complex questions regarding health, growth, and adaptation. Without an understanding of bone modeling drift, for example, ontological development of a single curve in a long bone can be achieved in 5 differing ways (Figure 70), involving more drift at the mid-diaphysis than either distal or proximal aspect, the reverse or net lateral or medial drifts on top of these options.

Figure 59: A) Schematic of immature cortical (black) and trabecular (grey) regions destined for modeling alteration during long bone extension. B) Bone is resorbed at the metaphyseal periphery and within the medullary cavity, affecting both compact and trabecular tissue alike (white checkering). New compact tissue must also be constructed, both within trabecular voids during lamellar compaction, and at the periosteum via diametric apposition (grey bars). C) Even in the adult there remains evidence of these changes as the bone extends in length through endochondral ossification. Adapted from Enlow, 1962a.
None of those considerations rule out the 5th possibility that curvature is achieved with no net drift according to the element at large because the metaphyses drift exactly as much but opposite the drift at the mid-shaft. Variance hidden among these options could turn out to be useful for bioarchaeological inquiry, for example on physical activity or growth patterns.

7.6 Summary

Overall, drift in the human humerus seems to be similar to that reported in McFarlin and colleague’s (2008) analysis of non-human primate humeri although they do report a more medial tendency that that measured here. The general agreeance between the $I_{\text{max}}$ and the lagging drift cortex in the current study, indicates that net drift direction is one of the several components (including primary periosteal depositional asymmetry, and non-linear drift components) that account for how $I_{\text{max}}$ was achieved. Interesting variation in $I_{\text{max}}$ achievement is revealed when observing or measuring modeling processes directly, as indicated by the sexual difference in drift direction observed in the Xcambó sample.

Net linear drift is well summarized by a consideration of the position of the ELP relative to the orientation of the element itself. This is because despite the more complex demands on the periosteum (including general diametric growth, regional limitations in depositional direction due to muscle insertions, or the pressure applied to the membrane
by muscle bellies), the endosteal deposition accompanying drift results from the repositioning of the medullary cavity. In this sense, endosteal depositional direction summarizes all drift vectors and functions as a simple indicator of net linear drift, useful for bone growth and adaptational comparisons between individuals or subpopulations.

The two techniques compared in this investigation offer different strengths for different circumstances. Point-count technique, though time consuming, is facilitated by the use of a rapid data entry program and provides more well-balanced information including osteon population density and distributions of any other histomorphic features as well as simultaneous consideration of primary tissue distributions from both membranes. In comparison, however, hand-drawn analysis is rapid and provides a simple means of summarizing net linear drift in the element, even when only endosteal tissue is considered. More inclusive techniques, however, could combine results of periosteal and endosteal hand drawn regions, but it is important to consider that this would be a much more time intensive form of data collection, requiring the hand outlining of nearly all tissues across the transection.

Sexual dimorphism in growth at the endosteum and periosteum indicated here has been observed before as well, particularly as it is best documented in Garn’s (1970) longitudinal studies; the current work confirms that females engage more deposition of endosteal tissue than males. This body of knowledge is expanded here where it is also shown that in general females have more of both types of primary tissue, and less
remodeling. More investigation though is necessary to explain the differences in statistical power for this observation between the two sites examined here, however.
CHAPTER 8: CONCLUSION

The research presented here both qualified and statistically analyzed distributions of each major bone tissue type among eight regions of interest. This was done by considering bone from a dynamic tissue perspective over development and considering mechanical adaptation to physical activity. Results indicate that there are relatively undescribed, biologically meaningful, patterns of variation in modeling drift among sexes, age-groups, and archaeological and modern populations. The study also tested the employment of a mid-diaphyseal histomorphological meta-feature, the endosteal lamellar pocket (ELP), for its potential to represent certain aspects of bone modeling, a process responsible for bone size, shape, robusticity, adaptation, growth, sexual dimorphism, and orientation. Results demonstrate the ELP is an effective indicator of net drift, differentiating this aspect of growth and adaptation from diaphyseal growth at the global level or more local limitations on periosteal bone deposition. The ELP is present in a large majority (80%) of individuals from the estimated age of 5 to 65, and is easily differentiated from either secondary (Haversian) tissue or primary periosteal lamellar tissue for the purposes of discussing tissue distributions across the cross-section. Important indicators of bone adaptation to mechanical loading, as measured through
cross-sectional geometrics, were found to be comparable to histomorphological variance by using the ELP position for comparison with $I_{\text{max}}$, the axes most resistant to bending. Two different techniques were developed and successfully employed that are both suitable for measuring ELP position and area, permitting comparisons among subgroups for the first time.

In consideration of the projects original predictions and hypotheses, the following conclusions can be made:

A) Indeed, the tendency for the ELP to provide a summary of drift that is simpler than consideration of all cortices simultaneously is now clear. The endosteal tissue deposited during drift is a side-effect of the necessary tendency for the medullary cavity to re-center itself in response to the mechanical environment as it changes during growth and adaptation.

$H_A$: The alternative hypothesis should be accepted: when all tissues were used to weight a drift direction the statistical strength of generated vectors was nearly 20x less than when endosteal tissue alone was used. In addition, comparison of the distributions of angular data collected by including a summary of all primary tissue was more highly variable with ranges in average position of the ELP that dampened out any meaningful pattern in its ability to describe drift (see Figure 48).
This conclusion follows from many sources but the particular acceptance of this alternative hypothesis is still preliminary. Of course, considering more information (i.e., both tissues) is “better” and more descriptive of total drift phenomena. It is important though to remember that until that can be done in a way that permits comparisons between individuals statistically the ELP alone suffices as a summary, providing important information for use in new experimental designs as well as new variables for direct use in bioarchaeological applications explaining and permitting variance in how bone morphology was achieved.

B) The original expectation that geometric analysis of the ELP would be significantly easier using hand-drawn techniques is not upheld by results of the research presented here.

\( H_A: \) The alternative hypothesis should be **rejected**: Variability was not noticeably higher or less patterned in ELP area or angular measures taken using one or the other method.

In actuality, both techniques, point-count and hand-drawn, had different strengths for different types of analysis, but point-count data in combination with frequency weighted vector calculation and circular distributional statistics actually turned out to be highly contributive. This is especially true when one considers that the technique collects data on many more potential variables than hand-drawn efforts ever could. One highly
important caveat is that without a data entry program like the one developed here: “μ Count Histo.” the time investment and complexity of tracking a significant number of variables using point-count is highly prohibitive. It is not always the case, however, that so many collected variables are necessary. In conclusion: When considering smaller sample sizes and/or a focus only on endosteal tissue distribution for the purpose of investigating net drift direction, hand-drawn techniques are more efficient. However, for complete assessment of all tissue types and OPD on a distributional basis, point-count technique becomes a preferred option, using weighted vectors to describe position and angular comparisons with $I_{\text{max}}$.

C) According to results here there is no \emph{mathematical} reason to hold the null hypothesis that ELP indicated drift direction and $I_{\text{max}}$ axis are statistically similar.

\textbf{H}_0: The null hypothesis should be \textbf{rejected}: There was indeed a statistically significant difference between drift and $I_{\text{max}}$ by these measures.

This conclusion is made with considerable disclaimer: variance was so different between $I_{\text{max}}$ and ELP indicated drift, that additional testing and methods may be necessary to make a clear argument as to what the finding of statistical difference between these axes means. For now though, the reliable and statistically significant trend
is that drift is more M-L oriented than $I_{\text{max}}$ is. There is room for the testable hypothesis that $I_{\text{max}}$ is summarizing the element’s biomechanical circumstance and that the ELP is more affected by local strain applied by the deltoid. However, there is equal room for the testable hypothesis that $I_{\text{max}}$ has such low variation because of some other constraint and that the ELP indicated drift represents general mechanical adaptation of the element. Clearly further research on this matter is extremely important and would probably benefit from experimental methods using non-human fluorochrome labeled bone.

D) The current data suggests that it is true that basic variance in ELP position and size differs significantly between members of subgroups defined by a) age, b) sex, and c) skeletal population of origin (modern versus archaeological).

$H_A$: The alternate hypothesis should be accepted: There was indeed a statistically significant difference in ELP variables among individuals of differing a) age, b) sex, and c) skeletal population samples.

This conclusion is a general summary of the data and is not as mundane as it first might appear. At the outset of this investigation it was quite likely that the ELP could be governed by evolutionary constraints similar in most individuals, violating all of these alternate hypotheses. Similarly, the ELP could have been more weakly connected to age than it was, or could have been completely similar between modern and ancient Yucatecâns. As the data suggest, however, not all comparisons among these subgroups
were statistically significant. One of the more interesting differences found in this section of the study was the modern sexual dimorphism compared to the ancient lack thereof. The specifics of significant differences found in drift among basic demographic subgroups is far too detailed to go permit a full review here. However, it suffices to say, that in general many of the patterns seen here are biologically important and potentially useful for bioarchaeological inquiry and verification of or rejection of certain archaeological hypotheses at the sites involved here or in other sites where similar techniques might be used.

Each observed trend here can be further verified by the collected data compared to other studies on similar questions and to the archaeological questions, important for understanding Xcambó. A full application of data collected here will take years of organized effort, which is of course the intention for future development of techniques and applied understanding regarding modeling drift and its relation to differential growth and physical adaptation.

Future efforts will also be aimed at seven major areas of the current project. First, the same techniques used here will be replicated for other elements, particularly the femur as an example of a weight bearing bone that has been extensively involved in histomorphometric study. There is great need, however for this type of population approach to tissue distributions in the femur, particularly because of its importance in activity pattern analysis and age estimation. Second, the statistical techniques will be modified to better provide evidence accounting for the strength of calculated vectors
based on tissue distributions. Third, the sample size will be expanded, especially for modern controls, in order to verify the current results with even greater statistical strength and to permit the inclusion and consideration of younger and older aged individuals.

Fourth, future work will continue the development of automated means of identification and quantification of tissue types across the bone transection. This will be accomplished by the averaging of areas delineated by computerized assessment of birefringent intensity, hue under compensated light, Haralick texture analysis, and distributional parameters and may eventually even succeed in discerning between primary lamellar bone from the periosteum versus the endosteum. Fifth, other important variables collected here, including humeral torsion, carrying angle, total humeral length, drifted osteon distribution, porosity, measures of torsional resistance, and ovality, will all be applied to aid the interpretation of the various results reported here in order to identify additional informative trends in tissue distribution. Sixth, proposals will be made for how a consideration of tissue-age-at-formation can be controlled for during other types of histomorphometric tests such as those on osteon morphotypes, targeted remodeling, or even age estimation. Finally, and perhaps most exciting, investigations will be made into the targeting of ELP tissue for stable isotopic data in comparison with other regions of the bone, other elements, or dentition, in order to provide finer resolution in our ability to connect diet, migration, growth, and adaptation in order to understand the true breadth of what it means to be human.
This portion of the project began with a curious question regarding a particularly pretty spot on a polished bone excision and ended with a particularly pretty quartz compensated 45° merged micrograph, extensive quantitative documentation and comparison, and a nearly infinite set of questions and interests to follow onwards from here. The next step will be documenting major drift patterns in other elements and considerations of what drift variation means for archaeological interpretations at sites like Xcambó. In this case, for example, complexity is added to prior cross-sectional geometric analyses at the site in that the sexual dimorphism observed could be arising from patterns of development and adaptation that begin much earlier in life in addition to differences in physical activity with the onset of adult working behavior. Particularly interesting would be whether the economic transition hypothesized for Xcambó in the Late Classic can be corroborated and made more visible by mechanically adaptive changes in drift direction between samples from before and after this point in the port’s history. Continued examination of tissue distributions throughout the diaphysis generates one more way to connect bones and behavior across the boundaries of modern biological understanding and ancient civilizations.
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