EXPLORING PRE-COLUMBIAN HEALTH AND LIFEWAYS IN THE GREATER COCLÉ REGION, PANAMA

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ABSTRACT

Steph M. Berger: Exploring Pre-Columbian Health and Lifeways in the Greater Coclé Region, Panama

This research combines an investigative life history approach with bioarchaeological methods to assess the developmental origins of health and disease in the Greater Coclé region of Panama during the Early and Middle Ceramic Periods (2480 BCE- 1200 CE). This work incorporates interdisciplinary frameworks and focuses on the role of pro-inflammatory immune activity as a physiological pathway linking early life exposures to later life mortality risk. Since developmental stress has consistently been associated with later life morbidity and mortality in modern populations, a deep time perspective is needed to better understand the nature of these relationships and underlying physiological pathways throughout human history. While bioarchaeological studies have increasingly incorporated evolutionary developmental theory over the last two decades, these studies encompass a limited range of geographical and historical contexts. This study provides new theoretical and methodological contributions to the study of developmental origins of health and disease in past populations in the understudied but rich archaeological context of the Greater Coclé region.

First, I explored biocultural kinship systems and postmarital residence practices at the Greater Coclé sites Cerro Mangote and Sitio Sierra. The dental evidence indicates a mix of cultural continuity and innovation in the region. While both biological and social relationships are represented in complex mortuary practices at Cerro Mangote, the shift towards single primary interments at Sitio Sierra appears to predominantly reflect social relationships. I also identified considerable gene flow between the communities, and these results provide insight

into potential Greater Coclé interactions, such as the existence of a special regional cemetery site at Cerro Mangote.

Second, I identified three different developmental phenotype classes from an analysis of standard osteological markers of growth disruption and developmental stress. Low to moderate developmental stress was common in the Greater Coclé region during the Early and Middle Ceramic Periods, and I found that phenotypes characterized by increasing developmental stress were associated with greater mortality risk and earlier age at death. These results support the developmental origins of health and disease framework, although Greater Coclé cultural buffering systems may be dampening the adverse effects of developmental stress.

Last, I found that developmental stress does interact with pro-inflammatory immune activity to increase mortality risk. I identified four immune phenotypes representing a range of pro-inflammatory immune activity, and among older age cohorts, individuals who experienced both development stress and later life chronic, systemic inflammation died at earlier ages than individuals who did not experience developmental stress. Although greater pro-inflammatory immune activity was paradoxically associated with older age death, these results fit with the inflammaging and allostatic load frameworks and provide novel bioarchaeological evidence of senescence-related frailty. Together, these findings contribute novel insights into Pre-Columbian health and lifeways in the Greater Coclé region and reinforce the need to use biocultural approaches that contextualize archaeological interpretations with skeletal remains.

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LIST OF ABBREVIATIONS

APC	Antigen presenting cells
AMTL	Antemortem tooth loss
BCE	Before Common Era
BLR	Buccolingual (labiolingual) root dimension
C	Carbon
CE	Common Era
DC	Dendritic cell
DOHaD	Developmental origins of health and disease
FA	Fluctuating asymmetry
ICC	Interclass correlation coefficient
IL	Interleukin
L3-L5	3rd-45h lumbar vertebrae
LCA	Latent class analysis
LEH	Linear enamel hypoplasia
LHT	Life history theory
PAR	Predictive adaptive response model
MDR	Mesiodistal root width
MDS	Multidimensional scaling
MHC	Major histocompatibility complex
mm	Millimeters
N	Nitrogen
n	Total number of observations

NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OA	Osteoarthritis
PCA	Principal components analysis
PD	Periodontal disease
RA	Rheumatoid arthritis
RANKL	Receptor activator of nuclear factor kappa-β ligand
SD	Standard deviation
Sr	Strontium
T_H	Effector memory T Helper cells
T10	10^{th} thoracic vertebrae
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
VNC	Vertebral neural canal
WHO	World Health Organization
Z	Standard score

CHAPTER 1: INTRODUCTION

1.1 Study Summary

Social inequality is a defining characteristic of the human experience, and modern population research has identified social inequality as a leading cause of global health disparities. Investigations into the development of social inequality, as well as the origins of modern human society and health patterns, therefore represent a "grand challenge" in archaeology, and skeletal datasets provide critical insight into the complex, variable relationship between social processes and health in various contexts throughout human history (Kintigh et al. 2014; Krieger 2005; Marmot et al. 2008).

Following the excavation of the elaborate Sitio Conte mortuary complex during the 1930s, Pre-Columbian Panamanian cultures have long served as important examples of tropical chiefdoms, and considerable archaeological research has been devoted to tracing the development and nature of social complexity in the region (Berrey 2015; Hoopes 2005; Isaza 2007). Archaeological investigations of Pre-Columbian sociopolitical organization, however, have relied predominantly on indirect evidence (Berrey 2015; Berrey 2018; Briggs 1993; Isaza 2007). The existence of complex mortuary rituals and lavish burials, for example, appear to correspond to Gaspar de Espinosa's 16th century record of the burial of chief *Parita,* and as a result, other ethnohistoric accounts have been used to interpret archaeological remains despite biases by Spanish and other European chroniclers (Briggs 1989, 1993; Espinosa 1994:63-64; Lothrop 1954; Smith-Guzmán and Cooke 2018).

The reliance on indirect evidence to understand Pre-Columbian lifeways in Panama stems in part from a lack of osteological analysis of skeletal remains. Similar to other non-European contexts, the analysis of skeletal remains has rarely been included in the archaeological interpretations of Pre-Columbian Panama due to challenges with skeletal preservation and a dearth of trained osteologists (Larsen 2006; Tayles and Oxenham 2006). However, the expansion of bioarchaeology has demonstrated the central importance of skeletal remains for investigations into social dynamics of past societies (Cox and Mays 2000; Larsen 2002). Recent bioarchaeological studies have also highlighted the uncertainty regarding scholarly understanding of Pre-Columbian lifeways in Panama. Smith-Guzmán and Cooke (2018) reviewed Samuel Lothrop's (1954) examination of skeletal remains from the Playa Venado site (550- 850 CE) along the Pacific coast of Panama. Lothrop claimed the skeletal remains exhibited clear evidence of ritual killing and sacrifice, and his interpretation of the Playa Venado burials has long served as evidence of general violence in Panama (Bishop and Knüsel 2005; Creamer and Haas 1985; Drennan 1993; Ibarra Rojas 2012). Smith-Guzmán and Cooke (2018), however, found no evidence of perimortem trauma and instead attribute the skeletal damage, burial positioning, and demographic composition to normal taphonomic processes and mortuary rituals, such as secondary burial and burial re-use.

Smith-Guzmán and Cooke's (2018) work demonstrates the pressing need to reevaluate previous interpretations and more fully explore the role of human health in shaping Pre-Columbian lifeways (Díaz 1999; Huard 2013; Norr 1995). Investigations of social complexity need to be contextualized with analysis of skeletal remains in order to understand the biocultural consequences of emerging stratification and inequality in Pre-Columbian Panama (Briggs 1993). Recent bioarchaeology research programs at the Smithsonian Tropical Research Institute (STRI)

have provided the opportunity for new projects, and this study contributes to current efforts to address fundamental gaps in knowledge of human experience in Pre-Columbian Panama by focusing on the relationship between human health and social organization. By employing an interdisciplinary, investigative life history framework, this project utilized novel techniques to examine the developmental origins of health and disease and create a foundation for further investigation into kinship as a social determinant of health at two Early and Middle Ceramic sites (2480 BCE- 1200 CE) in the Greater Coclé region of Panama. The goals of this study are:

Objective 1: to reconstruct kinship groups through a biological distance analysis and assess the importance of biological and social relationships in structuring social identities to determine the role of kinship in shaping health disparities following the emergence of inequality during the Ceramic Period in the Greater Coclé region

Objective 2: to reconstruct developmental phenotypes from osteological markers of developmental stress and assess how developmental exposures impact later life mortality outcomes in the Greater Coclé region

Objective 3: to assess the range of pro-inflammatory immune activity in Pre-Columbian Panama and explore the role of inflammation as an important physiological pathway linking developmental stress to later life mortality outcomes

1.2 Article 1: Pre-Columbian Kinship Systems and Complex Mortuary Tradition

Kinship is thought to play a central role in shaping Greater Coclé identities throughout the Ceramic Period (2500 BCE-1520 CE) in Panama, but kinship systems and their manifestation in Pre-Columbian mortuary practices remain unexplored. This study examines phenotypic variation to evaluate the role of kinship in structuring burial and cemetery organization and postmarital residence practices at Cerro Mangote (2476 BCE- 231 CE) and Sitio Sierra (39 BCE-561 CE, 978-1158 CE). A biological distance analysis was used to compare dental odontometric and morphological traits among 74 individuals at these sites. The analysis identified significantly smaller within-group distances for several multiple burials at Cerro Mangote, indicating that biological relationships determined placement within these multiple burials. No close biological relationships were identified at Sitio Sierra even as odontometric variability decreased over time. These results suggest a shift in mortuary practices where social, rather than biological, relationships determined placement. Comparable phenotypic variation among sexes indicates bilocal or unilocal postmarital residence was practiced at both sites. Cluster analysis identified considerable gene flow between the populations and provides additional evidence of Greater Coclé interaction networks. The results demonstrate that complex biocultural kinship systems with dynamic levels of affiliation served the changing needs of Pre-Columbian populations.

1.3 Article 2: Reconstructing Developmental Phenotypes and Mortality Risk

Objectives: The developmental origins framework has highlighted the critical role of developmental plasticity in responding to external cues and shaping later health and disease outcomes. This study provides historical evidence of this relationship by exploring

developmental stress and mortality risk in a novel context in Pre-Columbian Panama. *Methods:* This study uses burials (2480 BCE- CE 1158) from Cerro Mangote and Sitio Sierra, two Greater Coclé sites. A latent class analysis (LCA) of the following osteological markers was used to reconstruct developmental phenotype classes and explore developmental stress: linear enamel hypoplasia; vertebral neural canal dimension; dental size; dental fluctuating asymmetry). Developmental phenotype class associations with biocultural factors (age, sex, and site) were assessed with Chi-square tests, and phenotype class was modeled as a covariate affecting the Gompertz hazard of mortality. *Results:* LCA identified three classes of increasing developmental stress within the Greater Coclé sample. No significant associations were found between developmental phenotype class and biocultural factors. Although developmental phenotype class was not significantly associated with mortality risk, individuals in Classes 2 & 3, who experienced greater developmental stress, had a 60% increase in mortality risk. *Conclusion:* The results demonstrate the utility of LCA for assessing complex constructs with osteological markers. The hazards analysis suggests several biocultural factors may be affecting the relationship between development and later life mortality risk observed in the sample, including the timing of developmental insults and the Greater Coclé disease burden. Another possible cause may be Pre-Columbian cultural buffering systems that ensured adequate resources and social support for community members. Although further testing with a larger sample size and osteological markers that capture critical periods of development is necessary, this study demonstrates how modeling techniques can be used to explore the developmental origins of mortality risk in skeletal assemblages.

1.4 Article 3: Pre-Columbian Immune Phenotypes: The Role of Inflammation in the Developmental Origins of Mortality Risk

Objectives: The immune system and inflammation are critical physiological pathways that links adverse developmental experiences to later life morbidity and mortality risk. This study explores the relationship between developmental stress and pro-inflammatory immune phenotypes in adulthood through the use of skeletal proxy markers in order to examines the role of inflammation in the developmental origins of mortality risk in a novel context in Pre-Columbian Panama. *Methods*: This study uses burials (2480 BCE- CE 1158) from two Greater Coclé sites. A latent class analysis (LCA) of osteological proxy markers of pro-inflammatory immune activity, including bone remodeling, oral inflammation, and joint inflammation, were used to determine individual immune phenotypes. Immune phenotype associations with biocultural factors (developmental phenotype, sex, and site) were assessed with multinomial logistic regression, and the modifying effect of immune phenotype class on the relationship between developmental stress and mortality risk was assessed with linear regression. *Results:* LCA identified four classes of immune phenotypes along a spectrum of pro-inflammatory activity within the Greater Coclé sample. Phenotypes characterized by greater pro-inflammatory activity were significantly associated with older age at death but not developmental stress. When comparing older age cohorts, active systemic inflammation was associated with earlier age at death than local healed inflammation. Pro-inflammatory phenotypes also increased the mortality risk for developmentally stressed individuals compared to individuals with average developmental experiences. *Conclusions:* The results demonstrate that pro-inflammatory activity can be assessed with osteological proxy markers, but the age distribution of oral and joint inflammation markers is skewed towards older cohorts likely due to senescence-related

inflammaging. The multinomial regression did not provide evidence that developmental stress was associated with greater pro-inflammatory activity, but the linear regression demonstrated that later life pro-inflammatory activity increased the mortality risk for developmentally stressed individuals. These results suggest that the costs and allostatic burden of inflammation may be greatest later in the life course in Pre-Columbian Panama, although further testing with a larger sample size is necessary.

CHAPTER 2: PRE-COLUMBIAN PANAMA

2.1 Archaeology in Panama: A Historical Overview

Following the "gold rush" in the Chiriquí province of Panama and the destruction of thousands of Pre-Columbian graves for gold artifacts, early archaeological work began at the end of the 19th century (Cooke and Sanchez 2004). This period of antiquarian archaeology was guided primarily by foreign interests in recovering artifacts for North American and European museums and collectors and by possible profit in the looting and melting of gold artifacts. The materials recovered from mortuary sites also sparked academic interest in Pre-Columbian Panama. Archaeological investigation spread to the Coclé province and resulted in the excavations of important regional sites El Caño and Sitio Conte in the 1920-1930's (Briggs 1989; Lothrop 1937; Mason and Oesher 1942; Mayo Torné et al. 2020). Initial scholarly work laid a foundation that shaped conceptions of Pre-Columbian Panama for decades. Researchers like William H. Holmes (1919) focused on the existence of static cultural areas linked to "tribes" and used diffusionist ideas to explain the emergence of local Chiriquí stylistic traditions. The existence of complex, sumptuous mortuary artifacts and rituals appeared to correspond to Spanish ethnohistoric accounts, such as Gaspar de Espinosa's account of the burial of the chief *Parita*, and archaeologists argued that mortuary evidence proved the existence of rigid hierarchical chiefdoms (Cooke and Sanchez 2004, Isaza 2007; Jopling 1994:63-64; Oviedo 1944).

Archaeological excavation proliferated after WWII and expanded the established research program in new, positive directions. Researchers traced the gradual evolution of

regional pottery in order to build chronological sequences for each region. In the rebuttal of earlier theories, investigations by Willey and colleagues (1954) and Ladd (1964) deepened the chronology of the region by confirming the occupation of areas adjacent to Parita Bay from 5,000 BC onward. With the development of radiocarbon dating, Olga Linares established the first confirmed radiometric cultural sequence for the province (Linares 1968). Interest in reconstruction of past environments, including settlement patterns, pushed analysis beyond mortuary contexts to better understand pre-Columbian lifeways (Cooke and Sanchez 2004). However, the irreversible damage and destruction of many archaeological sites continued due to the activities of U.S. amateur "archaeologists" in the Archaeological Society of Panama, a front for looting ceramics and gold to sell to private collectors and museums. The significant destruction and sale of Panamanian cultural patrimony resulted in strong, negative public sentiment regarding archaeology and archaeologists (Cooke and Sanchez 2004).

In response to widespread looting and foreign interests, a growing nationalist sentiment in the 1960's lead to the development of the National Institute of Culture and Sports and the appointment of Reina Torres as director of the Historical Heritage Department. Torres oversaw the dramatic improvement of archaeology in Panama, and the "new" archaeology became more interdisciplinary, rigorous, and involved in the diachronic study of humanity (Cooke and Sanchez 2004). Subsequent researchers like Olga Linares and Richard Cooke pushed the field to study all aspects of Panama's Pre-Columbian history. Programs like the Santa Maria Project (1981-1985) reconstructed the indigenous colonization of the Santa Maria basin, and attempted to answer questions regarding the domestication and cultivation of crops (Bush et al. 1992; Piperno and Holst 1998; Piperno et al. 2000), endogenous development of technologies, and the development of sociopolitical hierarchies (Cooke and Ranere 1984). Current research continues

to challenge early assumptions of Pre-Columbian Panama based on biased ethnohistoric accounts and colonial, antiquarian theories (Isaza 2007; Smith-Guzmán and Cooke 2018; Smith-Guzmán et al. 2018). The resulting picture of Panama is one of an incredibly diverse context with heterogeneous physical environments, a long history of permanent indigenous inhabitants (Cooke 2005) and lifeways until Spanish contact (Cooke and Sanchez 2004). The following cultural chronology focuses on the central Greater Coclé region of Panama, the most extensively studied region in Panama and the location of the study sites.

2.2 The Pre-Ceramic Period (9,500 BCE- 2,500 BCE)

Although evidence of Paleoindian hunter-gatherers in Panama is visible as early as 9,500 BCE, human activity in the Gran Coclé region did not intensify until the climactic changes that mark the beginning of the Holocene period (Cooke et al. 2013; Cooke 2005). In the Early Preceramic period (9,500- 6,000 BC), some aspects of lifeways continued, such as stone tool technology, but microbotanical remains recovered from sediment cores and artifacts suggest that inhabitants broadened their subsistence strategies through the cultivation of plants like bottle gourd and arrowroot in small house gardens or stream alluvia (Cooke 2005; Dickau 2010; Piperno and Pearsall 1998; Piperno et al. 2000). These early experiences with cultivation presage a major development in the historical trajectory of Panama, and by 5,000 BCE, paleoecological reconstructions detail the disappearance of primary forests and the emergence of secondary forests due to the introduction of slash-and-burn agriculture (Cooke 2005; Cooke and Ranere 1992; Piperno and Pearsall 1998).

Evidence from changing stone tool assemblages and archaeobotanical data provide additional evidence of the growing reliance on plant cultivars, as well as the diversification of

plant cultivars after the introduction of new species, such as maize and manioc (Dickau 2010; Ranere and Cooke 1996; Piperno and Pearsall 1998). While Late Preceramic populations continued to hunt, they also increased their exploitation of diverse coastal resources as sea levels rose and marine habitats changed (Clary et al. 1984; Cooke and Ranere 1999).

These climactic, environmental and subsistence changes likely contributed to population growth that resulted in an increase in the size and number of settlements in the region. Late Pre-Ceramic populations settled primarily in coastal, littoral areas that overlooked streams and rivers (Norr 1991). With a greater population density, groups may have initiated trade and exchange relationships. Archaeological evidence in the form of a manatee bone recovered from Cerro Mangote suggests that Caribbean goods were brought into the area from across the cordillera, and within the Greater Coclé region, Cooke (2005) argues that marine resources were moved inland from the coast (Cooke and Ranere 1999). Although there is no evidence that any of the human burials date to the Pre-Ceramic period, the Early to Middle Ceramic burials at Cerro Mangote (2480 BCE- 317 CE) provide evidence of complex mortuary rituals, such as the recognition of social identities tied to age, that may represent continuity with Pre-Ceramic mortuary traditions (Huard 2013; Ranere 1980).

2.3 The Ceramic Period (2,500 BCE- 1520 CE)

A continuation of Pre-Ceramic lifeways defines the Early Ceramic period (2,500- 200 BCE), in addition to the development of new technologies. Archaeological evidence of ceramics and polished axes appear during this period, and researchers have tied these tools to subsistence changes (Cooke 2005). Although the diversity of important crops increased, the depletion of soil nutrients led populations to move to more fertile areas in alluvial bottomlands (Piperno and

Pearsall 1998). Polished axes may have enabled the clearing of new land, and ceramics may have enabled new means of preparing and storing foodstuffs to meet the needs of a growing population (Cooke 2005; Cooke 1998). By 2470 BCE, permanent villages become archaeologically visible and represent a shift towards more sedentary lifeways (Cooke 1995). The inhabitants of sites like Monagrillo farmed and exploited marine resources from the nearby coastal lagoon, and inhabitants likely continued to trade and/or travel to obtain food types from diverse coastal and inland habitats. Estimates suggest that the population size of Monagrillo reached 50-500 people, a drastic increase enabled by agricultural productivity, interaction, technological innovation, and sedentism (Cooke 2005; Isaza 2007).

The shift towards nucleated settlements in alluvial bottomlands continued into the Middle Ceramic Period (200 BCE- 700 CE), and the emergence of village lifeways marks another important development in the historical trajectory of Panama. In addition to an increased reliance on intensive agriculture, craft specialization appears to develop in village settlements (Cooke 2005; Isaza 2007). Funerary artifacts and evidence of open air workshops from Middle Ceramic sites suggest that some individuals performed the specialized production of utilitarian tools and non-utilitarian items, like marine shell jewelry. These localized production centers contributed to a wider variety of material goods, decorative motifs and technological improvements, such as better ceramic firing techniques. Trade networks helped move various goods, like lithic and shell tools and deep water fish, between inland and coastal sites to meet regional needs.

These long-standing interactions between populations likely shaped the rise of larger sites with more central or specialized roles within the region (Cooke 2005; Isaza 2007). Several theories have been proposed to explain the rapid social and technological innovation that occurred during the Middle Ceramic period, such as environmental crises related to prolonged

dry periods and volcanic eruptions or the long-distance interactions with changing Mesoamerican cultures. As Hoopes (2005) suggests, the possible explanations all focus on the introduction of risk and change to the Middle Ceramic environment as a catalyst for the emergence of sociopolitical differentiation and inequality in the Middle and Late Ceramic Periods (AD 200-1520). Cooke and Ranere (1992) propose that social and environmental tension resulting from the risks of population growth, increased territoriality, and limited resources, created opportunities for groups and individuals to compete over control and access to these resources. While descriptions of powerful chiefs and centralized chiefdoms from Spanish chronicles shaped early archaeological ideas regarding sociopolitical differentiation and organization, recent consideration of more heterarchical, corporate strategies provide alternative narratives (Hoopes 2005).

The excavations of rare, special function sites indicate the importance of ritual activity, especially mortuary traditions, during the Late Ceramic Period (Cooke 2005; Haller 2008; Locascio 2010; Mayo Torné et al. 2020). At Sitio Conté and El Hatillo, the cemeteries contain the richly provisioned burials of elite individuals, primarily adult men, accompanied by multiple individuals in the same interment (Cooke 2005; Cooke 1984; Locascio 2010). The remains of monumental architecture, including columns and altars, and pottery motifs suggest that ritual activities like the *balseria* game may have occurred at El Caño and El Hatillo (Cooke 1984; Ichon 1980). These few sites likely served as meeting places for performing important activities, including the burial and honoring of influential individuals, that reinforced the ties that structured social groups within the region (Hoopes 2005; Isaza 2007).

Figure 2.1 Map of Panama

Important archaeological sites are marked, including the two sample sites included in this study, which are indicated by bold font.

2.4 Summary

Overall, the Greater Coclé region of Panama provides a rich archaeological record detailing the diverse, complex lives of indigenous peoples. The earliest visible human activity appears by 9,000 BCE, and inhabitants developed mixed subsistence strategies to exploit the abundant terrestrial and marine ecosystems. Trade networks connected burgeoning populations, and following climactic, environmental, and demographic changes, Ceramic Period communities adopted intensive flood plain cultivation and settled in more nucleated and permanent villages. The emergence of village lifeways served as a catalyst for the development of new technologies and craft specialization and new forms of interaction, including increasing social hierarchy. The growing complexity of Pre-Columbian social relationships and cultural practices is manifest in

the development of special elite sites like El Caño, El Hatillo, and Sitio Conte, which likely served as regional centers for burials and other community events (Figure 2.1).

The history of archaeology in Panama, however, has contributed to many challenges that impact current research endeavors, from site looting to the persistence of inaccurate interpretations of Panamanian lifeways based on biased ethnohistoric accounts (Lothrop 1954). Skeletal remains, in particular, have received minimal scholarly attention, despite the potential of osteological data to contextualize archaeological interpretations and provide insight into lived experiences (Smith-Guzmán and Cooke 2018; Smith-Guzmán and Cooke 2019). As a result, there is a gap in our understanding of Pre-Columbian health and a need for rigorous bioarchaeological research to answer questions regarding lifeways in the Greater Coclé region.

CHAPTER 3: THE SKELETAL EMBODIMENT OF HEALTH AND DISEASE

3.1 Evolutionary Perspectives: Life History Theory and Developmental Origins

A key finding of modern biology is that genotypes can give rise to numerous different phenotypes, and considerable research has demonstrated that environmental conditions during development drive the phenotypic variation in a diverse array of organisms (Bateson et al. 2004). While natural selection takes generations to solidify genetic responses to the environment, developmental plasticity offers a more immediate timescale for "adaptations" that can enhance Darwinian fitness. During gestation, maternal environmental experiences are communicated through the placenta by endocrine and nutrient signaling pathways, and these inputs influence the development and function of the fetus' organs and physiological systems through various mechanisms, including epigenetic changes to gene expression (Gluckman et al. 2007; Kuzawa 2005).

Environmental cues continue to shape developmental trajectories throughout infancy, and the physiological responses can include stable and heritable changes (Agarwal 2016; Worthman and Kuzara 2005). Organisms can deviate from the phenotypic development of their evolved lineage in response to environmental changes, giving each generation the opportunity to re-adjust their developmental trajectory and phenotype to their own environmental conditions. Organisms who are better matched and equipped to survive in their postnatal environments have increased chances of successfully reproducing. Therefore, developmental plasticity provides an evolutionary advantage selected for throughout human evolution (Gluckman et al. 2010; Hanson and Gluckman 2014).

While environmental cues during developmentally sensitive periods guide phenotypic outcomes, the subsequent phenotypic variation is not inherently adaptive (Bateson et al. 2004). Considerable modern health research has established the relationship between developmental experiences and later life disease risk, and this body of work has resulted in the "developmental origins of health and disease" (DOHaD) hypothesis (Barker et al. 1989; McDade 2005; Gluckman et al. 2005; Godrey et al. 2010). Several conceptual frameworks have proposed the potential pathways and mechanisms linking developmental plasticity and early environmental exposures to later life disease. The Predictive Adaptive Response (PAR) model, developed by Gluckman et al. (2005), differentiates between two different types of plastic responses to environmental cues. Immediate adaptive responses involve responses that cope with severe developmental cues. These responses often sacrifice later function and health for immediate survival, and as a result, these phenotypic decisions can have negative health consequences later in life (Hanson and Gluckman 2014). Predictive adaptive responses are defined as responses to more neutral cues regarding later environments, and these responses alter the phenotype to match the individual's predicted future environment and maximize fitness.

The PAR model states that predictive adaptive responses are evolutionarily selected for because they confer individual fitness advantages through the reproductive period. These responses can become maladaptive, however, if the developmental trajectory and resulting phenotype do not match the actual later life environment. For instance, an individual exposed to nutrient restriction in utero will develop a phenotype adapted to a scarce environment, and the individual will have a fitness advantage if they live in a scarce environment throughout their life. If the individual experiences a resource-rich environment after development, their phenotypic responses will prohibit them from adapting to their new environment and lead to an increased

risk of disease in the post-reproductive period (Gluckman et al. 2010; Hanson and Gluckman 2014). This notion of "mismatch" between expected and actual postnatal environments is the causative agent behind the increased risk of chronic disease (Bateson et al. 2004; Hanson and Gluckman 2014).

Critiques informed by life history theory have reframed the adaptive responses as "tradeoffs" regarding resource allocation. Human physiological systems enable individual growth, reproduction, and maintenance, the three key determinants of individual survival and evolutionary fitness. Life history theory posits that organisms have limited energy to allocate to these processes, and energy allocation decisions result in "tradeoffs" that allow organisms to respond to environmental cues in ways that enhance evolutionary fitness (Kaplan et al. 2000). When individuals experience adverse environmental cues early in development, the prioritization of selective organ or system development for survival and evolutionary fitness over the development of other systems results in a tradeoff that increases an individual's vulnerability to morbidity and mortality later in life (Gluckman et al. 2007; Kuzawa 2005). For example, Thayer and Kuzawa (2014) noted that energetic stress during pregnancy led to fetal tradeoffs that constrained kidney development and increased the individual risk of hypertension in adulthood.

Much of the early DOHaD literature focused on the effects of nutritional stress. Seminal research on the health impacts of the Dutch Hunger Winter demonstrated that intrauterine exposure to famine increased the risk of later cardiovascular and metabolic disease later in life (Stein et al. 1975). Barker and colleagues' (1989) later study on ischemic heart disease found that individuals with low birth weight, a measure of energetic and nutritional stress experienced during gestation, had increased risk of mortality from ischemic heart disease. Recent work has demonstrated that early psychosocial stress burdens energetic resources and impacts later life

outcomes as well. Prenatal psychosocial stress can impair the development of the hypothalamic pituitary adrenal (HPA) axis, and subsequent dysregulated glucocorticoid function has cardiometabolic, neuroendocrine and behavioral consequences (Seckl and Holmes 2007). Overall, the DOHaD framework suggests that adverse environmental cues resulting in energetic stress during development leads to tradeoffs that shape long-term morbidity and mortality risk. As a result, one's developmental phenotype represents an important source of frailty.

3.2 Developmental Plasticity and Heterogeneous Frailty

Individual frailty is defined as the risk of death at a given age relative to others within the population, and a variety of biocultural factors such as genetics, epigenetics, socioeconomic status, and sex influence variation in frailty (Vaupel et al. 1979; Wood et al. 1992). In modern populations, researchers can identify and control for sources of frailty, like high blood pressure or obesity. Many of these sources, however, are unobservable or unknown in skeletal assemblages. Heterogeneous frailty is therefore considered "hidden," and the inability to assess sources of frailty in skeletal assemblages greatly complicates bioarchaeological interpretations (DeWitte and Stojanowski 2015; Wood et al. 1992).

In their seminal article on the "osteological paradox" caused by hidden heterogeneity of frailty, Wood and colleagues (1992) proposed several strategies to deal with the challenges inherent in skeletal assemblages: reduce heterogeneous frailty by studying egalitarian, homogeneous assemblages; use juveniles as non-survivors to determine how to interpret osteological data; and model frailty distributions with demographic tools to make sense of mortality hazards. An important addition to this list is the use of current public health and

epidemiological frameworks to better understand the interaction between sources of frailty, osteological markers, and selective mortality.

Considerable DOHaD and LHT studies demonstrate that developmental responses to early environmental cues shape morbidity and mortality risk, and bioarchaeologists are increasingly incorporating these frameworks to address developmental plasticity as a source of frailty (Armelagos et al. 2009). Temple's (2014) analysis of linear enamel hypoplasias (LEH) and age at death revealed that individuals who formed LEH at earlier ages experienced an increased risk of later health insults that lead to more hypoplasias and an increased risk of dying earlier compared to individuals who formed LEH later in development. He interpreted this relationship as evidence of developmental tradeoffs that increased allostatic load and compromised individual ability to respond to future stressor. Wilson (2014) also used LEH as an indicator of non-specific stress during development for his survival analysis of over 2,000 individuals from the Central Illinois River Valley (AD 1000-1500). The results indicated a significant association between LEH presence and reduced survivorship.

Additional research has demonstrated the utility of other osteological indicators, including stature, vertebral neural canal dimensions, and childhood pathological conditions, to indicate developmental disruption and tradeoffs that increase risk of morbidity and mortality later in life (DeWitte and Wood 2008; Watts 2013; Watts 2015; Weisensee 2013). While these studies point to the increasing interdisciplinary focus of bioarchaeology, additional engagement with public health and human biology discourse can continue to challenge bioarchaeological research. In particular, modern health frameworks can help bioarchaeological studies to expand beyond associative studies to address the potential physiological pathways that link early

environmental cues to later life morbidity and mortality risk and assess the effect of developmental phenotypes on morbidity and mortality outcomes.

3.3 The Immune System: A Pathway Connecting Early Life Experiences and Later Life Health Outcomes

The immune system plays central roles in cellular renewal and repair, and it is the key defense against microbial invasion and uncontrolled cellular replication. While these functions are essential to somatic maintenance, the development and function of the immune system requires considerable energetic resources (McDade 2003; Urlacher 2018). During development, the thymus, an important immune system organ, creates 20-25% of its total maturing T cell count (10^{11} cells) each day, and yet over 95% of these T cells are destroyed before their release (McDade 2003).

Animal studies have shown that immune activation was associated with a 29% increase in resting metabolic rate among birds, and in humans, acute phase immune responses including fever and protein synthesis have been shown to increase the metabolic rate as well (McDade 2003; Urlacher et al. 2018). Given the high energetic costs of the immune system, as well as its importance for evolutionary fitness, local environmental cues help calibrate immune system development to meet environmental risks with available resources (McDade et al. 2016).

McDade and colleagues (2016) propose a framework to explain variation in immune system investment based on these local environmental cues. Innate immune responses mobilize quickly and deal effectively with novel pathogens. Due to the non-specific nature of innate responses, the developmental cost is low, but activation costs are relatively high. In contrast, acquired immunity defenses use immunological memory to deal effectively with repeat pathogen

exposures. While the costs of acquired immunity maintenance and activation are relatively low, acquired immunity requires a high energetic investment in development (McDade et al. 2016). Based on the different benefits and costs of these systems, investment should vary based on local environments. Developmental environments characterized by nutritional abundance, high pathogen exposure and low signals of extrinsic mortality should favor higher levels of investment in acquired immunity, whereas environments characterized by undernutrition, low pathogen exposure, and high mortality risk should favor innate immune defenses (McDade et al. 2016). However, interactions between these domains can shape immune outcomes in additional ways. Due to the high energetic cost of the immune system and the limited nature of energetic resources, tradeoffs between immune system development and other competing life tasks should significantly impact immune function (McDade 2003; Urlacher et al. 2018). While environments with high pathogen exposure may favor higher levels of investment in acquired immune defenses, nutritional constraints may result in increased investment in low cost innate immunity during development (McDade et al. 2016; Urlacher et al. 2018).

A shift towards innate immune responses has numerous negative consequences as innate immune responses cause more immunopathology due to the greater cost of activation and the non-specificity of the response. For example, an experiment on the cost of innate immune defenses found that basal metabolic rate increased an average of 20% during the activation of acute phase response (McDade et al. 2016). Urlacher and colleagues (2018) examined the tradeoffs between immune function and childhood growth among the Shuar, a forager-horticulturalist population that experiences dietary energy constraints and high rates of infectious and parasitic disease. They found that acute-phase protein response and systemic inflammation had the greatest energetic cost and consequence to growth, and their results demonstrate that

innate immune function exacts a heavy energetic burden that compromises other life tasks. The long term, chronic activation of innate immune responses has also been implicated in the etiology of numerous diseases, such as coronary artery disease and depression (Hansson 2005; Maes 2011).

Inflammatory processes have been tied to the development of cancer, as the production of inflammatory cytokines and prostaglandins can suppress cell processes and lead to cell proliferation, inhibition of apoptosis, and carcinogenic mutations (O'Byrne and Dalgleish 2001). In addition to specific impacts of innate immune response, the concept of allostatic load suggests that the body's response to challenges, like pathogen exposure, exact a physiological toll. The toll accumulates throughout the life course and leads to the impaired function of multiple physiological systems (Danese and McEwen 2012; McEwen 1999a, 199b). Given the greater energetic costs and physiological damage caused by response non-specificity, predominantly innate immune responses may contribute more to allostatic load and increase the risk of disease and mortality.

3.4 Osteoimmunology

The skeletal system is deeply impacted by immune activity due to the integrated nature and mutually dependent regulation of these physiological systems (Walsh et al. 2018; Tsukasaki and Takanayagi 2019). Following key discoveries, such as osteoclast differentiation from hematopoietic stem cells, that indicated potential immune and skeletal interactions (Ash, Loutit, and Townsend, 1980; Walsh et al., 2018), the field of osteoimmunology was formally designated in 2000 (Arron and Choi 2000). Subsequent clinical and experimental research has identified

numerous shared signaling and transcriptional mechanisms and deciphered the role of osteoimmune interactions in contributing to disease states.

Although the master regulator of osteoclastogenesis and bone resorption in the skeletal system, RANKL (receptor activator of nuclear factor kappa-β ligand) plays a similarly important role in immune development and function (Takayanagi 2012; Walsh et al. 2018). RANKL signaling regulates dendritic cell (DC) survival; although activated DCS have short lifespans, they play a critical role in initiating adaptive T cell-mediated immunity (Josien et al., 2000; Kamath, Henri, Battye, Tough, and Shortman, 2002). RANKL prolongs DC survival and supports the waning phase of an immune response by upregulating T_H1 cytokine production and T cell memory formation (Josien et al., 2000; Walsh et al., 2006).

The immune and skeletal systems share a number of additional molecular parallels (Table 3.1). For example, TNF-α (tumor necrosis factor alpha) cytokines play a critical role in initiating the acute phase of the immune response and inflammation by stimulating dendritic cell differentiation (Walsh et al., 2006). TNF-α also initiates osteoclast differentiation through the upregulation of RANKL expression in osteoblasts (Lam et al., 2000; Steeve, Marc, Sandrine, Dominique, and Yannick, 2004). TNF-α and other pro-inflammatory cytokine signals converge by activating NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), a transcription factor critical for immune response and other cellular functions (Courtois and Gilmore, 2006; Karin and Greten 2005). In osteoclasts, NF-κB activates genes and upregulates the expression of osteoclastic molecules like cathepsin K (Boyce, Yao, and Xing, 2010). Although the role of NF-κB in osteoblasts is less clear, NF- κB activation appears to downregulate bone formation by inhibiting osteoblast differentiation and function (Chang et al. 2009; Li et al., 2007).

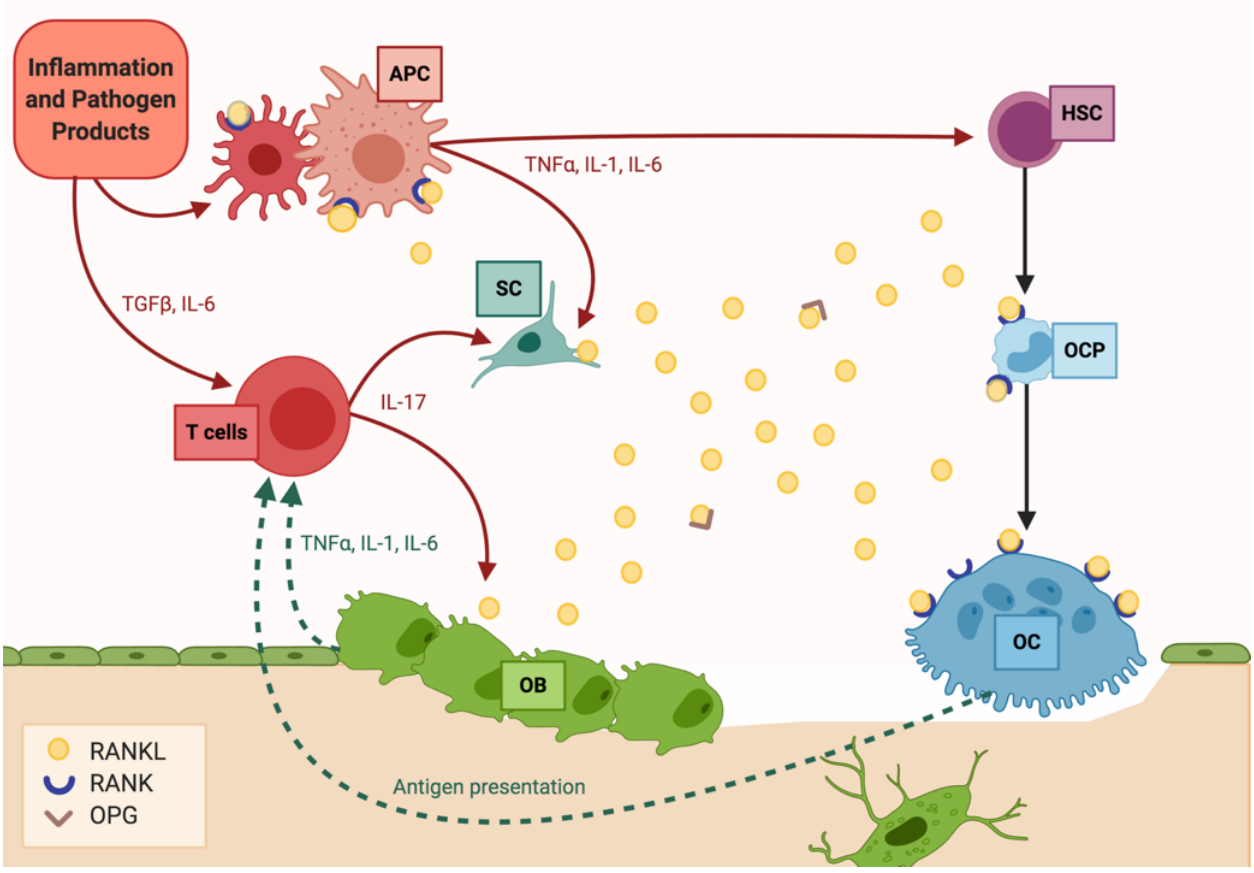
While toll-like receptors (TLR), a primitive receptor family, are highly expressed on antigen-presenting sentinel cells, TLR expression has also been detected on bone cells including osteoblasts and osteoclasts (Bar-Shavit, 2008). Ligation of these receptors by microbial molecules or endogenous factors activates NF- κB and other signaling cascades to initiate the innate immune response (Kawai and Akira, 2010). Interestingly, TLR ligation in osteoblasts, osteoclasts, and osteoclast precursor cells also upregulates the cellular expression of pro-inflammatory cytokines, chemokines, and prostaglandins that further drive local and systemic immune activity (Bar-Shavit, 2008; Itoh et al., 2003; Marriott, 2004).

Given the integration of the skeletal and immune systems, bone microenvironments are greatly impacted by immune activity (Figure 3.1). Regulatory T cells, T_H1, and T_H2 cells inhibit osteoclastogenesis through the expression of IL-4 and IL-10 (interleukin 4 and 10), which downregulate RANKL signaling in osteoclast precursor cells (Martin et al., 1998; O'Gradaigh and Compston, 2004). In contrast, $CD4^+$ lymphocytes, T_H1, and T_H17 cells stimulate osteoclastogenesis and resorption by significantly upregulating the expression of pro-inflammatory cytokines like TNF-α, IL-1, IL-6, and IL-17 (Takayanagi, 2007). Numerous immune cells, including B lymphocytes, DCs, and macrophages, can also express RANKL and can induce osteoclastogenesis and resorption (Karmakar, Kay, and Gravallese, 2010; Graves, Li, and Cochran, 2011). T lymphocyte signaling pathways, however, serve as the main link between immune activity and skeletal remodeling, and the predominantly osteoclastic effects of immune activity, especially inflammation, result in many pathological skeletal phenotypes.

In rheumatoid arthritis (RA), an autoimmune disease characterized by chronic inflammation and the destruction of joint cartilage and bone, researchers determined that the upstream actions of T_H17 cells, a pathogenic subset of T cells, were critical for the development

of RA (Hirota et al., 2007; Karmakar et al., 2010; Komatsu et al. 2014). In response to inflammatory cytokine signaling, T_H17 cells increase in number and express IL-17, which stimulates osteoclastogenesis by upregulating RANKL expression on immune and synovial cells and initiating the expression of pro-inflammatory cytokines and matrix-degrading enzymes (Sato and Takayanagi, 2006; Takayanagi, 2007). IL-11 and TNF-α simultaneously inhibit osteoblastic activity by upregulating inhibitors of the bone-forming Wnt pathway (Heiland et al., 2010; Karmakar et al., 2010). The amplification of RANKL expression in RA creates a feed-forward loop of inflammation, bone resorption, and joint destruction.

Figure 3.1. Immune Effects on Bone Remodeling



Summary of skeletal and immune system interaction; immune effects denoted by red arrows, and bone effects denoted by green dashed arrow. Inflammatory cytokine signaling through APCs and T cells leads to immune activation and the differentiation of T_H17 cells. T_H17 cell expression of IL-17 stimulates RANKL expression in osteoblasts and other stromal cells. RANKL and inflammatory signaling from additional immune cells further support osteoclast differentiation and activity, resulting in increased bone resorption. Osteoblasts and osteoclasts also perform immune functions by amplifying inflammatory signaling to boost the immune response and filling APC roles to improve immune clearance. Abbreviations: APC, antigen presenting cells; SC, stromal cell; HSC, hematopoietic stem cells; OCP, osteoclast precursor; OC, osteoclast; OB, osteoblast; RANKL, receptor activator of nuclear factor kappa-β ligand; RANK, receptor activator of nuclear factor kappa-β; OPG, osteoprotegrin. Originally published in Berger, Griffin, and Dent (2020).

Importantly, bone cells can also directly impact immune function in response to pathogenic stimuli and immune signaling (Figure 3.1). Osteoblast TLR ligation with microbial molecular products has been noted to increase expression of RANKL and other pro-inflammatory cytokines, chemokines, and prostaglandins that both promote osteoclastogenesis and initiate the immune activity by amplifying inflammation (Itoh et al. 2003; Kikuchi et al., 2001; Marriot, 2004). In mature osteoclasts, TLR ligation promotes cell function and survival, as well as upregulating the expression of pro-inflammatory cytokines (Bar-Shavit, 2008; Li et al., 2010). TLR ligation also stimulates osteoclast expression of MHC (major histocompatibility complex) genes, which allow osteoclasts to perform antigen presentation roles similar to macrophages (Li et al., 2010).

Overall, these findings suggest that the immune pathways, specifically inflammation, greatly impact the skeleton during disease states by decoupling normal physiological bone remodeling (Figure 3.1). Predominantly IL-17 and IL-6 signaling results in net increases in bone resorption as seen with RA, periodontitis, sepsis, chronic malaria, lupus, fungal infections, and some neoplastic diseases. Other cytokines contribute to both pathological bone resorption and formation. For example, TNF- α signaling in osteoporosis can also increase bone resorption and

formation, whereas IL-1, IL-6, and TFN-α signaling in osteoarthritis can result in either bone resorption and formation depending on the state of lesion progression (Tsukasaki and Takanayagi 2019). The skeletal system directly contributes to immune activity and subsequent effects on bone remodeling by amplifying inflammation and performing immune functions. Given the intimate ties between the immune and skeletal system defined by the field of osteoimmunology, skeletal phenotypes serve as useful indicators of immune activity and inflammation, a key determinant of allostatic load and morbidity and mortality risk.

3.5 Developmental Embodiment and Kinship

Embodiment refers to the concept of how humans literally incorporate the material and social world through biological processes (Krieger 2005). The embodiment of environmental cues during development promotes evolutionary fitness by shaping phenotypic variability to meet the constraints and challenges of an individual's environment. However, the embodiment of adverse environmental cues can result in maladaptive phenotypes characterized by increased risk of morbidity and mortality (Krieger 2005).

The idea that social factors shape health and disease trends is quite established in many human biology fields. In the 1830's and 1840's, African American physicians proposed that the reason black Americans had poorer health outcomes than white Americans was due to the trauma of enslavement (Krieger 2001). Everson and colleagues (2002) compiled data from four large epidemiologic studies and found strong associations between low socioeconomic status and chronic disease, with the greatest risk of poor mental and physical health seen in individuals who experienced sustained economic hardship. Experiences of marginalization have been identified by studies of past and present human populations as one important social determinant of health.

Marginalization generally refers to processes that render individuals unimportant or powerless within a group or society at large. Due to social statuses or social networks that limit their ability to cope with or mitigate scarcity, uncertainty, and conflict, marginalized individuals are more vulnerable to challenges (Klaus 2010; Lynam and Cowley 2007). Hall (1999: 95) argued that marginalization increases health risks resulting from "discrimination, environmental dangers, unmet subsistence needs, severe illness, trauma, and restricted access to health care." For example, a study on the impacts of marginalization found that experiences of social marginalization were positively associated with risky sexual behavior and HIV infection in young transgender women (Brennan et al. 2012). Bioarchaeological studies have found similar consequences of marginalization; in colonial Peru, native populations suffered from well-documented marginalization at the hands of Spaniards, and the prevalence of periosteal inflammation and degenerative joint disease greatly increased during this period (Klaus 2012). However, it is important to recognize marginalization occurs on multiple levels that can intersect to produce morbidity and mortality (Bauer 2014; Warner and Brown 2011). As a result, different components of social identities, such as age and sex, must be considered when examining the health consequences of marginalization.

Recent studies have demonstrated that morbidity and mortality outcomes shaped by social determinants of health, like marginalization, can be transmitted to subsequent generations through several mechanisms: persistence of abnormal, adverse environments across generations; maternal effects; and epigenetic transmission across the germline (Drake and Liu 2009; Thayer and Kuzawa 2011). The result is a cycle that enables the intergenerational persistence of health disparities, where poor parental health begets poor offspring health and onward (Adelson 2005;

Gravlee 2009; Kuzawa 2008). In turn, the biological consequences of marginalization can shape culture as well.

While modern research has demonstrated that the health disparities between white and black Americans are largely the result of historic differences in discrimination, power, education, and resource availability, early schools of thought considered that black Americans were genetically inferior. Ideas of genetic inferiority supported and reinforced the continued marginalization of black Americans and persistence of health disparities (Krieger 2001). This example demonstrates the powerful ways in which social experiences shape biology and how the intergenerational transmission of poor health outcomes can have long term, biocultural impacts.

In the field of bioarchaeology, a potential first step in engaging with the heritable nature of health and disease is the reconstruction of kinship groups (Gowland 2016). Kinship is a fundamental unit of human relation and interaction, and kinship systems serve as the foundation of social structures in societies past and present. Kinship reconstruction, therefore, is fundamental to both determining biological relationships within a skeletal assemblage and assessing an important social determinant of health.

Given the central importance of kinship in shaping human experience, anthropological research has long explored expressions of kinship and variation in kinship systems (Evans-Pritchard 1951; Lévi-Strauss 1969; Malinowski 1913; Radcliffe Brown 1930-1931). Early ethnographic work focused primarily on biological relatedness, and Western notions of biologically defined kinship were accepted as the normative kinship ideology until the 1960s when critics pushed back against the biological determinism and Eurocentrism inherent to anthropological conceptualizations of kinship (Collier and Yanagisako 1987; Klapisch-Zuber 1991; Schneider 1984). Sociocultural anthropologists have since called for more nuanced and

culturally relativistic conceptions of relatedness that emphasize socially defined relationships as much as biological relationships (Carsten 2000; Lévi-Strauss 1984; Sahlins 2013).

Although biological and sociocultural approaches to kinship have diverged due to different emphases, new theoretical and methodological frameworks aim to incorporate the diverse, complex biological and social relationships that structure kinship systems (Chapais et al. 2014; Ensor et al. 2017; Johnson and Paul 2016). Bioarchaeology is particularly well-suited to kinship studies as osteological evidence can be combined with archaeological remains and interpreted in light of ethnographic research and sociocultural theoretical frameworks to reconstruct the biological and cultural aspects kinship systems and broader social structures.

The theoretical model underlying bioarchaeological kinship studies relies on evolutionary mechanisms (Stojanowski and Schillaci 2006). Human phenotypic variation reflects underlying genetic variation, and as populations exchange mates and increase gene flow, the increased genetic similarity results in greater phenotypic similarity. Kinship analysis, also known as intracemetery or intragroup analysis, utilizes skeletal or dental phenotypic similarities to identify biological distance (biodistance) in mortuary contexts (Alt and Vach 1995, 1998; Alt et al. 1997; Stojanowski and Shcillaci 2006).

For clearly delimited mortuary contexts, such as a tomb or cave, biological variation is compared within the small grave to determine whether the burial group represents biological kin (Alt and Vach 1998; Sjøvold 1976; Sjøvold 1977). Similarly, structured spatial analysis uses broader cemetery structures, such as burial clusters or distinct sectors, in conjunction with cultural attributes and phenotypic variation to identify biological relationships (Howell and Kintigh 1996; Jacobi 1997; Velasco 2018). Unstructured spatial analysis does not require *a priori* assumptions about biological relatedness based on mortuary features; instead, clusters of

phenotypic similarity that may represent biological relationships are identified, although further validation is required (Alt and Vach 1995; Vach and Alt 1993).

Small grave and structured spatial analysis have dominated bioarchaeological kinship studies (Gamba et al. 2011; Meyer et al. 2012; Stojanowski and Larsen 2013) and due to clearer mortuary patterning, these types of analysis have great potential for revealing both biological and social relationships that constitute kinship systems (Stojanowski and Schillaci 2006). At Çatalhöyük, a Neolithic site in modern Turkey, Pilloud and Larsen (2011) found that biological distance did not influence residential burial practices underneath house floors. Instead, these burial practices may have reflected fictive relationships that pulled together individuals throughout the community for various economic, sociopolitical, and/or religious practices.

A nuanced analysis of Late Intermediate Period (CE 1100-1450) Andean above-ground sepulchers revealed that different aspects of kinship systems were emphasized through different symbolic practices (Velasco 2018). Mortuary traditions reinforced biological relationships through the burial of biologically distinct groups in separate sepulchers, whereas patterns of cranial modification cut across these boundaries and may have represented higher levels of *ayllu* or socially defined affiliation. These studies demonstrate the ability of bioarchaeological approaches to integrate multiple lines of evidence and rigorously test hypotheses to fully understand the complexities of kinship structure and broader social structures in past populations.

Phenotypic variation can also reveal differential movement between the sexes as a result of marriage and postmarital residence practices (Corruccini 1972; Lane 1977). While marriage and postmarital residence practices are defined by cultural traditions, they shape biological relationships by determining gene flow within and between populations, and population genetics

models have demonstrated that these patterns of movement can result in measurable phenotypic differences (Ensor et al. 2017; Konigsberg 1987; Konigsberg 1988). For example, greater variation among males than females indicates uxorilocality, whereas greater variation among females than males suggests virilocality (Konigsberg 1988; Schillaci and Stojanowski 2003). Bioarchaeologists use ethnographic analogies to make further inferences about social structures, such as gendered divisions of labor, regional interactions, and resource control (Schillaci and Stojanowski 2003; Velasco 2018). Recent work by Ensor and colleagues (2017) has coalesced ethnographic observations of marriage systems and postmarital residence patterns into clear models for interpreting kinship systems from patterns of phenotypic variation.

Bioarchaeological analyses have increasingly leveraged biological relatedness to answer questions about systems governing social relatedness (Ensor et al. 2017). Cook and Aubry (2014) explore diachronic postmarital residence practices in the Middle Ohio Valley. Differences in phenotypic variation among the sexes over time indicate shifting uxorilocal, virilocal, and multilocal residence patterns, and Cook and Aubry (2014) argue that postmarital residence practices changed depending on individual opportunity at the time.

At the Neolithic and Early Bronze Age cemetery of Tsepi, Attica, Prevedorou and Stojanowski (2017) used biological distance analysis to determine how biological relationships and postmarital residence patterns shaped cemetery spatial organization. Results suggest biological relatedness, especially among women, determined burial placement, whereas multiple burial reflected both biologically and socially defined relationships. Prevedorou and Stojanowski (2017) interpret low female variation as evidence of exogamous marriage and uxorilocal postmarital residence, which correlates with ethnographic evidence of male mobility in coastal

communities. These demonstrate how careful consideration of biological and material evidence, in conjunction with ethnographic analogies, offers insight into multiple levels of social structure.

3.6 Summary

In this interdisciplinary framework, the embodiment of environmental cues during early life shapes the development of major organ and physiological systems. Insults and physiological stressors, such as malnutrition, lead to energetic tradeoffs that compromise development and lead to impaired functioning. Given its importance to organism survival, as well as the high energetic cost of development and activation, the immune system is particularly sensitive to environmental cues and can be greatly impacted by adverse development.

Studies have demonstrated that adverse early life experiences are associated with impaired immune function later in life and the development of pro-inflammatory phenotypes. Inflammation exacts significant immunopathological and energetic costs that contribute to individual allostatic load, and considerable research has linked inflammation to the etiology of numerous chronic diseases and increased mortality risk. This research uses osteological markers as proxies to measure developmental experiences and immune activity to examine the pathways contributing to the developmental origins of mortality risk in Pre-Columbian Panama. Additionally, biocultural kinship was determined through an analysis of dental metric and non-metric traits to provide a foundation for future studies on the embodiment of social determinants and the intergenerational transmission of health and disease.

Table 3.1 Summary of Molecular Functioning in Skeletal-Immune System Cross-talk

Molecule	Main Producer	Immune Effects	Skeletal Effects
IL-1	Macrophages, DCs	Induces immune response and pro-inflammatory signaling	Promotes osteoclastogenesis and RANKL signaling
IL-4	T_H2, natural killer T	Induces humoral immunity	Inhibited osteoclastogenesis and RANKL signaling, promotes OPG expression
IL-6	T_H2, DCs	Pro-inflammatory signaling, induces T_H17 cell differentiation	Promotes osteoclastogenesis and RANKL signaling
IL-7	Stromal cells, DCs	Induces T and B cell activation, pro-inflammatory signaling	Promotes osteoclastogenesis and RANKL signaling
IL-10	T_H2	Anti-inflammatory signaling	Inhibited osteoclastogenesis and RANKL signaling
IL-12	Macrophages, DCs	Induces T_H1 cell differentiation and immune response	Inhibited osteoclastogenesis
IL-17	T_H17, memory T cells	Pro-inflammatory signaling	Promotes osteoclastogenesis, RANKL signaling, and bone resorption
IL-18	Macrophages, DCs	Induces T_H1 cell differentiation and immune response	Inhibited osteoclastogenesis and RANKL signaling, promotes OPG expression
IL-23	Macrophages, DCs	Induces T_H17 differentiation, pro-inflammatory signaling	Promotes osteoclastogenesis and RANKL
RANKL	T cells, osteoblasts	Induces DC differentiation and survival, promotes B cell proliferation, pro-inflammatory signaling	Induces osteoclastogenesis and bone resorption

TNF-α	Macrophages, T_H1	Induces immune response, pro-inflammatory signaling	Induces osteoclastogenesis, promotes RANKL signaling; Inhibits osteoblastic activity and bone formation
TGF-β	Macrophages	Inhibits T cell activation, anti-inflammatory signaling	Promotes osteoblastogenesis and promotes bone formation; Inhibits osteoclastic activity
M-CSF	Stromal cells, osteoblasts	Induces monocyte and macrophage differentiation	Promotes osteoclastogenesis and RANKL signaling
GM-CSF	T_H1	Induces granulocyte differentiation	Inhibited osteoclastogenesis and RANKL signaling
IFNγ	T_H1, natural killer	Induces cellular immunity and immune response	Inhibited osteoclastogenesis and RANKL signaling

Adapted from (Ginaldi and De Martinis, 2016; Takayanagi, 2007)

CHAPTER 4: STUDY DESIGN, DATA COLLECTION, MEASURES, AND ANALYSIS

4.1 Study Aims

This project aims to examine the pathways through which environmental cues and social identities, such as kinship, shape developmental plasticity and in turn determine immune activity and subsequent mortality risk later in life. This project, focused on skeletal assemblages from the Greater Coclé region of Panama, has three specific aims:

Aim 1: to reconstruct kinship groups through a biological distance analysis and assess the importance of biological and social relationships in structuring social identities to determine the role of kinship in shaping health disparities following the emergence of inequality during the Ceramic Period in the Greater Coclé region

Aim 2: to reconstruct developmental phenotypes from osteological markers of developmental stress and assess how developmental exposures impact later life mortality outcomes in the Greater Coclé region

Aim 3: to assess the range of pro-inflammatory immune activity in Pre-Columbian Panama and explore the role of inflammation as an important physiological pathway linking developmental stress to later life mortality outcomes

In alignment with these aims, this project employs a range of osteological and statistical methods, which are described in detail in this chapter.

4.2 Study Sample

Cerro Mangote (CO-40)

The Cerro Mangote site is a shell midden on the northern slope of a large hill, on the north bank of the Santa Maria River (Figure 2.1). Radiocarbon dating of charcoal (6014-5215 cal BCE) from the earliest cultural deposit confirmed Cerro Mangote was one of the earliest habitation sites in the Panama (Ranere 1980). When Charles R. McGimsey III first excavated the site in 1955 and 1956 as part of a program of survey and test excavations in southwestern Panama, Cerro Mangote was the only Preceramic site identified in Panama, and it greatly broadened the understanding of Pre-Columbian development (McGimsey 1956; McGimsey et al. 1987). Re-excavations led by Temple University took place in 1979 to acquire additional data and further refine the understanding of the site (Ranere 1980).

Initial occupation of the site began around 6,000 BCE, a decision likely impacted by changes in coastal ecology that gave rise to mudflat, mangrove swamp, and lagoon habitats easily exploited by inhabitants (Huard 2013; Ranere 1980). Despite evidence of significant marine resource utilization in midden contexts, phytolith remains and plant processing tools indicate that cultivars, including maize, contributed to the diet of Cerro Mangote inhabitants (Ranere 1980). Initial interpretations proposed Cerro Mangote was a seasonal site for the collection of marine resources during the dry season (Griggs 2005; Norr 1995). However, recent analysis of white-tailed deer remains recovered from Cerro Mangote demonstrate that hunting occurred at Cerro Mangote during the wet season and indicate that year round occupation was

possible at the site (Martínez-Polanco et al. 2020). Evidence from contemporaneous sites like Cueva de los Ladrones also suggest that trade routes connected inland and coastal sites and moved goods between the two habitat zones, and the recovery of a manatee bone from Cerro Mangote indicates that these networks may have extended to communities on the Caribbean coast (Carvajal-Contreras and Hansell 2008; Cooke 2005; Cooke and Sanchez 2004; Huard 2013). Cooke (2005) argues that the supply of diverse dietary and material resources likely enabled the year-round occupation and mixed subsistence strategies of Cerro Mangote and other coastal sites. Eventually, the site was abandoned as coastal progradation, due to heavy sediment flow down the Santa Maria River, pushed the coast further away and other locations became more favorable (Ranere 1980).

McGimsey's excavations of the Cerro Mangote site recovered the remains of 76 individuals, and an additional 18 individuals were recovered from various contexts during the 1979 excavations (McGimsey 1956; Ranere 1980). The burial program at Cerro Mangote was variable and complex; burial types ranged from primary flexed burials and a range of secondary burials containing disarticulated, flexed, and bundled individuals often in large numbers (McGimsey 1956; McGimsey et al. 1987). These different burial types appeared together in many contexts, and primary flexed burials were frequently associated with secondary burials. The small number of funerary items recovered from the site were carved shell beads or ornaments, and they mostly accompanied juvenile individuals (McGimsey 1956; Norr 1991).

While the bulk of interpretations regarding Cerro Mangote have relied on archaeological evidence, Lynette Norr (1991) did examine a sample of the Cerro Mangote osteological assemblage as part of her dissertation research on subsistence strategies and nutritional status of prehistoric, tropical populations in Central America. She performed an analysis of carbon and

nitrogen stable isotope ratios on 36 individuals, as well as a limited paleopathological analysis of 75 individuals. The stable isotope evidence indicated a maize-heavy diet with contributions from terrestrial and marine protein. Despite the abundance of faunal remains at the site, Norr (1991) notes a moderate prevalence of porotic hyperostosis, linear enamel hypoplasias, and periosteal lesions indicative of malnutrition, infection, and physiological stress. She interpreted these results as evidence of a population experiencing low to moderate levels of stress likely related to seasonality, diet, and health status (Norr 1991).

Aimee Huard's (2013) reanalysis of the assemblage reached similar conclusions, and she attributed the low to moderate prevalence of pathological conditions and physiological stress markers to a population of average health. However, Huard (2013) argued the lack of caries indicates a low-carbohydrate diet, and she combined this evidence with a reinterpretation of Norr's isotopic data to suggest that Cerro Mangote inhabitants had a marine-based diet with more limited maize contribution than previously accepted (Norr 1991; Norr 1995).

Ashley Sharpe and colleagues (2021) recently published their preliminary study of carbon, nitrogen, oxygen, and strontium isotopes at several Panamanian sites to explore diet and mobility patterns. They sampled two individuals from Cerro Mangote dated to 2476- 2293 BCE. Sharpe and colleagues (2021) resolved a key technical issue with the thiourea standard used in Lynette Norr's original analysis, and their new results show that Cerro Mangote inhabitants had the lowest $\delta^{13}C_{co}$ and $\delta^{15}N$ values, which indicates that individuals consumed little to no marine food in comparison to other communities. Instead, Cerro Mangote inhabitants consumed primarily terrestrial meat, although at lower levels than other communities, and maize, as well as some C_3 plant foods such as tubers, fruits, and squash.

The strontium and oxygen isotopes also revealed unexpected results. The two tested individuals had a higher than expected $^{87}Sr/^{86}Sr$ value, indicating the individuals may not have been born in the Santa Maria floodplain area. Although the human remains at Cerro Mangote represent a lengthy period of time (2480 BCE- 317 CE) and multiple possible occupations by differing groups, these results lend further support to the hypothesis that the site functioned as a specific cemetery site for communities within the Santa Maria River region during the Early and Middle Ceramic period.

Sitio Sierra (AG-3)

The Sitio Sierra site occupies a low hill above the Santa Maria River floodplain (Figure 2.1) Richard Cooke undertook excavations at the site in 1971, 1973, and 1975, and a total of thirteen contexts were opened in three different areas of the site to determine the site's occupation history and position within the cultural trajectory of the Gran Coclé region (Cooke 1972, 1977). Excavators found two sequences of occupation at the site containing numerous domestic structures, middens, and burial contexts.

While faunal remains suggest that inhabitants hunted and traded for a diverse range of terrestrial and marine protein, the macrobotanical evidence, including the remains of three different maize varieties, highlights the important role of cultivars in the diet of Sitio Sierra inhabitants (Cooke 1984; Cooke 1995; Dickau 2010). Numerous manos and metates used to process corn further emphasize the agricultural focus of the community. The cane and palm thatched houses and intensive agricultural practices of the Sitio Sierra inhabitants represent the Middle Ceramic period cultural shift towards nucleated settlements on fertile river floodplains (Cooke 1984).

Burial contexts were found in both sequences of occupation. The cemetery in Sequence 1 contained 34 individuals, and radiocarbon dating suggests the burial context was utilized from 39 BCE- CE 561. The later cemetery contains another sixteen individuals dated to the end of the site occupation from CE 978-1158 (Cooke 1972, 1977, 1979). The disturbance of lower interments indicates potential burial reuse by social or kin groups and cultural continuity with the burial practices observed at Cerro Mangote. Funerary items were mostly utilitarian items, and Cooke (1979, 1984) interpreted the presence of certain tools like chisels and sharpeners as evidence for special activities or roles within the community that preceded more intensive craft specialization. Similar to the Cerro Mangote mortuary assemblage, the few elaborate funerary items consisted of shell and pyrite beads, as well as ceramics with painted or plastic decorative motifs and worked faunal bone.

Most published examinations of the Sitio Sierra skeletal remains were not performed by trained osteologists until Lynette Norr's (1991, 1995) inclusion of the assemblage in her dissertation research. She performed a stable isotope analysis on a sample of 34 individuals, as well as a limited paleopathological examination of periosteal lesions, porotic hyperostosis, and linear enamel hypoplasias on 37 individuals. Stable isotope ratios confirmed that the Sitio Sierra inhabitants pursued agricultural subsistence strategies and consumed a lot of maize, although the low prevalence of severe porotic hyperostosis in both burial contexts suggests that most inhabitants consumed enough protein in their diet. The moderate prevalence of periosteal lesions indicative of infection in the early and later burial contexts was comparable to the prevalence at Cerro Mangote. Norr (1991), however, found that linear enamel hypoplasia occurred in shorter intervals in the Sitio Sierra assemblage, and she argued that the inhabitants experienced a pattern

of moderate but more frequent physiological stress likely tied to changes in subsistence strategies, population size, and settlement trends.

Eight individuals from both Sitio Sierra cemetery contexts were also re-tested by Sharpe and colleagues (2021). Compared to Cerro Mangote, the Sitio Sierra inhabitants consumed more meat and more maize, results consistent with archeofaunal remains and the agricultural focus of the community. Individuals from the earlier occupation have significantly different $\delta^{13}C_{co}$ values than the later occupation, however, and this difference may be due to greater maize and marine food consumption during 39 BCE- CE 561.

These results suggest that the early Sitio Sierra inhabitants consistently consumed marine resources and likely participated in exchange networks linking inland and coastal sites to acquire these food items. Strontium isotopes also distinguish the early and late populations; individuals from the earlier occupation have higher $^{87}Sr/^{86}Sr$ values, whereas individuals from the later occupation fall below the baseline average. These results indicate a degree of mobility within the Santa Maria River region, and Sharpe and colleagues (2021) suggest Sitio Sierra may have served as a cemetery for people living near the site similar to Cerro Mangote.

4.3 Age Estimation

Data Collection. Adult age estimation followed Boldsen and colleagues' (2002) transition analysis method, which involves the analysis of several features on the pubic symphysis and auricular surface, as well as cranial vault suture closure. A total of 19 features were assessed and scored using descriptions and images from the Transition Analysis handbook, included in Appendix A. Juvenile age was estimated using dental development and eruption (AlQahtani et al. 2010), as well as epiphyseal closure and post-cranial metrics (Cunningham,

Scheuer and Black 2016). Preference was given to dental age estimates due to the strong correlation between dental development and chronological age (Hillson 2014).

Data Analysis. Adult Transition Analysis scores were input into the Anthropological Database, Odense University (ADBOU) Age Estimation software, which generates point age at death estimates and confidence intervals using: a) prior distribution of age at death estimated from 17th century Danish rural parish records; and b) the conditional probability of age indicators given known age at death estimated from the Terry Collection (Milner and Boldsen 2012).

4.4 Sex Estimation

Data Collection. For all individuals older than 16 years of age, sex was estimated through cranial morphology, pelvic morphology, and post-cranial metrics. Following standards, five cranial morphological features, including nuchal crest, supraorbital ridge, mental eminence, supraorbital margin, and mastoid processes, were given a score of 1-5 (Buikstra and Ubelaker 1994). A similar score of 1-5 was assigned to describe the morphology of the ventral arc, subpubic concavity, and ischiopubic ramus of the pubis bone(s) following Klales and colleagues (2012), as well as the sciatic notch and preauricular sulcus (Buikstra and Ubelaker 1994). When possible, the following measurements were also taken: humeral head diameter; femoral head diameter; clavicle maximum length; and humeral midshaft diameter (Moore et al. 2016).

Data Analysis. Scores derived from the Buikstra and Ubelaker (1994) standards were averaged for a composite cranial morphology score and a pelvic morphology score. Pubic bone morphology scores were plugged into Klales and colleagues' (2012) logistic regression to calculate the probability of the individual being male or female, and a corresponding score of 1 (female), 3 (indeterminate) or 5 (male) was assigned. Post-cranial measurements were assessed

via sectioning point or discriminant function analysis using Moore and colleagues' (2016) standards for modern Colombian populations. Individuals were scored as 1 (female), 3 (indeterminate) or 5 (male). The scores for each category were compared to determine a final sex estimation, with preference for the pelvic morphology score, and individuals were designated as: female (1); probably female (2); indeterminate (3); probable male (4); and male (5).

4.5 Developmental Phenotype Markers

The following standard measures of developmental stress were collected in order to reconstruct the developmental phenotype of individuals aged two years or older at time of death who had therefore completed critical developmental stages (Barker 2012). Some of these indicators represent age-specific stress events, such as linear enamel hypoplasia, vertebral neural canal size, and dental root size, while measures like dental fluctuating asymmetry capture general developmental stress. By incorporating multiple skeletal indicators, this study provides a more complete representation of growth and developmental stress throughout infancy, childhood and adolescence.

Data Collection. Linear enamel hypoplasia, vertebral neural canal dimensions, dental size, and dental asymmetry were recorded for each individual in the sample when possible. Linear enamel hypoplasias (LEH) were identified on the labial surfaces of all mineralized and minimally worn anterior mandibular and maxillary teeth with a lighted stereomicroscope. Individuals were scored as "present" (1) if a defect was visually matched on two or more teeth. Vertebral neural canal (VNC) dimensions in the anterior-posterior diameter and the transverse diameter were measured to the nearest 0.01 mm for vertebrae T10-L5 (Clark et al. 1986; Hinck et al. 1966; Watts 2013, 2015). Vertebrae with postmortem or pathological damage were

excluded (Watts 2015). Maximum mesiodistal and buccolingual root dimensions (Aubry 2014; Hillson et al. 2005) at the cemento-enamel junction were recorded to the nearest 0.01 mm with Mitutoyo 573 Absolute Point calipers for all non-polar teeth (maxillary second incisors, mandibular first incisors, and mandibular and maxillary fourth premolars and second molars), excepting in cases of extreme wear or incomplete mineralization. These measurements were used in both the size and fluctuating asymmetry analysis.

Data Cleaning. All developmental marker variables were turned into binary categorical variables due to the small sample size and data scarcity resulting from preservation issues. No data transformation was required for LEH as a binary categorical variable. To calculate a composite score of VNC growth, the sample was first split into two age groups: juveniles less than 14 years of age, whose spinal development was not complete, and individuals 15 years of age or older. Each diameter measurement (anterior-posterior and transverse) per vertebrae was first assessed for normality and heterogeneity of variance using Shapiro-Wilks and Levene's tests, respectively.

When assumptions were satisfied, Student's independent two sample t-tests were used to identify any significant differences in average diameter scores based on site and sex per age group sample, and Mann-Whitney non-parametric tests were used for variables with non-normal distributions. The adult L3, L4, and L5 anterior-posterior variables had to be sorted by sex. Following another normality test and log-transformation of necessary variables (adult T10 anterior-posterior measurement), Z-scores were calculated for each vertebral diameter per age group, and individuals received one of the following binary categorical scores: 1 if one or more VNC dimensions were below average size (> 1 SD below mean for sample); and 0 if all VNC dimensions were average or above average size (< 1 SD below mean for sample).

A similar procedure was used to create a composite score for root size. The left side measurements were used preferentially, and antimere measurements were substituted when necessary. After splitting the sample into juvenile (0-14 years) and adult (14.1+ years) categories, each root measurement (mesiodistal and buccolingual) per tooth was assessed for normality and heterogeneity of variance using Shapiro-Wilks and Levene's tests, respectively. Student's t-tests and Mann-Whitney tests were used to identify significant differences in measurement scores based on site and sex per age group.

The adult Upper 4th Premolar mesiodistal root (UPM4-MDR), Lower 2nd Molar mesiodistal root (LM2-MDR), Lower 2nd Molar buccolingual root (LM2-BLR), Lower 4th Premolar mesiodistal root (LPM4-MDR), and Lower 4th Premolar buccolingual root (LMP4-BLR) measurements had to be divided by sex. Following another normality test, the adult female UPM4-MDR and male LPM4-MDR measurements were log-transformed. Z-scores were calculated for each root measurement per age group, and individuals received one of the following binary categorical scores for their anterior teeth (incisors) and posterior teeth (premolars, molars): 1 if one or more root dimensions were below average size (> 1 SD below mean for sample); and 0 if all VNC dimensions were average or above average size (< 1 SD below mean for sample).

To calculate dental asymmetry, the difference between left and right non-polar antimeres (d= |L-R|) was calculated for each measurement per tooth. The differences were averaged to provide an estimate of mean FA per individual (Garn, Lewis, and Kerewsky 1966, 1967; O'Donnell and Moes 2020). The variable distribution was non-normal, even following transformations, so a univariate sectioning point based on the average of individual mean FA

scores was used to assign binary categorical scores for each individual; 1 for above average asymmetry, 0 for low or average asymmetry.

Data Analysis. The five total developmental marker scores (LEH, VNC, anterior root size, posterior root size, and dental fluctuating asymmetry) were assessed with a latent class analysis in Mplus, a comprehensive mixture modeling program that uses maximum likelihood estimation to handle missing data common in bioarchaeological datasets (Muthén and Muthén 2019). Three models with different specified latent classes were compared, and goodness of fit measures (Adjusted Bayesian Information Criterion, loglikelihood, and entropy) were used to determine the most appropriate model for the data. See Appendix D for scores input into Mplus and the posterior probabilities generated for each individual, as well as their final class assignment.

4.6 Immune Phenotype

Inflammation impacts the function of numerous physiological systems, and the predominant skeletal effect of immune activation and inflammation is decoupled bone remodeling (Berger, Dent, and Griffin 2020; Klaus 2014). During periods of active inflammation, circulating pro-inflammatory cytokines stimulate osteoclast differentiation and local and systemic bone resorption, while simultaneously inhibiting osteoblast activity (Matzelle et al. 2012; Walsh et al. 2018). The resolution of inflammation promotes osteoblast differentiation and local bone formation, but continued systemic inflammation can lead to recurrent episodes of inflammatory bone loss and formation (Klaus 2014; Matzelle et al. 2012). Immune phenotypes will be reconstructed from multiple skeletal indicators that reflect oral, joint, and bone inflammation.

Data Collection. Individual remains were first examined for periosteal lesions. Following a differential diagnosis (Ragsdale and Lehmer 2012), non-specific periosteal lesions were scored to reflect the inflammatory activity: 0 for lesion absence; 1 for periosteal lesions of active or remodeled proliferative bone, which is formed after the resolution of inflammation; and 2 for periosteal lesions with active resorption OR mixed lesions with multiple phases of bone resorption and formation, indicative of the active, destructive phase of inflammation (Buikstra and Ubelaker 1994; Matzelle et al. 2012). The location of lesions was also noted; individuals were scored as 1 if they had systemic non-specific periosteal lesions affecting two or more bones throughout the body and 0 if they had no lesions or lesion(s) affecting only one bone.

Four measures of oral inflammation were collected: periodontal disease (PD) progression; carious lesions; pulp exposure; and antemortem tooth loss (AMTL). Periodontal disease was scored as present (1) if alveolar crest reduction exposed more than 50% of a single tooth root and/or subgingival calculus was observed on a single tooth (Lieverse 1999; Listi 2011; Lukacs 1989). Following standards (Buikstra and Ubelaker 1994; Hillson 2018), caries presence (1) was scored if an individual exhibited a carious lesion on any tooth, and pulp exposure was scored as present (1) if a clear cavity penetrated the pulp chamber of the tooth. AMTL was marked as present (1) if one or more teeth were absent and showed evidence of alveolar bone remodeling, excluding individuals with dental trauma, congenital tooth absence, and impacted teeth (Hillson 2018). For all analyses, damaged alveolar bone and teeth were not assessed.

To assess osteoarthritis (OA), all observable joints were examined and differential diagnosis of joint changes followed Waldron (2009). OA was recorded as present (1) if one or more non-vertebral joints were affected. The number of OA sites was also recorded to capture the scale of inflammatory joint activity. The spinal joints, including all costal, transverse, and

articular facets, were compiled into one site to minimize preservation biases, and all other joints counted as a single site.

Data Cleaning. The OA site number variable was the only continuous variable that required cleaning. A Shapiro-Wilks test was used to assess normality, and the distribution was non-normal even after data transformation. As a result, a univariate sectioning point was used to dichotomize scores as average (1= 6-10 affected joints) or above average (2= 11+ affected joints). Individuals without OA were scored as 0.

Data Analysis. The eight immune marker scores (periosteal lesion presence, periosteal lesion location, PD presence, caries presence, pulp exposure, AMTL presence, OA presence, OA site number) were also assessed with a latent class analysis in Mplus (Muthén and Muthén 2019). Three models with different specified latent classes were compared, and goodness of fit measures (Adjusted Bayesian Information Criterion, loglikelihood, and entropy) were used to determine the most appropriate model for the data. See Appendix E for scores input into Mplus and the posterior probabilities generated for each individual, as well as their final class assignment.

4.7 Biological Distance

Biodistance analyses have been employed widely in skeletal samples around the world, and research has demonstrated that dental phenotypes are strongly correlated proxies for neutral genetic markers (Pilloud and Kenyhercz 2016). Due to shared genes and shared environments, family members are more phenotypically similar and share a similar dental size, shape, and presence of morphological anomalies or variants (Stojanowski and Schillaci 2006). While close

relationships cannot be accurately determined, biodistance analysis can differentiate broader patterns of genetic affiliation (Stojanowski and Hubbard 2017; Stojanowski and Schillaci 2006).

Data Collection. The biodistance analysis included the following polar teeth: maxillary first incisors; mandibular second incisors; maxillary and mandibular canines; maxillary and mandibular third premolars; and maxillary and mandibular first molars. The left dentition was used preferentially, but antimeres were substituted when necessary. Heavily worn or damaged teeth were excluded from the analysis. Thirty-eight morphological traits were scored using the Arizona State University Dental Anthropology System (Turner et al., 1991), and 32 mesiodistal and buccolingual root dimensions at the cemento-enamel junction were measured to the nearest 0.01 mm with Mitutoyo 573 Absolute Point Calipers (Hillson et al, 2005; Stojanowski 2007). The complete list of all dental variables and scoring ranges are provided in Appendix B. To assess intraobserver error, metric and nonmetric observations were repeated for five individuals each week for three weeks at the beginning and middle of the data collection (Appendix C).

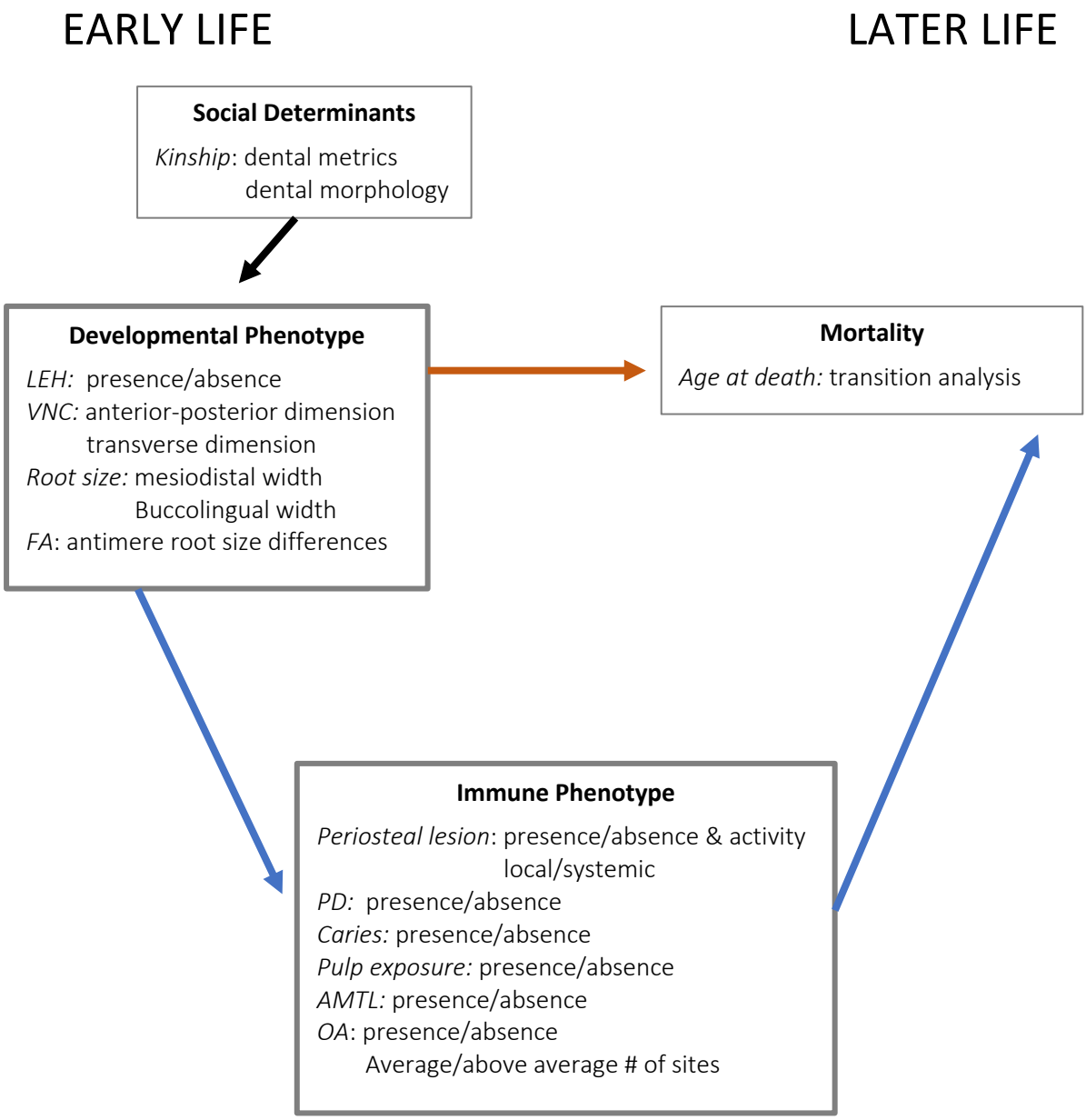
Data Cleaning. Following standards, variables with less than 75% observations were removed, as were individuals with fewer than 50% of their teeth assessed (Stojanowski and Hubbard 2017). The remaining missing observations were computed with multiple imputation, which uses regression models of observed values to predict values of data Missing at Random (MAR). The R packages *MICE* and *sjmisc* were used to compute missing metric and morphological values from the averages of appropriate predictive means matching (metric variables) and proportional odds models (morphological variables) (Collins, Schafer, and Kam 2001; Lüdecke, D. 2018; van Buren and Groothuis-Oudshoorn 2011).

Student's two sample t-tests and Levene's test of homogeneity of variance revealed no significant differences in the means or variances of the metric variables when comparing the

imputed and original datasets. Chi-square tests similarly found no significant differences in the distribution of the morphological variables in both datasets. The imputed metric variables were Q-mode transformed to eliminate the allometric and sexually dimorphic effects of tooth size (Corruccini 1973; Powell 1995). Measurements with non-normal distributions were removed (Appendix B contains final variable list), and a principal components analysis (PCA) was performed on the transformed metric variables to control for inter-trait correlation (Pilloud and Larsen 2011). Principal components (PC) with an eigenvalue greater than or equal to one were retained (n=5 PC Cerro Mangote, n= 4 PC Sitio Sierra) (Stevens 2012).

Data Analysis. Gower similarity was calculated for all pairwise combinations of individuals to measure phenotypic resemblance. The R *Cluster* package calculates Manhattan distance for continuous data and the Dice coefficient for categorical data, and the results were scaled between zero and one to generate a Gower coefficient (Gower 1971; Maechler et al. 2019). Gower coefficients were compared with Student's two sample independent t-tests (or Kruskal-Wallis tests of independent measures for non-parametric data) to test biological relatedness within sites and k-medoids cluster analysis to assess biological relatedness between the sample sites (Maechler et al. 2019). The square root of each coefficient was taken following Gower (1966, 1971) to plot distances in Euclidean space with multidimensional scaling (MDS). Finally, variance-covariance matrices were calculated using the retained principal component scores of the imputed and transformed metric variables (Konigsberg 1988; Morita et al. 2011; Schillaci and Stojanowski 2003).

Figure 4.1 Conceptual Model Summary



Note: Research Aim 1 denoted by the red arrow, Research Aim 2 by blue arrows, and Research Aim 3 by the black arrow. Bolded boxes are constructs that were defined by a latent class analysis of the listed observed variables.

CHAPTER 5: PRE-COLUMBIAN KINSHIP SYSTEMS AND COMPLEX MORTUARY TRADITION IN THE GREATER COCLÉ REGION, PANAMA

5.1 Introduction

Kinship is fundamental to social organization across cultures, and anthropological inquiry throughout the last century has explored the various ways in which kinship systems shape social structure, identity and power (Evans-Pritchard 1940; Sahlins 1961). Mortuary tradition provides an important arena for the manifestation and negotiation of both biological and cultural kinship ideologies (Gillespie 2001; Goldstein 1981; Joyce 2001).

In Pre-Columbian Panama, the nature of kinship and its role in mortuary tradition has been explored primarily through the excavation of elite cemetery sites like Sitio Conte, El Hatillo, and El Caño (Briggs 1989; Haller 2004; Locascio 2010; Torné et al. 2020; Stirling 1949). Those elaborate burials of richly adorned central figures surrounded by secondary individuals, as well as other mortuary practices like burial reuse, correspond to Spanish ethnohistoric accounts of chiefly burials (Jopling 1994:63-64; Linares 1977; Lothrop 1937; Mason 1942; Oviedo 1944). Subsequent interpretations of Pre-Columbian social systems have emphasized hierarchy and complex mortuary traditions centered on the veneration of high-status individuals who may have included regional ancestors, mythical figures, and religious or sociopolitical leaders (Cooke and Sanchez 2004; Isaza 2007).

Kinship ties have particular cultural and religious importance among modern Chibchan-speaking groups as well, and strict mortuary rituals require the burial of kin groups in communal graves (Hoopes 2005). Although no direct evidence ties the Pre-Columbian peoples to any

specific surviving ethnolinguistic group, archaeological and ethnolinguistic research suggests these ancient Panamanian populations shared certain cultural affinities with modern Chibchan-speaking groups who have long inhabited the Isthmo-Colombian Area (Hoopes 2005; Hoopes and Fonseca 2003). Researchers have argued that Pre-Columbian mortuary practices may represent similar forms of ancestral and kinship veneration to those practiced by Chibchan-speaking groups.

Although these interpretations of Pre-Columbian kinship systems and mortuary traditions have substantial archaeological and ethnographic support, these assumptions have yet to be tested in the Greater Coclé region with biological evidence. Capodiferro and colleagues' (2020) recent genomic analysis of Pre-Columbian skeletal remains in the Greater Darien region and the lack of genetic relatedness in two multiple burials from Panamá Viejo highlights the need to further examine the relationship between biological relatedness and mortuary traditions.

The bioarchaeology of kinship provides a robust framework for exploring the intersection of biologically and socially-defined relationships and the role of kinship systems in shaping mortuary tradition (Ensor et al 2017; Stojanowski and Schillaci 2006). This study explores patterns of relatedness in the Greater Coclé region of Panama and contextualizes current understanding of Pre-Columbian social structures with rigorous biological analysis. Intragroup variation in dental metric and nonmetric traits were analyzed alongside mortuary evidence to infer kinship and postmarital residence practices at two non-elite sites: Cerro Mangote (2476 BCE- CE 231) and Sitio Sierra (39 BCE- CE 561, CE 978-1158) (Figure 1). An analysis of intergroup variation between both sites provided insight into regional interactions. Given the destructive effects of acidic soil and tropical climate on DNA and skeletal preservation in the

humid Panamanian tropics, the dental phenotype approach outlined in this study provides a useful methodological framework for further biological distance analysis in the region.

Figure 5.1 Map of Panama and Archaeological Sites

Locations of study sample sites, Cerro Mangote (CO-40) and Sitio Sierra (Ag3), are noted in bold. Adapted from Wikimedia Commons map - Alexrk2 (Bathymetry: NGDC ETOPO2v2 (public domain); Topography: NASA Shuttle Radar Topography Mission (SRTM30 v.2) (public domain); Shoreline and additional data: VMap-0 (public domain).

5.2 The Bioarchaeology of Kinship

Kinship is one of the most fundamental units of human relation and interaction, and anthropological research has long explored expressions of kinship in past and present societies (Evans-Pritchard 1951; Lévi-Strauss 1969; Radcliffe Brown 1930). While early work focused primarily on normative Western ideologies of biologically-defined kinship, critiques have led to more culturally relativistic conceptions of relatedness that emphasize social relationships as much as biological relationships (Carsten 2000). Synthetic biocultural approaches in

bioarchaeology are particularly well suited to exploring the full range of kinship systems in past societies (Johnson and Paul 2016; Meyer et al. 2012).

The theoretical model underlying bioarchaeological kinship studies relies on simple evolutionary mechanisms (Stojanowski and Schillaci 2006). Human phenotypic variation reflects underlying genetic variation, and as populations exchange mates, increasing genetic similarity results in greater phenotypic similarity. Kinship analysis, also known as intracemetery analysis, utilizes phenotypic similarities to identify biological distance (biodistance) in mortuary contexts (Alt and Vach 1995, 1998; Alt et al. 1997; Stojanowski and Schillaci 2006). The most common type of intracemetery analysis is structured spatial analysis, which utilizes broader cemetery structures, such as burial clusters, in conjunction with cultural attributes and phenotypic variation to identify biological relationships (Goldstein 1980; Howell and Kintigh 1996; Jacobi 1997; Velasco 2018).

Although inherently cultural, marriage and postmarital residence practices shape biological relationships by determining gene flow within and between populations. Subsequent phenotypic differences can reveal differential movement between the sexes (Corruccini 1972; Lane 1977; Konigsberg 1987, 1988). For example, greater variation among males than females indicates uxorilocality, whereas greater variation among females than males suggests virilocality (Konigsberg 1988; Schillaci and Stojanowski 2003). Bioarchaeologists use ethnographic analogies to make further inferences about social structures, such as gendered divisions of labor, regional interactions, and resource control (Ensor et al. 2017; Schillaci and Stojanowski 2003).

5.3 Pre-Columbian Panama and Kinship

Archaeological evidence suggests that human activity in the Greater Coclé region first expanded during the Holocene (Cooke et al. 2013). Paleoecological and subsistence reconstructions indicate that inhabitants began slash-and-burn agriculture in the Early Preceramic Period (9500-6000 BCE) and combined an increasingly diverse range of cultivars, including maize and manioc, with foraged resources (Clary et al. 1984; Cooke and Ranere 1999; Dickau 2010; Piperno and Pearsall 1998; Piperno et al. 2000). Late Preceramic populations also exploited diverse coastal resources as sea levels rose and marine habitats changed (Clary et al. 1984; Cooke and Ranere 1999).

These climactic, environmental and subsistence changes likely contributed to population growth that led to increased settlement size and density in the region. Late Preceramic populations settled primarily in coastal, littoral areas that overlooked streams and rivers (Norr 1991). Due to greater population density, groups may have initiated trade and exchange relationships (Carvajal-Contreras et al. 2008; Cooke 2005; Cooke and Ranere 1999; Zohar and Cooke 1997).

The depletion of soil nutrients, exacerbated by population growth and intensified cultivation, led populations to move to more fertile alluvial bottomlands and develop new means of clearing land and preparing and storing foodstuffs (Cooke 2005; Cooke 1998; Piperno and Pearsall 1998). By 2470 BCE, permanent villages become archaeologically visible and represent a transition towards sedentary lifeways. The growth of nucleated settlements continued into the Middle Ceramic Period (200 BCE- CE 700) as populations increasingly relied on intensive agriculture, craft specialization, and regional trade (Cooke 1995; Isaza 2007; Martín-Rincón and Sánchez Herrera 2007; Martín et al. 2016). Long-standing interactions between populations

likely shaped the rise of large Late Ceramic sites, like Sitio Conte and El Caño, that served specialized roles within the region (Cooke 2005).

Case Studies

Given the challenges with Pre-Columbian skeletal preservation and a dearth of trained osteologists in Panama, comparative bioarchaeological kinship studies are limited. Most biodistance studies in the Isthmo-Colombian Area focus primarily on large scales of biological relatedness related to population movement, such as the peopling of the Americas and the African Diaspora (Huffman 2014; Morales Arce 2017). Intracemetery biodistance analyses that focus on kinship are considerably rarer.

Notably, Huard (2013) performed a spatially structured intracemetery biodistance analysis of 20 individuals from Cerro Mangote and found no evidence of biological relatedness based on burial location within rows of stacked stone columns. There were several methodological issues with this study, however. First, the assumption that the stone columns and burials were associated is not supported by the original excavators (McGimsey 1956; McGimsey et al. 1987), who did not regard the stone column as structural artifacts. The sole use of dental crown measurements also reduced the sample size and limited the amount of phenotypic variation captured by the analysis. As Huard (2013: 215) notes, the model was incorrect and other mortuary features should be considered in future analysis.

Cerro Mangote

The Cerro Mangote site is a shell midden on the northern slope of a large hill along the Santa Maria River. It was first excavated by Charles McGimsey in 1955 and 1956 and re-

excavated by Temple University in 1979 (Ranere 1980). Initial occupation of the site began around 6018-5212 cal BCE as changes in coastal ecology gave rise to new mudflat, mangrove swamp, and lagoon habitats (Huard 2013; Ranere 1980). Evidence from phytolith remains, stable isotope ratios, and processing tools indicate that cultivars, including maize, contributed to the diet (Norr 1991, 1995; Piperno et al. 2011; Ranere 1980). Interestingly, skeletal isotope data from two of the earliest directly dated individuals at Cerro Mangote indicates low meat and marine food consumption, but further testing is needed to assess the diets of individuals from later burials (Sharpe et al 2021). The site was later abandoned as heavy sediment from the Santa Maria River pushed the coast further away and other locations became more favorable (Ranere 1980).

A total of 110 individuals, dated to 2476 BCE- CE 231, were recovered from various contexts (Figure 2). The burial norms at Cerro Mangote were variable and complex, with a range of burial types that compare with other diverse Pre-Columbian mortuary sites in Panama and allude to a regional cultural tradition maintained over a broad timespan (Briggs 1989, Cooke et al. 1998, Rojas-Sepúlveda et al. 2011, Smith-Guzmán and Cooke 2018). The small number of funerary items recovered from the site comprised carved shell beads or ornaments that accompanied mostly juvenile individuals (Norr 1991).

Sitio Sierra

Sitio Sierra encompasses a low hill above the Santa Maria River floodplain, approximately 6 km upstream from Cerro Mangote. Led by Richard Cooke, excavations in 1971, 1973, and 1975 revealed two sequences of occupation containing domestic structures, middens, and burial contexts (Cooke 1972, 1977). Zooarchaeological and archaeobotanical evidence

suggest that inhabitants hunted and traded for a range of terrestrial and marine protein, and diverse cultivars, including beans (Fabaceae) and three maize varieties, made important dietary contributions (Cooke 1984; Cooke 1995; Dickau 2010). Numerous manos and metates used to process maize further emphasize the agricultural focus of the community, and evidence for cane and palm thatched houses represent an important cultural shift towards nucleated settlements on fertile river floodplains during the Middle Ceramic period (Cooke 1984).

Burial contexts were found in both sequences of occupation (Figure 3). The early cemetery contained 34 individuals, and radiocarbon dating suggests the burial context was utilized from 39 BCE- CE 561. The later cemetery contains another 16 individuals and dates to the later period of the site occupation from CE 978-1158 (Cooke 1972, 1977, 1979). Burial disturbance in several instances indicates burial reuse and possible cultural continuity with the mortuary traditions observed at Cerro Mangote. Funerary goods were mostly utilitarian items, and Cooke (1979; 1984) interpreted the presence of tools as evidence for special roles preceding more intensive craft specialization. The few elaborate funerary items consisted of shell and pyrite beads, as well as ceramics with painted or plastic decorative motifs and worked faunal bone.

Study Expectations

Despite limited bioarchaeological kinship analysis in Pre-Colombian Panama, several mortuary features have been attributed to kinship systems, specifically: burial co-interment, secondary burial, and burial reuse. Ethnohistorical accounts document practices of co-internment, the reuse of mortuary space, and the manipulation of remains in mortuary houses (Carvajal et al. 2006; Espinosa 1994:63-64; Martyr D'Anghera 1912:219-220). Archaeological evidence demonstrates that co-interment, secondary burial, and burial reuse were widely

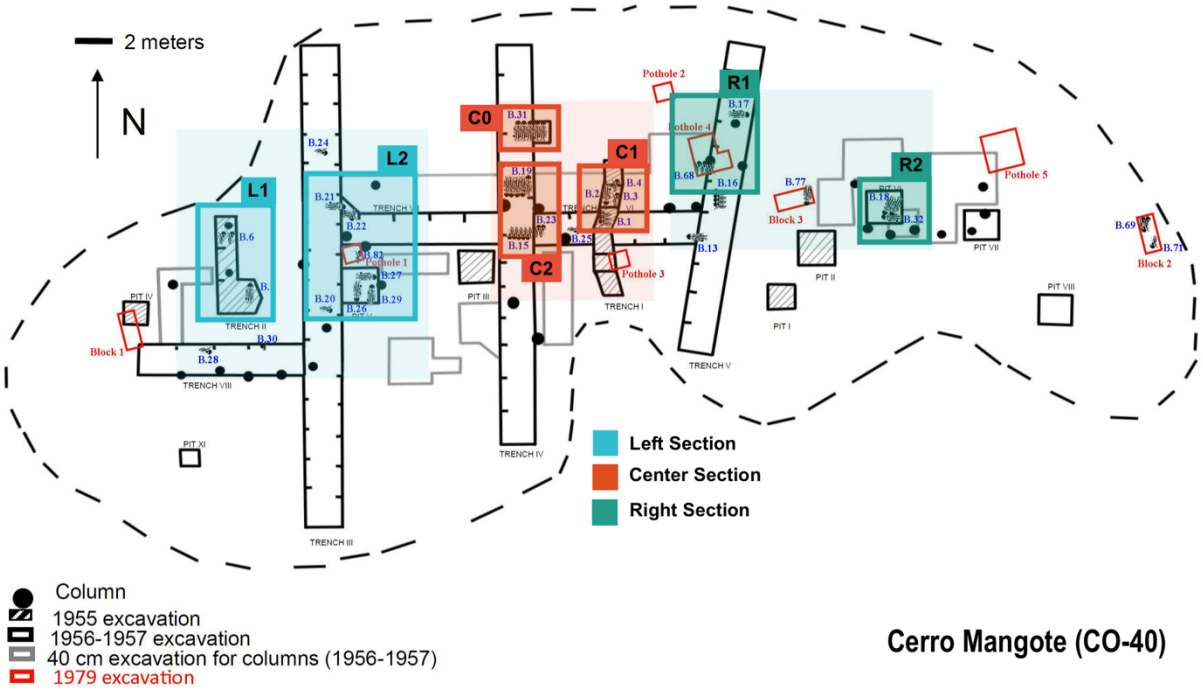
practiced throughout the Greater Coclé region at cemetery contexts as early as 2480 BCE at Cerro Mangote and as late as CE 1500 at Cerro Juan Diaz (Cooke et al. 1998; Isaza 2007; McGimsey 1956; Ranere 1980). Scholars argue that these Pre-Columbian mortuary rituals align with models of ancestor veneration, and the continued interaction with and maintenance of the dead likely reinforced important relationships and identities (Cooke 2001, 2004; Smith-Guzmán and Cooke 2018).

This study utilizes a biodistance analysis of dental metric and nonmetric traits to test these hypotheses related to kinship systems and marriage and postmarital residence practices enumerated below:

1. If mortuary practices reinforced kinship through burial type, then biological distances within "multiple burials", defined as a burial involving co-interment, secondary burial, and/or burial reuse, should be smaller than biological distances between other burials in the cemetery assemblage.
2. If mortuary practices reinforced kinship through burial proximity, then biological distances within burial groups will be smaller than biological distances between burial groups. Burial groups were identified based on spatial patterning (Figures 2 & 3) and chronology (Table 1), and they are outlined in Tables 2 and 3 for Cerro Mangote and Sitio Sierra, respectively.
3. If mortuary practices included exogamous marriage of outsiders, then women will be migratory and display greater within-group heterogeneity (Konigsberg 1988; Stojanowski and Schillaci 2006). Several lines of evidence suggest that men in the Greater Coclé region experienced elevated status, which may be indicative of patrilineal and patrilocal kinship systems. Men predominantly procured important

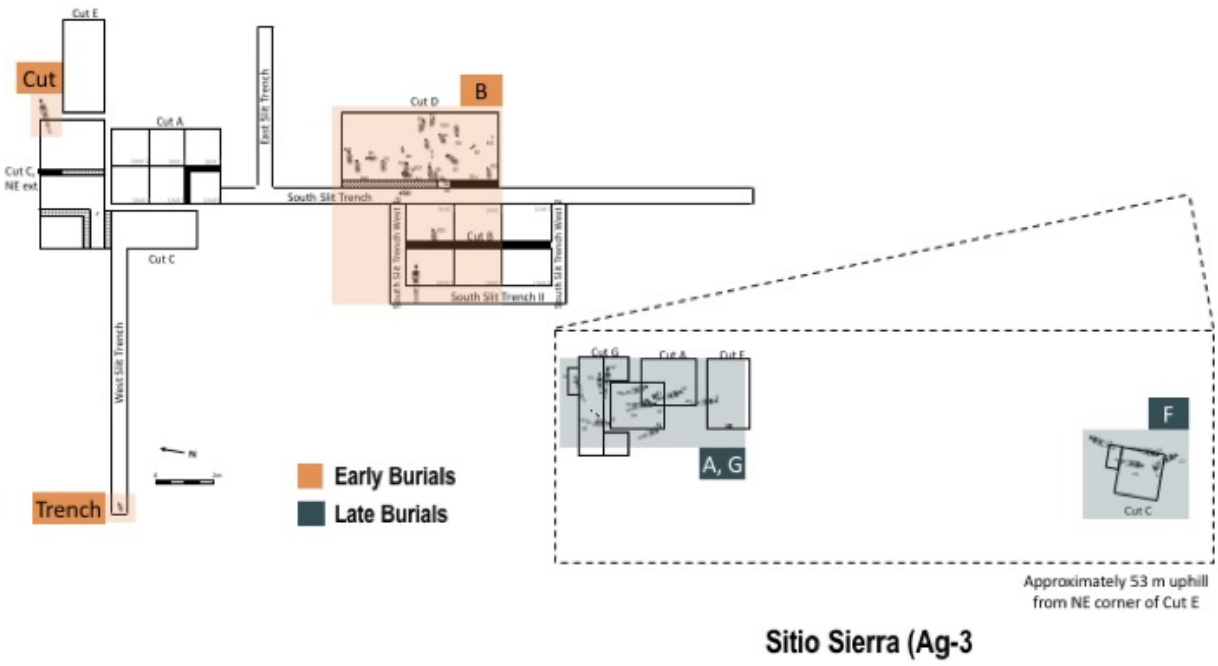
sumptuary resources like marine shells, and elite cemeteries like Sitio Conte and El Caño have a strong male bias (Lothrop 1937; Torné et al. 2020; Smith-Guzmán and Cooke 2019).

Figure 5.2 Site map of Cerro Mangote (CO-40)



Map adapted from Huard (2013) with input from Anthony Ranere (personal communication, 2018) and guided by original unpublished field documentation by Charles R. McGimsey III on file at STRI. The dotted line shows the extent of the shell-bearing midden, which encloses the cemetery.
Note: Burial groups and cemetery sections highlighted with colored outlines; Burials 69 and 71 were not included in analysis due to field limitations (COVID-19 Pandemic). The date ranges for burial groups include: L1= 2480- 2299 cal BCE; L2= 166 cal BCE- cal CE 317 CE; C0= 401- 203 cal BCE; C2= 162 cal BCE- cal CE 209; R1= 920- 550 cal BCE; R2= 1396- 1021 cal BCE.

Figure 5.3 Site Map of Sitio Sierra (Ag-3)



Map adapted from maps and original unpublished field documentation by Richard Cooke on file at STRI. *Notes*: Burial groups and cemetery sections highlighted with colored outlines. Date ranges for cemetery sections include: Early (B, Cut, and Trench Burials)= 39 BCE- CE 561; Late (A, G, and F)= CE 978-1158.

5.4 Study Sample

In order to explore normative Pre-Columbian kinship ideologies, one hundred and two skeletal remains from Cerro Mangote (n=62) and Sitio Sierra (n=39) were assessed (Table 4). These non-elite sites represent a broad temporal range (2480 BCE- CE 561 and CE 978-1158) and provide insight into changing kinship systems through the Ceramic Period in the Greater Coclé region.

A final sample size of 74 individual dentitions were included (Tables 2 & 3). Standard bioarchaeological methods were used to estimate sex (Buikstra and Ubelaker, 1994; Klales et al., 2012). Adult age was determined through transition analysis, which uses a combination of

cranial suture closure, pubic symphyseal features, and iliac auricular surface changes to estimate age with the ADBOU software (Boldsen et al. 2002; Milner and Boldsen 2012). Juvenile age estimations were based on dental development, diaphyseal length, and epiphyseal fusion, with a preference for dental age estimates (AlQahtani, Hector, and Liversidge 2010; Buikstra and Ubelaker 1994; Cunningham, Scheuer, and Black 2016). Juvenile individuals were included in the final sample, although only permanent teeth with fully developed crowns and roots were measured and scored.

5.5 Methods

Thirty-eight morphological traits were scored using the Arizona State University Dental Anthropology System (Turner et al., 1991). When both sides of the dentition were observable, the antimere, or paired tooth, with the greatest trait expression was recorded in order to capture maximum genetic potential (Scott and Turner 1997). Heavily worn or damaged teeth were excluded from analysis. Ordinal trait values were used to maintain the sensitivity necessary for intracemetery analyses (Mayhall 2000, Stojanowski and Hubbard 2017).

In addition, 32 mesiodistal and buccolingual root dimensions at the cemento-enamel junction were measured (Hillson et al, 2005; Stojanowski 2007). Only the polar teeth, or most mesial tooth in each tooth class, were included in the analysis as these teeth are more ontologically stable (Dahlberg 1945; Garn et al. 1965a, 1965b; Stojanowski 2005). The left dentition was used preferentially, but antimeres were substituted when necessary, and teeth with worn or damaged roots were not measured. Measurements followed positioning guidelines from Aubry's (2014) revised root measurement methodology (Hillson et al. 2005) and were taken to

the nearest 0.01 mm with a Mitutoyo 573 Absolute Point Caliper. Appendix B contains a list of all dental variables.

Intraobserver Error

To assess intraobserver error, metric and nonmetric observations were repeated for five individuals each week for three weeks at the beginning and middle of the data collection. Intraclass correlation coefficients (ICC), which accommodates continuous and ordinal data, were used to measure the strength of repeated rating agreement and correlation. ICC determines the reliability of ratings by comparing the variability of different ratings of the same subject to the total variation across all ratings and subjects in the sample (Koo and Li 2016).

The metric and nonmetric intraobserver error data were each analyzed using a two-way mixed effects model with absolute agreement, which computes the reliability of the specific rater (S.M.B) to assign the same score to the same subject (Koo and Li 2016). The results are presented in Appendix C. The ICC for both intraobserver error models are above 0.9, which is considered excellent agreement and indicative of intra-rater reliability (Koo and Li 2016). ICC analysis was completed in R using the *Psych* package.

Estimating Phenotypic Distance

Morphological and odontometric variables with less than 75% observations were removed, as were individuals with fewer than 50% of their teeth assessed. The remaining missing observations were computed with multiple imputation, which uses regression models of observed values to predict values of data Missing at Random (MAR). The R packages *MICE* and *sjmisc* were used to compute missing metric and morphological values from the averages of appropriate predictive means matching and proportional odds models, respectively (Collins, Schafer, and Kam 2001; Lüdecke, D. 2018; van Buren and Groothuis-Oudshoorn 2011). The

imputed matrices fell below the threshold of 25% data imputation limit set by previous studies (Stojanowski 2003), and no significant differences were found in the means or variances of the metric variables or the distribution of the morphological variables when comparing the imputed and original datasets.

The imputed metric variables were Q-mode transformed to eliminate the allometric and sexually dimorphic effects of tooth size (Corruccini 1973; Powell 1995). Measurements with non-normal distributions were removed, and a principal components analysis (PCA) was performed on the transformed metric variables to control for inter-trait correlation (Pilloud and Larsen 2011) (see Appendix B for final variable list). Principal components (PC) with an eigenvalue greater than or equal to one were retained (n=5 PC Cerro Mangote, n= 4 PC Sitio Sierra) (Stevens 2012).

Gower similarity was calculated for all pairwise combinations of individuals to measure phenotypic resemblance. The R *Cluster* package calculates Manhattan distance for continuous data and the Dice coefficient for categorical data, and the results were scaled between zero and one to generate a Gower coefficient (Gower 1971; Maechler et al. 2019). The square root of each coefficient was taken following Gower (1966, 1971) to plot distances in Euclidean space with multidimensional scaling (MDS). Gower pairwise distance coefficients were further compared with Student's two sample independent t-tests (or Kruskal-Wallis tests of independent measures for non-parametric data) to test hypotheses on biological relatedness and mortuary practices.

Variance-covariance matrices were used to explore marital strategies and mortuary practices (Konigsberg 1988; Morita et al. 2011; Schillaci and Stojanowski 2003). The analysis utilized the retained principal component scores of the metric variables, and pairwise differences between men and women at each site were computed to test the null hypothesis that the

variability in one skeletal sample, |H|, was less than or equal to the variability in a second reference sample, |W|. The statistical significance of the resultant determinant ratios, |H|/|W|, were assessed with Zhivotovsky's *F* ratio and non-parametric bootstrapping, which involves calculating the *p* value after randomly shuffling and resampling the data (999 iterations) to generate a randomized distribution of determinant ratio values (Konigsberg and Frankenberg 2016; Petersen 2000). Analysis was completed in R with scripts provided by Lyle Konigsberg.

The Cerro Mangote and Sitio Sierra datasets were combined following the procedures listed above, and the transformed Gower distance matrix was plotted with MDS. A k-medoids clustering analysis was also performed on the combined Gower distance matrix with the R package *Cluster* (Maechler et al. 2019). K-medoids clustering is a robust form of non-hierarchical clustering that minimizes the sum of pairwise dissimilarities to generate clusters (Harikumar and Surya 2015). Differences in phenotypic variability at both sites were compared by calculating the determinant ratio of a variance-covariance matrix, as described above.

5.6 Results

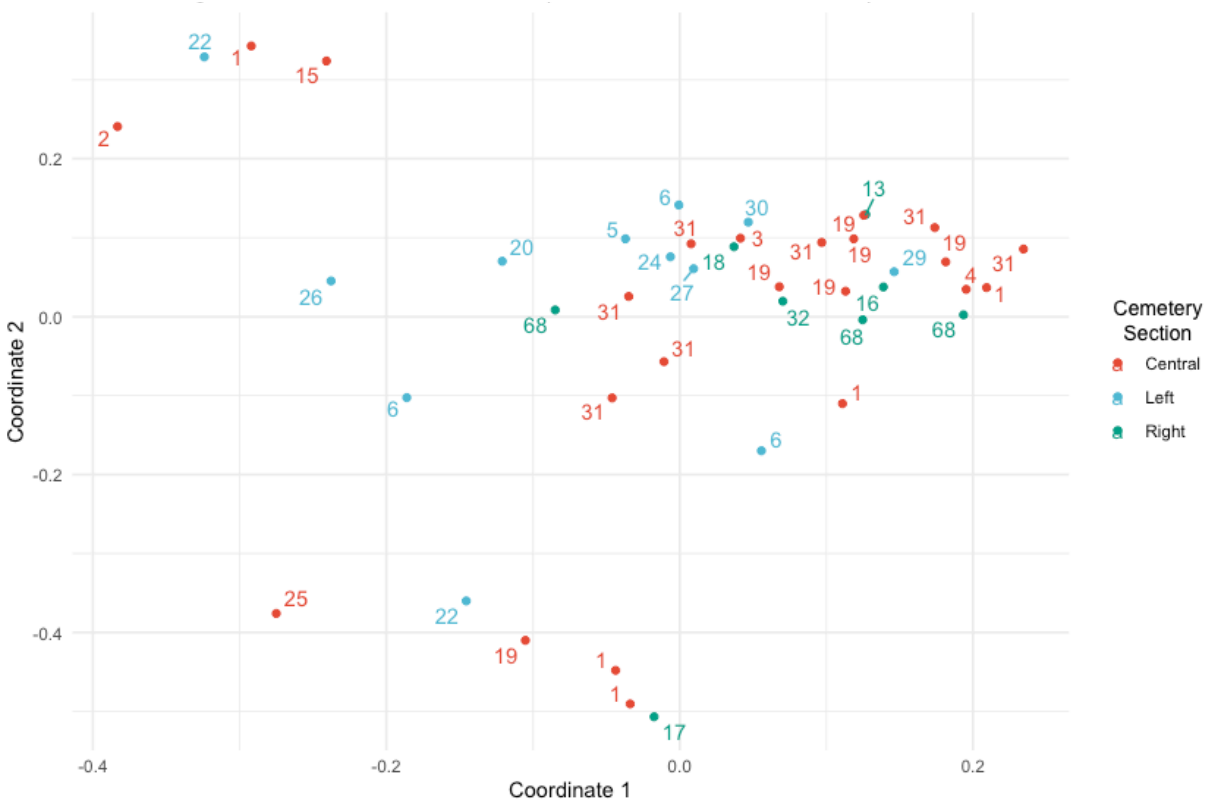
Cerro Mangote

Many individuals in multiple burials plot close in the MDS (Figure 4), particularly those in Burials 19 and 31, although much of the assemblage is clustered together. The mean pairwise distance in the entire assemblage was 0.35.

Multiple burial mean pairwise distances were compared against burial group, cemetery section, and total cemetery assemblage pairwise distances (Table 5). The mean pairwise distance in Burial 31 (C0) was significantly smaller than other central burial groups (C1 and C2), the central cemetery section, and the total assemblage. Burials 19 and 68 mean pairwise distances

were smaller than the cemetery mean, whereas Burials 1 and 6 had greater mean pairwise distances. These burials represent different multiple burial types: Burials 1 and 6 contain bundled individuals, and Burials 19 and 31 contain individuals in flexed positions. The mean pairwise distance among bundled multiple burials (0.41) is significantly greater ($t= 2.88$, $p <0.01$) than the mean pairwise distance among flexed multiple burials (0.28). Additionally, several individuals within Burials 1, 19, and 31 were identified as potential close kin with significantly smaller pairwise distances (Table 5).

Figure 5.4 MDS of Cerro Mangote Pairwise Gower Distances



None of the remaining burial groups or cemetery sections contained significantly lower mean pairwise distances than other burial groups in their cemetery section or the rest cemetery

assemblage. Further, the cemetery sections themselves did not contain significantly smaller pairwise distances compared to the other cemetery sections or total cemetery assemblage.

The small, positive determinant ratio does not reject the null hypothesis (Table 6), and these results instead suggest equal or possibly a little more variability among women compared to men at Cerro Mangote, although this difference is not significant.

Sitio Sierra

The majority of the individuals in the cemetery assemblage cluster together in the MDS (Figure 5), especially the early cemetery burials. Some multiple burial individuals plot close together, particularly those in Burial 19/20. The mean assemblage pairwise distance was 0.33.

Figure 5.5. MDS of Sitio Sierra Pairwise Gower Distances

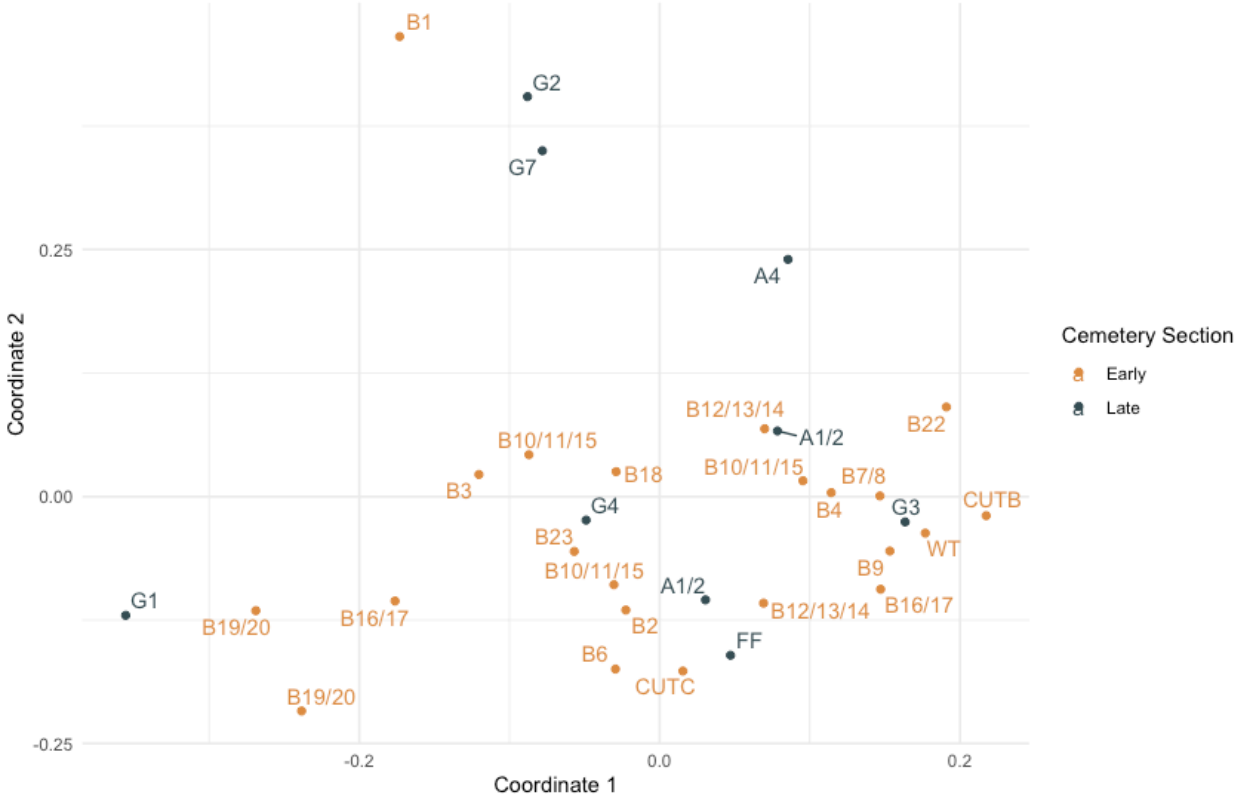


Table 5 contains the mean pairwise distances for each multiple burial. Given low individual count, the multiple burials were grouped together to facilitate statistical comparison. The mean pairwise distance of the combined burials was 0.29, which was not significantly smaller compared to the total cemetery assemblage.

The small sample size also precluded a comparison of burial groups, and only the mean pairwise distances between cemetery sections could be assessed (Table 5). The early burials, which include all B, Cut, and Trench burials, have a significantly smaller mean pairwise distance than the late A, G, and F burials (t value= -2.41, p= 0.02).

A positive determinant ratio indicates greater variability among women at Sitio Sierra, although this difference failed to reject the null hypothesis (Table 6). Additional determinant ratios were calculated for the early and late burials. Although the difference was not statistically significant, the early cemetery determinant ratio indicates much greater female phenotypic variability than the late cemetery.

Site Comparisons

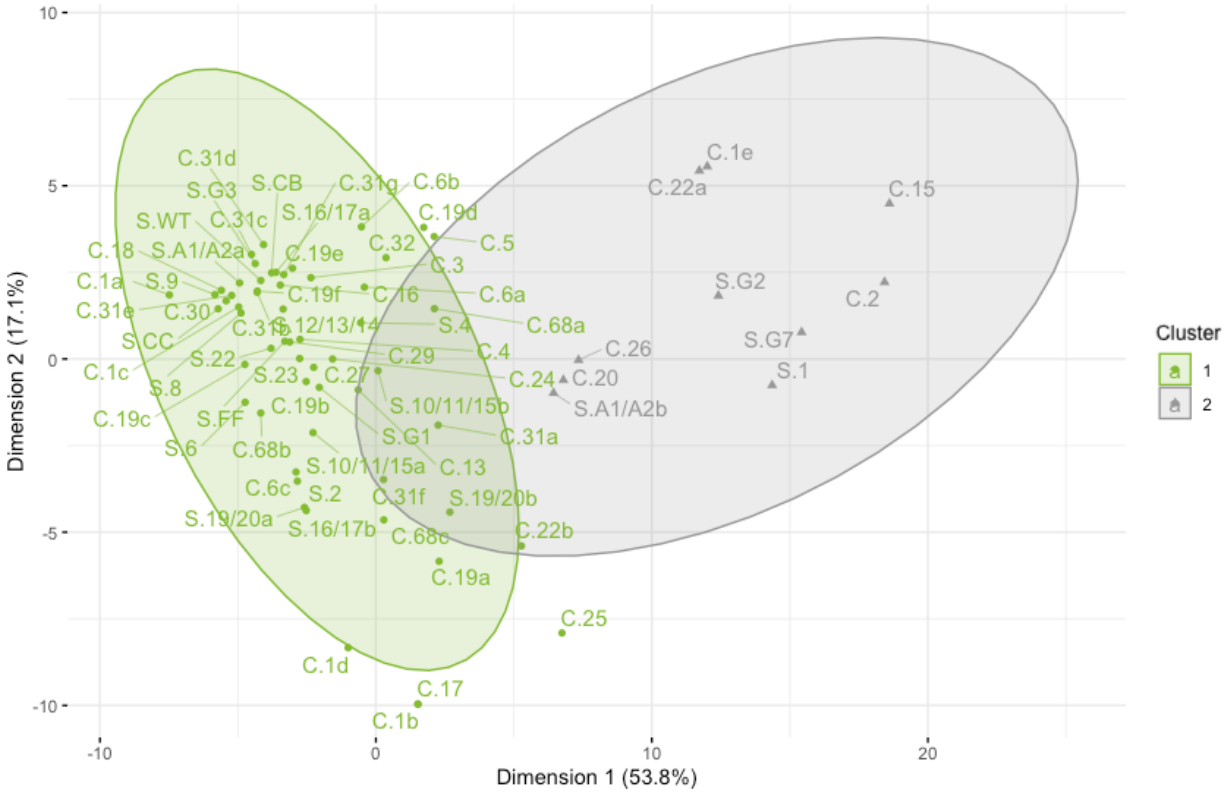
The sites overlap to a high degree in the center of the combined MDS in Figure 6. While the Cerro Mangote individuals cluster towards the positive end of the y axis, the Sitio Sierra individuals skew towards the negative end of the y axis. The mean pairwise distance for the combined sample was 0.31.

The k-medoid cluster analysis identified two distinct clusters containing individuals from both sites (Figure 7). Cluster 1 includes individuals with predominantly lower pairwise distances that plotted near the center of the MDS, whereas Cluster 2 is comprised of individuals with predominantly higher pairwise distances that plotted away from the MDS center. Both clusters contain the same approximate proportion of individuals from both sites (x^2= 0.02, p= 0.9).

The small positive determinant ratio does not reject the null hypothesis that variability at Sitio Sierra was equal to the variability at Cerro Mangote (Table 6). The non-significant results instead indicate similar variability at both sites.

Figure 5.6. MDS of Combined Pairwise Gower Distances

Figure 5.7 K-medoids Cluster of Combined Pairwise Gower Distances



Note: The average silhouette width is 0.5, which indicates average model fit. No individuals were wrongly assigned to a cluster. Cluster 1: n=37 Cerro Mangote, n= 21 Sitio Sierra. Cluster 2: n= 6 Cerro Mangote, n= 4 Sitio Sierra. There were no significant differences in site distribution between clusters (x^2= 0.02, p= 0.9). Each burial has a unique label with the following format: Site.Burial Number.indivdual letter.

5.7 Discussion

This study combined measures of phenotypic variation, a proxy for biological relatedness, with other material indicators of group affiliation to explore how kinship was constructed and symbolically represented through Pre-Columbian mortuary traditions (Johnson and Paul 2016). This biocultural approach offers new insights into social systems and the nature of kinship in the Greater Coclé region during 2480 BCE- CE 1158.

Kinship and Mortuary Tradition

The primary research aim of this study was to elucidate kinship from various aspects of Pre-Columbian mortuary traditions that may represent biological and/or social relatedness. We hypothesized that measures of biological distance would be significantly smaller within "multiple burials", defined as burials involving co-interment, secondary burial, and/or burial reuse. Additional potential kin-related burial groups were identified from cemetery spatial patterning and chronology and compared as well.

Cerro Mangote. The results demonstrate that biological relatedness determined burial inclusion and burial location in some instances but not uniformly throughout the cemetery. Biological relatedness appears to dictate inclusion in the largest multiple burial, Burial 31, and Burial 19 to a lesser extent, whereas phenotypic variation in the remaining multiple burials, burial groups, and cemetery sections does not indicate significant biological affiliation. Despite these results, multiple burials may still represent biological lineages. Although lineages are assumed to be biologically homogenous, marriage practices, genetic inheritance, and epigenetic mechanisms all result in varying clusters of homogeneity that could be spread throughout the cemetery instead of concentrated within multiple burials (Ensor et al. 2017; Stojanowski and Hubbard 2017).

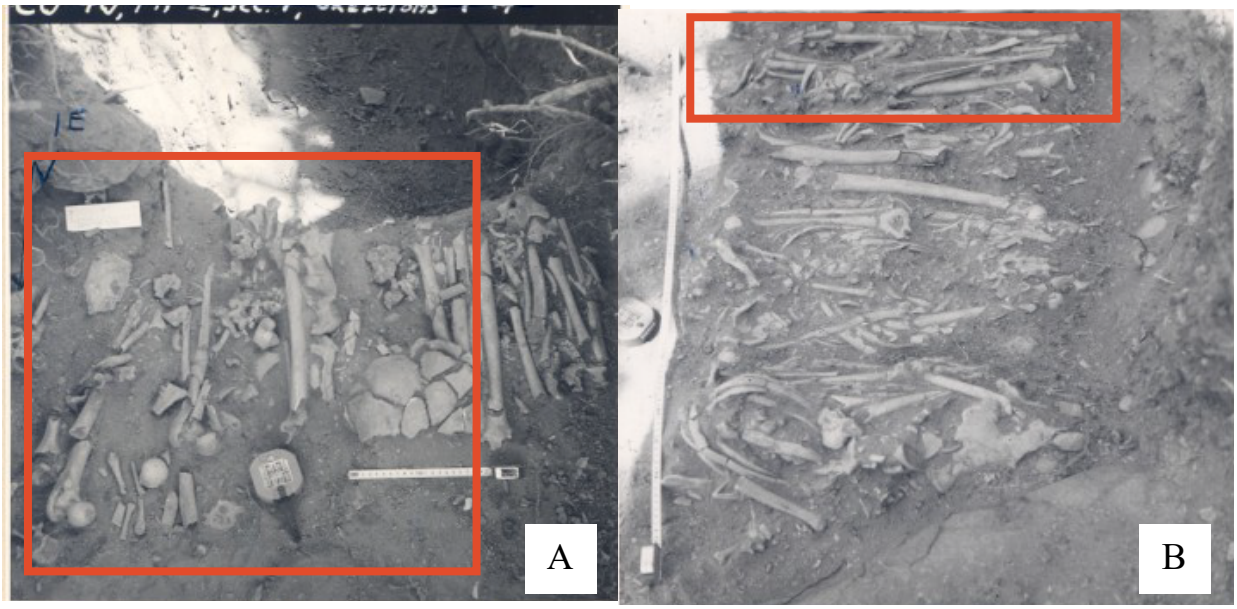
Multiple burial inclusion, particularly certain types of multiple burials, as well as broader cemetery organization may also represent socially constructed forms of affiliation. The Kogi, a modern Chibchan-speaking group, offer an ethnographic example of Isthmo-Colombian corporate groups (Hoopes 2005). In addition to a biologically oriented kinship system, the Kogi use corporate groups for important functions, such as the selection and training of *mamas* or priests who maintain traditions and provide village authority (Reichel-Dolmatoff 1976, 1985).

Pre-Columbian corporate group membership may have similarly shaped broader levels of affiliation that determined burial inclusion and cemetery organization (Carr 1995). Mortuary traditions may reflect other forms of fictive kinship related to the diverse socioeconomic activities pursued by the community, such as marine foraging and regional trade interactions.

Biological relatedness appears to be most strongly correlated with individual placement within multiple burials, particularly multiple burials with flexed individuals. Several close biological relations with significantly smaller mean pairwise distances were identified in Burials 1, 19, and 31 (Table 5), and burial photos (Figure 8) and excavation notes show these individuals were buried in close proximity in the multiple burial (Huard 2013; McGimsey n.d). Burial 1 contained a neat row of eight individuals, and the close biological relations were placed next to each other towards one side of the burial. In Burial 31, the remains of 13 individuals were organized into five piles arranged in a row, and the close biological relations were stacked on top of each other in single pile to the far side of the burial. While a similar intermingling of close biological relations in single pile appears to be present in Burial 19, the burial was damaged and its contents disturbed by looters before precise mapping and observation occurred.

The grouping of close biological relations suggests that community members had detailed knowledge of biological relationships, and biological relatedness played a significant role in shaping this aspect of funerary ritual. These results indicate that some elements of the kinship system were congruent with biological relatedness (Meyer et al. 2008; Meyer et al. 2012). Overall, the complex mortuary rituals at Cerro Mangote represent multiple layers of kinship, including immediate biological relationships and membership in broader corporate or practical kin groups.

Figure 5.8 Photos of Burials 1 and 31 from Cerro Mangote



Note: (A) Photograph of Burial 1, with individuals 1A-1C highlighted in red; they were buried next to each other in a line with other Burial 1 individuals on either side. (B) Photograph of Burial 31, with individuals 31E-31H highlighted in red. The individuals were buried together in a single pile of bones, which was placed in a line of similar bone piles.

Sitio Sierra. In comparison, there was minimal evidence of biologically structured mortuary patterning in either the early (39 BCE- CE 561) or late (CE 978-1158) cemetery contexts at Sitio Sierra. Although the mean pairwise distance of the early burials was significantly smaller than the late burials, the pooled multiple burial mean distance did not indicate close biological affiliation.

This analysis was limited by the smaller sample size of individuals, as well as a smaller sample of multiple burials and burial groups to test. Only nine of the 27 burials (33%) examined from Sitio Sierra are multiple burials, and the multiple burials were limited to three adult individuals. The most common burial mode was a single, primary flexed or extended

inhumation. With the exception of trench and cut burials, the majority of burials in both cemetery contexts were grouped close together without additional spatial organization.

The Sitio Sierra cemeteries represent a shift in mortuary tradition in the Greater Coclé region, possibly attendant to shifts in social structures and kinship systems. Following Cooke (1979; 1984), one explanation may be that the cemetery contexts at Sitio Sierra represent small household or single lineage cemeteries. The early burials partially underlie the floor of a round structure and are proximate to an ovate house (cal 39 BCE- CE 561), and the cemetery context may have served these households. The low average pairwise distance among early burials further supports this interpretation, as does the small number of burials and predominance of primary, single flexed interment style (Ensor et al. 2017). Skeletal isotope data also indicates that the diets of the early inhabitants were quite similar and involved heavy maize consumption (Sharpe et al. 2021).

Alternatively, socially defined relationships may have played a greater role in determining kinship and mortuary tradition. The early Sitio Sierra occupation coincides with the Middle Ceramic period shift from foraging to sedentary village lifeways centered on flood plain cultivation. These major transitions may have drawn individuals together and created the need for "practical kin" relations (Bourdieu 1977). Practical kin groups may have facilitated the intensive cultivation of domesticated plants through the organization of labor needed for land clearing, planting, and harvesting (Pilloud and Larsen 2011). As population and settlement density increased, practical kinship ties may have helped determine the inheritance of property and access to land. If these utilitarian kinship ties were more important in ensuring economic success or determining sociopolitical status, then these aspects of individual identity may have been preferentially reflected in burial traditions as Sitio Sierra.

Marriage and Postmarital Residence Patterns

Our second research aim analyzed patterns of phenotypic variation in order to explore Pre-Columbian marriage and postmarital residence practices. We hypothesized that female individuals may exhibit greater phenotypic variation due to mortuary traditions that reinforced male-based kinship and residence practices in the Greater Coclé region.

Cerro Mangote. The results of the postmarital residence analysis do not support the expected male-based model of postmarital residence and demonstrate that both sexes exhibited statistically similar variability. This pattern of equal variation could be attributed to several practices.

Community endogamy results in low sex-based phenotypic variation. Although Ensor and colleagues (2017) also demonstrate that exogamy between unilineal descent groups does not necessarily lead to significant differences in phenotypic variation between men and women, community endogamy should be considered as a potential explanation at Cerro Mangote. Some modern Chibchan-speaking groups like the Guna practice community endogamy, and the overall relatedness in the assemblage despite a long chronological period may indicate some degree of endogamy (Herrera 1972; Holloman 1976; Tice 1994). The results, however, may also point to a more complicated relationship between postmarital residence and postmortem location (Ensor 2013).

The cemetery size, biodistance patterning, and lack of significant difference in sex-based variation observed at Cerro Mangote correspond with Ensor and colleagues' (2017) model for bilateral descent. Postmarital residence is more flexible in communities practicing bilateral descent, which results in diverse household groups including spouses, cousins, offspring, and

non-biologically related individuals. As residence determines postmortem location, there is subsequently greater variation in postmortem location (Ensor et al. 2017). Among modern Chibchan-speaking groups, the Guna practice bilateral descent, but men are predominantly buried in their wife's natal community (Stout 1947). In contrast, the Bribri practice uxorilocal residence for an initial period after marriage often followed by neolocality (de Wille 1975).

Sitio Sierra. The phenotypic variability appears to be greater among female individuals during the early site occupation (determinant ratio= 81.66), and sex-based differences decrease during the late occupation (determinant ratio=1.15), resulting in an insignificant difference in female and male phenotypic variation (determinant ratio= 1.65).

Although it is unclear whether the burials represent a small household cemetery or a larger community cemetery, the mix of phenotypic homogeneity and heterogeneity observed suggests that Sitio Sierra inhabitants also practiced bilateral descent with bilocal residence patterns that allowed spouses to utilize a variety of beneficial kinship ties (Cooke 1979, 1984; Ensor et al. 2017). The greater female heterogeneity points to a possible preference for virilocal residence and postmortem location, particularly in the beginning sequence of Sitio Sierra occupation (40 BCE- CE 400). Although female heterogeneity appears to decrease over time, it is difficult to determine if this change is due to real social shifts or small sample size obscuring sex-based differences.

Overall, the existence of bilateral descent and bilocal postmarital residence practices at Sitio Sierra denotes a degree of cultural continuity with Cerro Mangote even as mortuary traditions shift towards single, primary burials and potentially smaller household or lineage cemeteries.

Greater Coclé Regional Interaction

The biological distance comparison of Cerro Mangote and Sitio Sierra further indicates interaction and continuity between these two Greater Coclé sites. There was no significant separation between individuals, and the average pairwise distance for the combined assemblage was similar to the average pairwise distances within each cemetery. Both assemblages contain similar amounts of phenotypic variation as well.

How do these results fit with our understanding of these sites? Both sites are located in the Santa Maria river valley, separated by six kilometers, and current estimates suggest that several Cerro Mangote burials overlap chronologically with the early Sitio Sierra occupation. Evidence of gene flow, therefore, is not unexpected. Given their proximity, these sites could have been part of extensive trade networks, documented by ethnohistoric sources and archaeological evidence, that connected inland and coastal sites (Contreras and Hansell 2008; Cooke 2005; Espinosa 1913). Exogamy and social exchange could have occurred alongside trade activities, resulting in regional gene flow. The outliers in the Cluster 2 (Figure 7) may represent exogamous individuals traveling from more distant locations, such as the Caribbean coast (Cooke and Sánchez 2004).

Additional archaeological, osteological, mortuary, and archaeological evidence, however, further complicates our understanding of how these communities may have been related. Scholars have previously argued that Cerro Mangote served as a seasonal site for the collection of marine resources for part of the year (Griggs 2005; Norr 1995). Skeletal strontium ratios from two of the earliest Cerro Mangote individuals suggests that they may have been born outside the area. Strontium values of the early Sitio Sierra inhabitants are also elevated above the area baseline, but this appears to be a result of marine food consumption as opposed to mobility

(Sharpe et al. 2021). Interestingly, the biodistance analysis indicates gene flow and comparable amounts of phenotypic variation at both sites, potentially derived from similar marriage, postmarital residence and postmortem location practices. The Cerro Mangote assemblage may represent an earlier mobile community whose descendants later settled at Sitio Sierra during the site's early and late occupations. The mean age at death at Sitio Sierra is significantly older, and this demographic difference could indicate distinct populations experiencing different mortality risks. Faunal isotope and faunal evidence also highlight subsistence differences between the communities. Terrestrial hunting greatly contributed to the diet at in the earlier Cerro Mangote and later Sitio Sierra occupations, but the early Sitio Sierra inhabitants appeared to rely predominantly on maize and marine resources (Martinez-Polanco et al. 2020; Martinez-Polanco and Cooke 2019; Sharpe et al. 2021)

The human occupation and cemetery contexts at Cerro Mangote, however, represent a lengthy period of time and may reflect the different activities of different populations at the site. Juveniles received special mortuary care, including burial with shell beads, throughout the Ceramic period, and Cerro Mangote may have served as a dedicated microregional cemetery for the ritual burial of young community members in multiple burials (Briggs 1989; Cooke and Ranere 1992). Descendant populations at Sitio Sierra may have continued the practice, resulting in the demographic and mortuary differences between the sites, such as the predominance of single primary burials and burial goods at Sitio Sierra (Cooke 1984; Huard 2013). Further mortuary analysis, radiocarbon dating, and stable isotope analysis is needed to untangle the relationship between these sites.

5.8 Conclusion

The biodistance analysis of dental metric and nonmetric traits from Greater Coclé sites Cerro Mangote and Sitio Sierra provides evidence of multiscalar kinship systems that incorporate biological and social notions of relatedness. At Cerro Mangote, phenotypic variation suggests that social relationships, such as corporate groups, largely determined burial inclusion and cemetery organization, whereas close biological ties determined the placement of individuals within certain multiple burials. The Sitio Sierra cemetery contexts represent a significant shift in mortuary traditions, and phenotypic heterogeneity suggests that burial practices may reflect household groups or social relationships, such as practical kin groups.

The absence of significant sex-specific differences suggests that both Greater Coclé communities practiced exogamy with bilateral descent and bilocal or unilocal postmarital residence, resulting in variable patterns of postmortem location. These Pre-Columbian practices align with ethnographic observations of modern Chibchan-speaking groups, but given the small sample size, further study is needed to support these interpretations.

The biodistance analysis identified considerable biological affiliation between the Cerro Mangote and Sitio Sierra assemblages and hint at potentially low genetic diversity within the region. The evidence suggests that related communities occupied these sites and may have been connected through regional interaction networks that moved resources and people between coastal and inland sites. Alternatively, communities may have utilized these sites for distinct mortuary purposes, particularly the ritual burial of juveniles. Future biodistance and mortuary analyses of surrounding sites may help to further tease apart regional relationships.

The recent growth of bioarchaeology in Panama has demonstrated the importance of contextualizing archaeological evidence with skeletal remains to better nuance our understanding

of Pre-Columbian lifeways, and this study demonstrates the potential of bioarchaeological kinship studies to contribute to these efforts (Smith-Guzmán and Cooke 2018, 2019). In the Greater Coclé region, multiple lines of evidence suggest that complex burial practices emphasized both biological ties and multiple levels of social relationships, such as religious sodalities, practical kin, or other corporate groups. Biocultural kinship systems likely provided multiple scales of affiliation to help meet the challenges of significant demographic, environmental, and subsistence changes attendant to the Early and Middle Ceramic Periods.

Table 5.1 Radiocarbon Dates

	Context	**Conventional Age**	**IntCal20 Range**
Cerro Mangote (BCE 2476- CE 231)	Burial 6	3920 +/- 30 BP	2476- 2293 BCE
	Burial 16A	3920 +/- 30 BP	2476- 2293 BCE
	Burial 20	2015 +/- 50 BP	153 BCE- CE 122
	Burial 23	1970 +/- 60 BP	61 BCE- CE 216
	Burial 26	1850 +/- 45 BP	CE 107- 256
	Burial 31	2260 +/- 50 BP	403- 197 BCE
	Burial 32	2983 +/- 66 BP	1400- 1042 BCE
	Burial 68	2630 +/- 60 BP	923- 746 BCE
	Burial 69	2150 +/- 30 BP	353- 54 BCE
Sitio Sierra (BCE 39- CE 561, CE 978- 1158)	Burial B0	1770 +/- 40 BP	CE 214- 401
	Burial B6	1770 +/- 40 BP	CE 214- 401
	Burial B17	1880 +/- 40 BP	CE 33- 244
	Burial B22	1950 +/- 40 BP	39 BCE – CE 204
	Burial B23	2840 +/- 40 BP	1125- 899 BCE
	Burial A4	1020 +/- 30 BP	CE 978- 1151
	Burial Ff	980 +/- 30 BP	CE 995- 1158

Table 5.2 Cerro Mangote Burial List with Biodistance Analysis Spatial Groupings

Cemetery Section	Burial Groups	Burial	Individuals
Left	L1	5	**5**
		6	**6A, 6B, 6C,** 6D^{+}
	L2	20	**20**
		21	**21**
		22	**22A,** 22B*, **22B Child**, 22C^{+}, 22D*, 22E*
		26	**26**
		27	**27**
		29	**29A**, 29B*
	Outliers	24	**24**
		28	**28**
		30	**20**
Center	C0	31	**31A, 31B,** 31B1^{+}, **31C,** 31D^{+}, 31D1^{+}, **31E, 31F, 31G, 31H,** 31I*
	C1	1	**1,** 1-Commingled*, 1A*, **1AB, 1B, 1C,** 1D*, **1E**
		2	**2**
		3	**3**, 3-1*
		4	**4**
	C2	15	**15A,** 15B*, 15D*, 15E*
		19	**19A, 19B, 19E, 19F, 19H, 19I,** 19J^{+}, 19K^{+}, 19L^{+}, **19M,** 19P*, 19R*
		23	23A*, 23B*
	Outliers	25	25*, **25 Child**
Right	R1	16	16A^{+}, 16B^{+}, **16C,** 16D^{+}, 16E^{+},
		17	**17**
		68	68C-Infant^{+}, **68C-1, 68C-2,** 68E-1^{+}, 68E Adult*, **68E Child,** 68W-Infant^{+}, 68W-1*, 68W-2*
	R2	18	18A*, **18B**
		32	**32A**, 32B^{+}
	Outliers	13	**13**

Bold denotes individuals included in the biodistance analysis.
*Individuals not included in analysis due to missing or unobservable teeth
$^{+}$Individuals not included due to age and lack of permanent dentition

Table 5.3 Sitio Sierra Burial List with Biodistance Analysis Spatial Groupings

Cemetery Section	Burial Groups	Burial	Individuals
Early	B	0	0
		1	**1**
		2	**2**
		3	**3**
		4	**4**
		5	**5**
		6	**6**
		7/8	7*, **8**
		9	**9**
		10/11/15	**10, 11, 15**
		12/13/14	**12, 13, 14**
		16/17	**16, 17**
		18	**18**
		19/20	**19, 20**
		22	**22**
		23	**23**
	Cut	C	**Cut C Child**, Cut C Adult*
		B	**Cut B**
	Trench	West	**WT Adult**, WT Child*
Late	A	A1/A2	**A1-A, A1,** A2*
		A3	A3*
		A4	**A4**
	G	G1	**G1**
		G2	**G2**
		G3	**G3**
		G4	**G4**
		G7	**G7**
	F	F-f & F-m	**F-f**, F-m*

Bold denotes individuals included in the biodistance analysis.
*Individuals not included in analysis due to missing or unobservable teeth
[+]Individuals not included due to age and lack of permanent dentition

Table 5.4 Demographic Profile of Cerro Mangote and Sitio Sierra

		< 20 yrs	20-34 yrs	35-49 yrs	50-64 yrs	65+ yrs	Total (%)
Cerro Mangote (n= 43)	male	1	11	1	0	1	32.6
	female	1	8	1	0	1	25.6
	undetermined	16	2	0	0	0	41.8
	Total (%)	41.8	48.8	4.7	0	4.7	
Sitio Sierra (n= 31)	male	0	3	4	0	4	35.5
	female	1	7	5	0	4	54.8
	undetermined	3	0	0	0	0	9.7
	Total (%)	12.9	32.3	29	0	25.8	

Fisher Exact test comparing age distributions, F= 1.7x e-4, $p < 0.05$
Fisher Exact test comparing sex distributions (males and females only), F= 0.28, $p > 0.05$

Table 5.5 Average Pairwise Gower Distance Coefficients

	Cemetery Section	**Burial Groups**	**Multiple Burial**	**Close Kin[a]**
Cerro Mangote	Left= 0.35	L1= 0.41 L2= 0.38	B6= 0.38	
	Central= 0.36	**C0= 0.28$^{+,\S}$** C1= 0.4 C2= 0.35	**B31= 0.28$^{+,\S}$** B1= 0.42 B19= 0.3	**B31 = 0.26§** **B1 = 0.26*,§** **B19 = 0.23*,§**
	Right= 0.31	R1= 0.23 R2= 0.28	B68= 0.33	
Sitio Sierra	**Early= 0.31§**	B=0.32 Cut= 0.26 Trench= 0	B10/11/15= 0.29 B12/13/14= 0.28 B16/17= 0.45 B19/20= 0.21	
	Late= 0.36	A= 0.35 G= 0.35 F= 0	A1/A2= 0.23	

*Significantly ($p < 0.05$) smaller mean pairwise distance compared to the rest of the multiple burial
$^{+}$Significantly ($p < 0.05$) smaller mean pairwise distance compared to the rest of the cemetery section
§Significantly ($p < 0.05$) smaller mean pairwise distance compared to the total assemblage
[a]Close kin members include: 31F, 31G, 31H, and 31E in Burial 31; 1, 1AB, 1B, and 1C in Burial 1; and 19B, 19H, 19I, 19M in Burial 19 from Cerro Mangote. While Burial 19/20 at Sitio Sierra likely contains close kin members as well, the sample size was too small (n=2) for statistical testing

Table 5.6 Results of Determinant Ratio Analysis

	Ratio	**F Ratio**	**P Value**	**Non-parametric P value**
Cerro Mangote *females (H), n*=9 *males (W), n*= 10	0.02	0.42	0.99	0.88
Sitio Sierra *females (H), n*=17 *males (W), n*=11	1.65	1.02	0.49	0.49
Early *females (H), n*=11 *males (W), n*=6	81.66	2.01	0.12	0.27
Late *females (H), n*=6 *males (W), n*=5	1.16	0.74	0.68	0.71
Regional Comparison *Sitio Sierra (H), n*= 28 *Cerro Mangote (W), n*= 19	0.07	0.52	0.99	0.99

The determinant ratio (|H|/|W|) compares the variation between a comparative (|H|) and reference (|W|) group. When equal variability exists between the comparative (|H|) and reference (|W|) group, the determinant ratio should equal one; when the comparative group is more variable, the ratio is positive and vice versa (Konigsberg 1988).

CHAPTER 6: RECONSTRUCTING DEVELOPMENTAL PHENOTYPES AND MORTALITY RISK IN PRE-COLUMBIAN PANAMA

6.1 Introduction

A key finding of modern biology is that genotypes can give rise to numerous phenotypes; environmental conditions during development drive phenotypic variation in diverse organisms (Bateson et al. 2004). Developmental plasticity enables the modification of individual phenotypes in order to enhance evolutionary fitness (Gluckman et al. 2007). During gestation, maternal and environmental cues are communicated through endocrine and nutrient signaling pathways.

These inputs influence the development and function of fetal organs and physiological systems through various mechanisms, including epigenetic changes to gene expression (Gluckman et al. 2007; Kuzawa 2005). Environmental cues continue to shape developmental trajectories throughout infancy, and physiological responses result in stable and heritable changes (Kuzawa and Quinn 2009; Thayer and Kuzawa 2014; Worthman and Kuzara 2005). Although environmental cues during developmentally sensitive periods guide phenotypic outcomes, the subsequent phenotypic variation is not inherently adaptive (Bateson et al. 2004).

Early theoretical models, such as the thrifty phenotype (Hales and Barker 2001) and the predictive adaptive response (Gluckman, Hanson, and Pinal 2005; Gluckman et al. 2010), related fetal nutritional stress to permanent changes that were adaptive in resource scarce environments but physiologically "mismatched" to later environments of surplus, resulting in increased morbidity risk. The negative consequences arising from developmental plasticity, however, can be understood more broadly as tradeoffs regarding resource allocation (Bateson et al. 2004).

Human physiological systems enable individual growth, reproduction, and maintenance, the three key determinants of evolutionary fitness.

Life history theory (LHT) posits that organisms have limited energy to allocate to these processes, and energy allocation decisions result in "tradeoffs" that allow organisms to respond to environmental cues in ways that enhance evolutionary fitness (Hill and Kaplan 1999; Kaplan, Hill, Lancaster, and Hurtado 2000). When individuals experience adverse environmental cues early in development, the priority of select organ or system development over the development of other systems results in a tradeoff that increases an individual's vulnerability to poor health outcomes later in life (Gluckman et al. 2007; Kuzawa 2005). For example, Thayer and Kuzawa (2014) noted that energetic stress during pregnancy led to fetal tradeoffs that constrained kidney development and increased the risk of hypertension in adulthood. Energetic deficiencies have also been linked to the impaired development of the hypothalamic pituitary adrenal (HPA) axis, and subsequent dysregulated glucocorticoid function has cardiometabolic, neuroendocrine, and behavioral consequences (Seckl and Holmes 2007; Van den Bergh et al. 2017).

These findings have coalesced into the Developmental Origins of Health and Disease (DOHaD) framework, and considerable research has demonstrated that developmental processes and subsequent phenotypes shape later life morbidity and mortality risk (Godfrey, Gluckman, and Hanson 2010; Kuzawa and Quinn 2009; Waterland and Michels 2007). Kermack and colleagues (1934) published one of the first studies to connect early life events with adult outcomes, and their investigation attributed a decrease in 18th century death rates to improved childhood conditions. Large cohort studies in the Netherlands (Stein et al. 1975) and the UK (Wadsworth, Cripps, Midwinter, and Colley 1985) found that intrauterine exposure to famine increased the risk of later cardiovascular and metabolic disease. Barker and colleagues' (1989)

seminal study on ischemic heart disease identified associations between low birth weight and increased risk of mortality from ischemic heart disease. Recent work has linked developmental stress with obesity (Reilly et al. 2005), diabetes (Li et al. 2010; Yajnik 2004), cardiovascular disease (Galobardes, Smith, & Lynch 2006), and mental health disorders (Heim and Binder 2012).

The prevalence of chronic diseases has reached epidemic proportions around the world. Globally, chronic diseases cause more deaths than all other causes of mortality combined, and over 388 million people are expected to die from chronic diseases in the next ten years (Daar et al. 2007; WHO 2015). In the United States, chronic diseases affect 60% of all adults (National Center for Health Statistics 2019). Developmental stress therefore represent an important source of frailty in modern populations, and the DOHaD framework raises the question of whether such associations exist in past populations (DeWitte and Stojanowski 2015; Wood et al. 1992).

In skeletal assemblages, many sources of frailty are unobservable or unknown, and heterogeneous frailty is therefore considered "hidden". While the inability to assess sources of frailty in skeletal assemblages greatly complicates bioarchaeological interpretations, the use of the DOHaD framework can help researchers model developmental stress as a source of frailty and identify the physiological pathways underlying these relationships.

Since the 1970s, bioarchaeological studies have steadily explored the interaction between osteological markers of developmental stress and selective mortality. Early studies (Cook and Buikstra 1979; Rose, Lallo, and Armelagos 1978; White 1978) utilized linear enamel hypoplasia (LEH) as a marker of non-specific developmental stress and found significant associations between LEH presence and early mortality. Updated statistical models and methodologies (Antoine, Hillson, and Dean 2009; DeWitte and Wood 2008; Goodman and Rose 1990)

improved our ability to explore developmental periods in skeletal remains, and many bioarchaeologists have advocated for the incorporation of DOHaD and life history theoretical frameworks (Agarwal 2016; Gowland 2015; Temple 2019). The increasing number of skeletal studies on developmental stress and mortality demonstrates the ability of bioarchaeology to test hypotheses and provide new insight into DOHaD in diverse contexts (Reitsema, Vercellotti, and Boano 2016; Temple 2014; Weisensee 2014).

The results of bioarchaeological studies, however, vary considerably, and the relationship between development and mortality risk depends on methodology. Some studies utilize single markers that may not capture the full range of developmental stress (Ham, Temple and Klaus 2020; Palubeckaité, Jankauskas, and Boldsen 2002), and other markers, such as tibia length, are difficult to interpret in cross-sectional skeletal studies due to physiological processes like catch up growth (Holder, Miliauskiené, Jankauskas, and Dupras 2020; Vercellotti et al. 2014). More holistic measures of developmental stress are needed to integrate multiple lines of evidence that represent different developmental periods and physiological pathways.

In an effort to extend current research in developmental bioarchaeology, this study analyzes the association between developmental stress and age at death with a parametric hazards analysis in Pre-Columbian Panama. This project uses latent class analysis, a common technique in social science fields, to reconstruct developmental phenotype classes that integrate multiple osteological indicators of developmental stress in order to explore the underlying relationships between these variables and their effect on development. Hazards analysis was used to assess developmental phenotype class associations with mortality risk in the Greater Coclé region of Panama during the Early to Late Ceramic Period (2480 BCE- CE 1158). Pre-Columbian Panama is an understudied context, and this study not only broadens our

understanding of health in the Isthmo-Colombian Area but also explores the effects of developmental plasticity on mortality risk in a tropical setting with a unique cultural trajectory and active infectious disease ecology. Our investigative life history approach contributes new theoretical and methodological insights to the study of developmental origins in bioarchaeology

6.2 Pre-Columbian Panama: Archaeological Context

Human activity in the Greater Coclé region of Panama expanded during the Holocene as climactic changes and environmental shifts improved subsistence (Cooke 2005; Dickau 2010). Inhabitants utilized slash-and-burn agriculture to grow an increasingly diverse range of cultivars and exploited coastal resources as sea levels rose and marine habitats changed (Clary, Hansell, Ranere, and Buggey, 1984; Cooke, Ranere, Pearson, and Dickau, 2013). An increase in the population and settlement density of the Greater Coclé region followed these shifts and resulted in new challenges.

Soil depletion and coastal progradation led populations to move to alluvial bottomlands in river valleys (Piperno and Pearsall, 1998; Ranere, 1980). By 2470 BCE, permanent villages become archaeologically visible in the Greater Coclé region and mark the beginning of sedentism and increasing reliance on intensive agriculture (Cooke, 2005; Isaza, 2007). The preparation and storage of food stuffs with new ceramic technology further supported population growth, and extensive trade networks between burgeoning communities moved food items and craft goods between inland and coastal sites (Cooke 2005; Isaza 2007). The remains of monumental architecture, elaborate burials, and pottery throughout Pre-Columbian Panama provide evidence of increasing stratification, and scholars have interpreted important regional sites like El Hatillo as meeting places for performing ritual activities, such as honoring ancestors

and feasting, that reinforced complex social ties (Cooke 2005; Hoopes, 2005; Ichon, 1980; Mayo and Mayo, 2013).

Following European contact, Spanish ethnohistoric accounts of Panama detail a chiefly society defined by conflict and inequality (Hoopes, 2005). The Greater Coclé cemetery at Sitio Conte contains some of the most elaborate burials in the Americas, with elite central figures surrounded by gold, material goods, and human retinues (Briggs 1989). Although Panama has long served as a nexus for the study of tropical chiefdoms and social stratification, few studies have been contextualized with the analysis of skeletal remains. Similar to other non-European contexts, the analysis of skeletal remains has rarely been included in the archaeological discussion of Panama due to preservation challenges, small sample sizes, and a dearth of trained osteologists (Larsen 2006; Tayles and Oxenham 2006). Recent bioarchaeological studies have demonstrated, however, the need to reevaluate earlier interpretations that relied heavily on biased ethnohistoric accounts and highlighted the uncertainty regarding scholarly understanding of Pre-Columbian lifeways (Smith-Guzman and Cooke 2018). In particular, comparatively little is known about the impacts of cultural shifts on patterns of development, health, and disease throughout the Pre-Columbian period in the Greater Coclé region.

6.3 Study Sample

This study explored the relationship between developmental phenotypes and mortality at two Greater Coclé region sites: Cerro Mangote (n=60) and Sitio Sierra (n=39). These skeletal assemblages represent a broad temporal range, from 2480 BCE– CE 561 and CE 978-1158, that includes important Ceramic Period cultural transformations and provides insight into the developmental origins of health and disease in Pre-Columbian Panama (Figure 1).

Figure 6.1 Map of Panama and Archaeological Sites

Locations of study sample sites, Cerro Mangote (CO-40) and Sitio Sierra (Ag3), are noted in bold. Adapted from Wikimedia Commons map - Alexrk2 (Bathymetry: NGDC ETOPO2v2 (public domain); Topography: NASA Shuttle Radar Topography Mission (SRTM30 v.2) (public domain); Shoreline and additional data: VMap-0 (public domain).

Cerro Mangote (CO-40). Occupied as early as 6018 BCE, Cerro Mangote is a shell midden on a large hill on the northern Santa Maria River bank and one of the oldest sites containing human remains in the Greater Coclé Region (McGimsey, 1956; Ranere 1980). Preceramic and Early Ceramic inhabitants exploited resources from nearby marine habitats, although cultivars like maize contributed to the diet (Huard, 2013; Norr, 1991, 1995). Initial interpretations considered Cerro Mangote a dry season site (Griggs 2005, Norr 1995), but recent evidence suggests that Cerro Mangote may have functioned as permanent habitation site served by extensive trade networks and diverse ecosystems may have supported permanent habitation at Cerro Mangote (Carvajal and Hansell, 2008; Martinez-Polanco, Ranere, and Cooke, 2020). A total of 110 individuals were recovered from the site, and the cemetery context demonstrates

complex mortuary traditions and burial practices that continue throughout the Pre-Columbian period and appear in later elite cemetery contexts at Sitio Conte and El Caño (Briggs, 1989; Smith-Guzmán and Cooke, 2018). Osteological analysis of the skeletal remains has been limited, but Norr (1991) and Huard (2013) both noted a moderate to high prevalence of nutrient deficiencies, infection, and skeletal stress markers.

Sitio Sierra (*AG-3).* Located approximately six kilometers upstream from Cerro Mangote, Sitio Sierra is a floodplain site comprised of two Middle Ceramic Period occupations (Cooke, 1972). Sitio Sierra was a nucleated settlement with cane and palm thatched houses, and the fertile alluvial soil supported intensive agriculture of numerous cultivars, including three different maize varieties (Cooke, 1984; Cooke, 1995). Although inhabitants still hunted and traded for terrestrial and marine protein, archaeological and archaeobotanical evidence emphasize the strong agricultural focus of the community characteristic of the Middle Ceramic Period cultural transformations (Cooke, 1984; Norr, 1995). Cemetery contexts were found in both sequences of occupation (39 BCE- CE 561 and CE 978-1158), and a total of 39 individuals were recovered (Cooke 1972, 1977, 1979). The mortuary traditions at Sitio Sierra represent a mix of cultural continuity and innovation, with single primary interments supplanting multiple burial and burial reuse (Berger and Smith-Guzmán, submitted; Cooke 1984). Norr (1991) noted minimal evidence of infection and malnutrition, although the periodicity of LEH suggest that inhabitants experienced mild but frequent stress attributed to changes in subsistence, settlement strategies, and population size.

6.4 Methods

The demographic profiles for each skeletal assemblage are presented in Table 1, and the sample includes all individuals for whom age at death and developmental phenotype could be determined using the methods described below. Standard bioarchaeological methods were utilized to estimate sex from pelvic and cranial morphology (Buikstra and Ubelaker, 1994; Klales, Ousley, and Vollner, 2012). Dental development, diaphyseal length, and epiphyseal fusion were used to estimate juvenile age, and preference was given to dental age estimates (AlQahtani, Hector, and Liversidge, 2010; Buikstra and Ubelaker, 1994; Cunningham, Scheuer, and Black 2016). Individuals younger than two years of age who have not completed critical developmental stages were excluded from the analysis (Barker 2012).

Adult age at death was estimated using transition analysis (TA). Transition analysis (TA) uses maximum likelihood estimation to overcome issues with traditional age estimation, particularly broad age categories and fragmentary remains, and produce point estimates of age, even for older individuals often under enumerated in skeletal assemblages (Boldsen, Milner, Konigsberg, and Wood, 2002; Milner and Boldsen, 2012). A combination of cranial suture closure, pubic symphyseal changes, and iliac auricular surface changes were scored for each individual following Boldsen and colleagues (2002). The Anthropological Database, Odense University (ADBOU) Age Estimation software generated age at death estimates from a prior distribution of age at death estimated from 17th century Danish rural parish records and the conditional probability of age indicators given known age at death estimated from the Terry Collection (Milner and Boldsen 2012).

Osteological Markers

The following osteological markers were assessed for each individual in order to capture age-specific growth disruptions and general developmental stress. By incorporating multiple skeletal and dental indicators, this study holistically assesses the effects of developmental stress throughout infancy, childhood and adolescence.

Linear Enamel Hypoplasia. LEH (linear enamel hypoplasia) is a type of defect caused by the disruption of enamel formation during crown development (Dahlberg 1991; Hillson 2018). These defects appear as horizontal grooves of varying width and depth on the surface of the tooth, and they can be caused by numerous insults, including infection and malnutrition, that disrupt normal growth rhythms (Masterson et al. 2018; Roberts and Manchester 2007). Considerable research in skeletal assemblages and modern populations has linked LEH with an elevated risk of later morbidity and mortality (DeWitte and Wood 2008; Masterson et al. 2018; Temple 2014; Wilson 2014).

LEH were identified on the labial surfaces of all mineralized and minimally worn anterior mandibular and maxillary teeth with a lighted stereomicroscope. These teeth represent relatively long developmental periods (1.1-6.2 years) and are highly sensitive to stressors (Goodman, Armelagos, and Rose, 1980; Hillson 2018; Reid and Dean 2006). LEH were scored as "present" if a defect was visually matched on two or more teeth to ensure the systemic nature of the developmental insult (Hillson 2018; Temple 2014).

Vertebral Neural Canal Dimension. The dimensions of lower thoracic and lumbar vertebrae provide evidence of adverse environmental exposures, including disease and malnutrition, that result in energetic tradeoffs and negatively impact skeletal growth (Clark et al. 1986; Watts 2011). During periods of physiological and psychosocial stress, energetic resources

are shunted away from growth to survival and maintenance demands (McDade 2003; Urlacher et al. 2018). Smaller vertebral neural canal (VNC) dimensions represent growth disruptions during different periods: the anterior-posterior diameter of the VNC in the lower spine is complete by two to six years, whereas the transverse diameter reaches adult size at approximately 14 years (Hinck et al. 1966; Scheuer and Black 2004; Watts 2013, 2015). VNC dimensions were recorded for the T10-L5 vertebrae for each individual using Mitutoyo digital calipers; vertebrae with postmortem or pathological damage were excluded (Clark et al. 1986; Watts 2015).

To determine a VNC score, the sample was first divided by age; juveniles less than 14 years of age were grouped together due to incomplete development. Each diameter measurement (anterior-posterior and transverse) per vertebrae was first assessed for normality and heterogeneity of variance using Shapiro-Wilks and Levene's tests, respectively. When assumptions were satisfied, Student's independent t-tests were used to identify any significant differences in average diameter scores based on site (both juveniles and adult samples) and sex (adult sample); non-parametric Mann-Whitney tests were used to assess non-normal distributions. The adult L3, L4, and L5 anterior-posterior variables had to be sorted by sex. Following another test of normality and log-transformation of necessary variables (T10 anterior-posterior measurement), Z-scores were then calculated for each vertebral diameter by sample. To avoid issues of preservation bias and measurement scarcity, each individual received a dichotomized score: 1 if one or more VNC dimensions were below average size (> 1 SD below mean for the population and/or sex); and 0 if all VNC dimensions were average or above average size (< 1 SD below mean for the population and/or sex).

Tooth Root Size. The field theory of dental development holds that non-polar or distal teeth in a tooth class are more ontologically unstable due to reduced genetic influence (Butler

1939; Corruccini and Potter 1980; Dempsey, Townsend, Martin, and Neale 1995; Tucker and Sharpe 2004). Developing non-polar teeth respond more to environmental cues as a result, and research has shown that non-polar dental size reflects physiological stress (Armelagos 2003; Hillson et al. 2000; Pilloud and Kenyhercz 2016). Root measurements were used to avoid issues of dental attrition and maintain sample size (Hillson, FitzGerald, and Flinn, 2005, Stojanowski 2007).

Maximum mesiodistal and buccolingual root dimensions at the cemento-enamel junction were recorded for the following non-polar teeth, excepting in cases of extreme wear or incomplete mineralization: maxillary second incisors, mandibular first incisors, and mandibular and maxillary fourth premolars and second molars. Measurements from the left side were used preferentially, but antimeres were swapped when necessary. Tooth crown and root size capture growth disruptions occurring from 6 months-8 years and 7-14 years of age, respectively (Hillson 2014; Moorrees, Fanning and Hunt, 1963). Root measurement followed Aubry's (2014) revision of Hillson and colleague's (2005) methodology, and measurements were taken to the nearest 0.01 mm with the Mitutoyo 573 Absolute Point Caliper. The left dentition was used preferentially, but antimeres were substituted when necessary.

A similar procedure was used to create a composite score for root size. The sample was first split into two age groups: juveniles less than 14 years of age, whose overall root development was not complete, and individuals 15 years of age or older. Each root measurement (mesiodistal and buccolingual) per tooth was assessed for normality and homogeneity of variance. Student's two sample t-tests were used to identify significant differences in average measurement scores based on site and sex within each age group, and Mann-Whitney tests used for variables with non-normal distribution.

The adult Upper 4th Premolar mesiodistal root (UPM4-MDR), Lower 2nd Molar mesiodistal root (LM2-MDR), Lower 2nd Molar buccolingual root (LM2-BLR), Lower 4th Premolar mesiodistal root (LPM4-MDR), and Lower 4th Premolar buccolingual root (LMP4-BLR) measurements had to be divided by sex. Following another normality check, the adult female UPM4-MDR and male LPM4-MDR measurements were log-transformed. Z-scores were calculated for each root measurement by sample, and individuals received a binary categorical score for their anterior teeth (incisors) and/or posterior teeth (premolars, molars): 1 if one or more root dimensions were below average size (> 1 SD below mean for sample); and 0 if all VNC dimensions were average or above average size (< 1 SD below mean for sample).

Dental Asymmetry. Fluctuating asymmetry (FA) is the phenotypic divergence from perfect bilateral symmetry (Parsons 1990, 1992). These deviations are caused by environmental and genetic stressors during development, and studies of phenotypic variation in numerous organisms, including humans, have demonstrated that increased levels of FA represent the cumulative effects of developmental disturbances and subsequent growth disruptions (Leary and Allendorf 1989; DeLeon 2007; Møller and Pomiankowski 1993). In addition to root size, the FA of all non-polar root dimensions was determined for each individual. FA was calculated by determining the size differences between left and right non-polar antimeres (d= |L-R|) for each measurement (Harris and Nweeia 1980). Scores were averaged to provide an estimate of mean FA per individual (Garn, Lewis, and Kerewsky 1966, 1967; O'Donnell and Moes 2020). Due to data non-normality, a univariate cut off point based on the average of individual mean FA scores was used to dichotomize individual scores as either high FA (1= above average asymmetry) or low FA (0= low or average asymmetry).

Latent Class Analysis

A subset of structural equation modeling, latent class models were first introduced by Lazarsfeld (1950). Similar to cluster or factor techniques, latent class models are widely used in social sciences for data exploration and classification (Hagenaars and McCutcheon 2002; Moustaki and Papageorgiou 2004). The principle underlying latent class analysis (LCA) is simple; a latent or unobserved variable(s) causes the covariation between observed variables, and identifying the number of classes of the latent variable will explain the relationship among the observed variables (McCutcheon 1987; Moustaki and Papageorgiou 2004; Vermunt and Magidson 2004). LCA uses the observed variables to estimate a parametric model and then generates conditional posterior probabilities of latent class membership for each case in the sample (McCutcheon 1987). Bioarchaeological and human biology studies have used LCA to explore constructs like status (Passalacqua 2012), lifelong physical activity (Salin et al. 2019), and sex (Passalacqua, Zhang, and Pierce 2013).

In this study, the latent variable of developmental phenotype was assessed through the standard osteological markers detailed above. As LCA requires the pre-specification of latent classes, three competing models with two, three and four potential classes were compared (McCutcheon 1987). Adjusted Bayesian Information Criterion (BIC) and loglikelihood will be used to assess model fit, and an entropy statistic will be used to measure delineation between latent classes (Celeux and Soromenho 1996; Nylund 2007). Following the identification of the most appropriate model, posterior probabilities were used to assign developmental phenotype classes to each individual (Celeux and Soromenho 1996; Nylund 2007). The LCA was completed with Mplus, a comprehensive mixture modeling program that uses maximum

likelihood estimation to handle missing data common in bioarchaeological datasets (Muthén and Muthén 2019).

The sample size used in this study is smaller (n=85) than recommended size of 200 (Muthén and Muthén 2019) due to data collection limitations resulting from the COVID-19 pandemic. Every effort was made to address this issue, however, such as the use of dichotomized categorical variables (Muthén 2004; Nylund, Asparouhov, and Muthén 2007). The goodness of fit measures and results were assessed carefully to ensure both statistical and practical validity of the results given theoretical expectations based on existing research.

Statistical Analysis

The relationships between developmental phenotype class and biocultural factors including sex, site, and burial type were examined with Chi-square tests. The association between age at death and developmental phenotype was first evaluated with a Kruskal-Wallis non-parametric test of independent measures, followed by a parametric hazards model with a Gompertz survival distribution.

Hazards models examine the effect of multiple variables on the time until the occurrence of a specific event, usually death (Cox, 1972). Semi-parametric models, such as the Cox proportional hazards model, are commonly used in bioarchaeology to estimate relative risk of death as they do not require a baseline survival distribution (Garland 2020; DeWitte 2014; Yaussy and DeWitte 2018). Parametric hazards models, however, have many advantages as baseline specification allows users to select appropriate survival distributions that match the data structure and predictable variations in hazards (Aalen et al., 2008; Reid, 1994). The Gompertz survival distribution, used by demographers and actuaries, better models human mortality risk by

reflecting the exponential increase in mortality risk that occurs with age (Gompertz, 1824; Preston, Heuveline, and Guillot, 2001; Vaupel, 1986). The RStudio package 'flexsurv' was used to generate the hazards model and survival curves for the combined Pre-Columbian dataset, as well as each individual site (Jackson, 2016). All other analyses were completed in RStudio, an integrated environment for the R programming language to facilitate statistical computing and graphics (RStudio Team 2020).

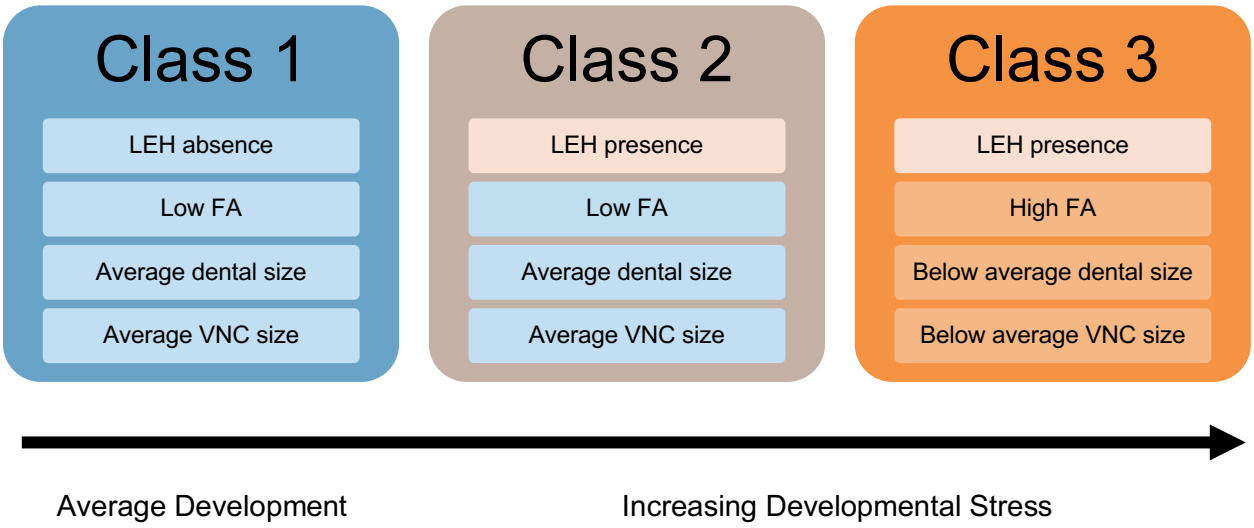
6.5 Results

Latent Class Analysis

Based on the goodness of fit measures, the three class model of developmental phenotypes best represents the data (Table 2). The three class model had the lowest Adjusted BIC and highest entropy values, indicating the best model fit and strongest class delineation relative to competing models. Given the small sample size, the following results should be interpreted cautiously, but the goodness of fitness measures and model parameters support the statistical and practical appropriateness of the three class model.

The results of the three class model are displayed in Table 3. The first phenotype class is categorized by minimal developmental stress, as individuals in this class have less than 10% probability of exhibiting any markers of impaired growth and development. In contrast, Classes 2 and 3 encompass individuals with differing degrees of disruption (Figure 2). The largest of the developmental phenotype classes, Class 2 is predominantly defined by LEH presence (100% probability), although individuals do exhibit other markers of developmental stress to a lesser extent. Class 3 reflects the highest level of developmental stress, and individuals in this class have more than 50% probability of exhibiting all markers of impaired growth and development.

Figure 6.2 Developmental Phenotype Latent Classes



Although the latent class model distinguishes between the degree of developmental stress represented by Classes 2 and 3, we combined these classes for further statistical analysis to prevent issues of unevenness due to the small sample size of Class 3 (n=4). This decision was justified by the overlap in posterior probabilities between Classes 2 & 3 and the performance of the two class model as the second best fitting model.

Basic Comparisons

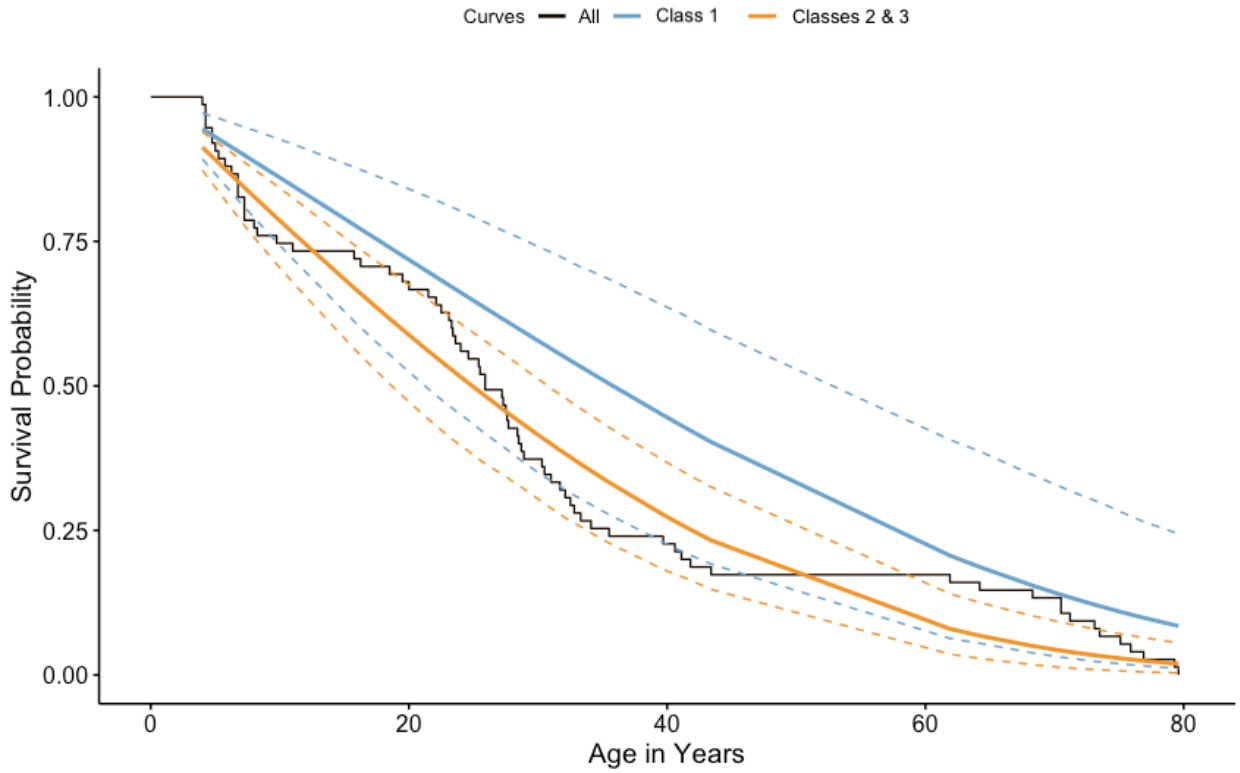
The Chi-square tests did not reveal any significant associations between developmental phenotype class and sex, site, or burial type (Table 4). Although Classes 2 and 3 were more common among individuals buried in single interments (n=24/27, 88.9%) compared to multiple interments (n=46/58, 79.3%), these differences in class distribution were not significant. Similarly, there were no significant differences in age at death distributions when comparing

Class 1 with Classes 2 & 3 (Kruskal-Wallis H=1.12, p= 0.29). The mean age at death for Class 1 was 39.2 years, and the mean age at death for Classes 2 & 3 was 29.1 years.

Gompertz Hazards Model and Survival Curves

The hazards analysis results are shown in Table 5, and corresponding survival curves are shown in Figures 3-5. In the total Pre-Columbian sample, the combined "disrupted" developmental phenotype classes (Classes 2 & 3) were associated with a 60.4% increase in mortality hazards. The difference in hazards, however, between individuals with "disrupted" developmental phenotypes compared to individuals with "average" phenotypes (Class 1) was not significant. A clear overlap of the 95% confidence intervals of mean survival times for each phenotype class is visible in the survival plot in Figure 3.

Figure 6.3 Gompertz survival curves for the combined Pre-Columbian sample



When analyzed separately, both Cerro Mangote and Sitio Sierra assemblages share a similar association between "disrupted" developmental phenotype and increased mortality hazards, although the difference in hazards between "disrupted" and "average" individuals is not significant. The survival plots for both sites (Figures 4 & 5) show significant overlap between the 95% confidence intervals, and in particular, the Sitio Sierra survival curves visibly diverge from the slope of the combined Kaplan-Meier survival curve average (black line). These issues of model instability are likely caused by the smaller sample size (n=44 Cerro Mangote, n=28 Sitio Sierra) and unevenness between phenotype class counts (e.g., 16% of Cerro Mangote assemblage in Class 1 and 84% in Classes 2 & 3) and ages, and therefore the results from the separate site hazards analyses should be interpreted with caution.

Figure 6.4 Gompertz survival curves for the Cerro Mangote (CO-40) site

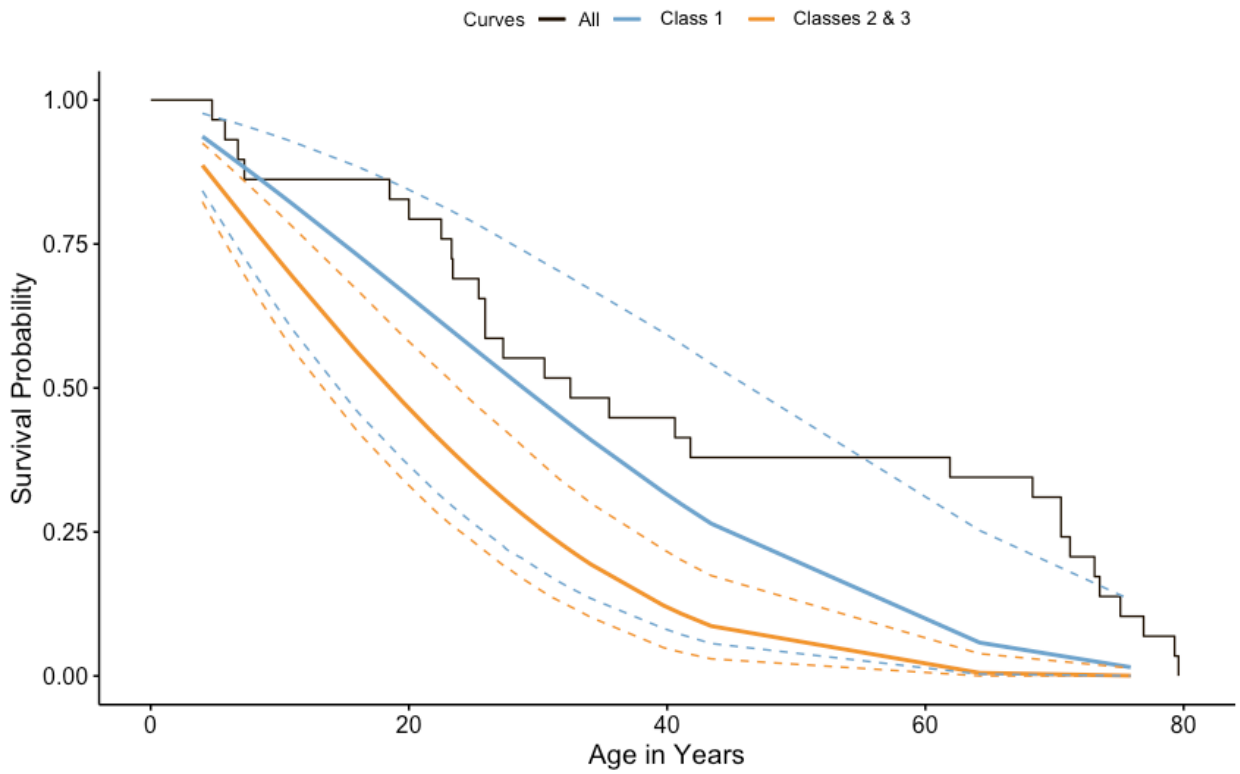
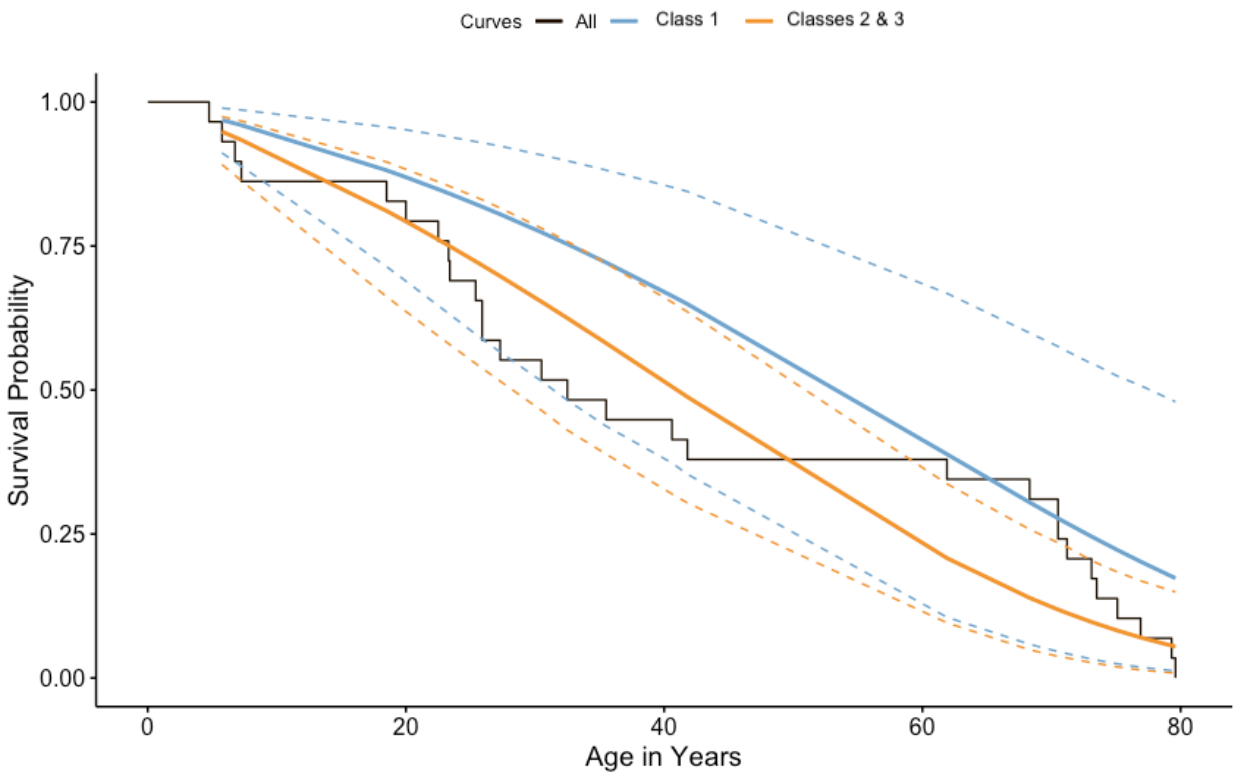


Figure 6.5 Gompertz survival curves for the Sitio Sierra (AG-3) site



6.6 Discussion

The DOHaD framework underscores the importance of development in shaping later life physiological function and health outcomes, and research in modern and skeletal populations continues to contribute to this scholarship. This study reconstructed developmental phenotypes through a latent class analysis of osteological markers in order explore the relationship between developmental stress and mortality in Pre-Columbian Panama. Pre-Columbian Panama (2480 BCE- CE 1158) provides a novel context and broad chronological range to test these associations.

Developmental Phenotype Latent Classes

Although the skeleton is a highly integrated physiological system involved in maintaining whole body homeostasis, bioarchaeologists face numerous challenges in extracting information from skeletal remains (Berger, Griffin, and Dent 2020; Ortner 2007). Bioarchaeological studies, as a result, often utilize non-specific osteological markers like porotic hyperostosis and LEH, to explore more complex, unobservable phenotypes of interest, such as frailty (DeWitte 2014; Marklein, Leahy, and Crews 2016; Yaussy 2019), malnutrition (Garland, Reitsema, Larsen, and Thomas 2018), and inflammation (DeWitte and Bekvalac 2011).

Latent class models provide a means of statistically assessing these unobservable constructs and their relationship to osteological markers. Through latent class analysis, we identified three unique developmental classes that represent different levels of developmental stress within our sample. Class 1 was comprised of individuals (n=15/85, 17.6%) with "average" developmental experiences who exhibited no or minimal osteological markers of growth disruption and developmental stress.

Class 2 membership was predominantly defined by LEH presence (100% probability). LEH appear to characterize a low to intermediate level of developmental stress common (n=66/85, 77.6%) during the Pre-Columbian Period in the Greater Coclé region of Panama. Finally, Class 3 represents the highest level of developmental stress, which is characterized by the presence of three or more osteological markers of growth disruption and developmental stress during childhood (LEH presence, high FA) and into adolescence (below average root size, below average VNC size). This level of pervasive developmental stress, however, was less common and only affected four individuals (4.7%) in the sample.

Although each osteological marker in our analysis has been independently associated with elevated mortality risk, LCA provides a means of incorporating multiple variables to better explore the interactions between different osteological markers and reconstruct developmental stress. Osteological markers of developmental stress represent the disruption of different growth processes, from the cessation of amelogenesis (Hillson 2019) to reduced periosteal bone apposition (Cunningham, Scheuer and Black 2016), occurring at different times throughout the body. The skeletal system, however, is tightly integrated and regulated by energy, mineral, and other homeostatic processes (Berger, Griffin, and Dent 2020). As a result, a holistic assessment of these osteological markers is needed to capture both acute growth disruptions and systemic developmental stressthat contribute to later life outcomes.

Latent class models also provide an important means of contextualization. The developmental classes identified in our model represent the specific range of developmental experiences in our Greater Coclé sample and provides important insight into the manifestations of developmental stress in this unique context. For example, LEH is commonly utilized as a single indicator of developmental stress in many studies (Armelagos, Goodman, Harper, and Blakey, 2009; Boldsen 2007; Ham, Temple and Klaus 2020). Our results demonstrate, however, that LEH presence alone may not be sufficient enough to identify developmental experiences that significantly increase future mortality risk in settings similar to Pre-Columbian Panama where intermediate forms of developmental stress are common. The results from "one size fits all" approaches may be misleading and should be contextualized with methods such as LCA.

The main limitation to adding LCA to bioarchaeological studies is the requisite sample size of 200 individuals. Skeletal preservation, assemblage size, archival space, and numerous other challenges result in smaller bioarchaeological samples. While sample size was an issue in

this study as well, our results suggest that careful LCA of smaller samples can still provide meaningful insights into biocultural phenotypes and experiences of past populations. Importantly, these results provide a useful foundation for future efforts to expand the Pre-Columbian sample and add additional osteological markers to better capture earlier developmental stress.

Developmental Origins and Mortality Risk

The parametric hazards analyses revealed a consistent positive association between developmental stress and increased mortality risk, and these results align with other bioarchaeological studies into the developmental origins of health and disease in past populations (Armelago, Goodman, Harper and Blakey 2009; Boldsen 2007; Godde, Pasillas, and Sanchez 2020). Although growth and developmental stress contributed to frailty and increased risk of mortality among Pre-Columbian populations living in the Greater Coclé region of Panama, this association was not statistically significant. These results provide an important reminder that geographical, temporal, and cultural differences between populations warrant caution when making general assumptions regarding the developmental origins of health and disease. Potential biocultural factors affecting the relationship between developmental stress and mortality risk are considered below.

Infectious Disease. The DOHaD framework arose out of research into the relationship between developmental stress and heart disease (Barker 2004; Barker et al. 1989), and studies in modern populations continue to explore the effects of development disruption on chronic diseases and impaired physiological function (Hoffman, Reynolds, and Hardy 2017; Stiemsma and Michels 2018). Infectious disease, however, has been the primary cause of mortality for the

last 10,000 years following the first epidemiologic transition, and comparatively less is known about the relationship between developmental experiences and infectious disease mortality risk throughout human history (Barret, Kuzawa, McDade, and Armelagos 1998).

Modern population studies have explored the relationship between development and infectious disease in diverse ecological settings (Ashworth 1998; Black et al. 2008). In Gambia, premature adult mortality from infectious disease was linked to developmental stress caused by seasonal and nutritional stress (Moore et al. 1999; Moore, Collinson, N'Gom, Aspinall, and Prentice 2006). Adverse developmental environments have also been associated with increased susceptibility to infectious disease (Fagundes, Glaser, and Kiecolt-Glaser 2013; McDade, Beck, Kuzawa, and Adair 2001a; Shirtcliff, Coe, and Pollak 2009). Impaired immune system development and subsequent immune function have been identified as the primary pathway linking early developmental experiences with increased risk of infectious disease morbidity and mortality (McDade 2005; McDade, Beck, Kuzawa, and Adair 2001b; Moore et al. 2012).

In the field of bioarchaeology, DeWitte and colleagues' (DeWitte and Wood 2008; DeWitte and Hughes-Morey 2012) exploration of medieval survivorship revealed that developmental stress was associated with excess mortality during the Black Death (*Yersinia pestis*) plague in London. Although the catastrophic Black Death does not represent normal infectious disease mortality, these results suggest that developmental stress still increases frailty and mortality risk in disease ecologies with a primarily infectious disease burden. In their study comparing 18-19th century cholera victims to a modern attritional population, Smith and colleagues (2020) found similar results. Developmental stress markers were significantly more prevalent among cholera victims, and they concluded that developmental stress was associated with infectious disease mortality.

Evidence from Pre-Columbian skeletal remains suggests that the tropical climate and disease ecology of Panama resulted in relatively frequent infectious disease exposure throughout the life course (Rojas-Sepúlveda, Rivera-Sandoval, and Martín-Rincón 2011; Smith-Guzmán and Berger, submitted). Given the results of comparative modern and past population studies, we expected infectious disease to be a common source of developmental stress that would also lead to increased infectious mortality risk later in life. Despite evidence of infectious disease exposure in the study samples (Smith-Guzmán, n.d), developmental phenotypes characterized by stress and disruption are not associated with increased mortality risk. These results are unexpected, and we argue that other factors may be affecting the relationship between developmental stress and infectious disease mortality risk.

Critical Developmental Periods. Developmental plasticity enables phenotypic variation in response to environmental cues in order to enhance evolutionary fitness (Bateson et al. 2004; Gluckman et al. 2007). Plasticity is energetically expensive, however, so physiological responses are limited to critical periods during the fetal and neonatal stages when maternal provisioning transmits important intergenerational phenotypic information (Bornstein 1989; Drake and Walker 2004; Wells 2007). Insults during critical periods, therefore, have the greatest adverse impact on future outcomes because they lead to permanent modifications to system function through epigenetic changes (Godfrey, et al. 2011; Heijmans et al. 2008; Weaver et al. 2004) and impaired organ or hormone axis development (Beijers, Buitelaar, and de Weerth 2014; Seckl 2007; Thayer and Kuzawa 2014).

Most osteological markers of development stress, however, represent responses to growth and developmental insults occurring after critical windows of developmental plasticity. For example, LEH can occur as early as the first postnatal year when permanent tooth crown

formation begins. However, the first 20-50% of crown growth and any potential amelogenesis disruptions during this period are hidden under cusp tips, and tooth wear can further obscure evidence of early hypoplasias (Hillson 2019). Without microscopic enamel or dentin analyses, most of the LEH observed in bioarchaeological analyses reflect later amelogenesis disruption occurring after the age of two. Similarly, VNC and root size reflect later postnatal skeletal growth after critical periods (Hillson 2014; Moorrees, Fanning, and Hunt 1963; Watts 2013).

As a result, the disruptions captured by the osteological markers in this study may not reflect the permanent epigenetic or system and axis developmental changes that lead to significantly impaired physiological function, allostatic load and increased mortality risk. Recent microscopic enamel and dentin studies (Garland 2020; King, Humphrey, and Hillson 2005; Temple 2014) show that defects formed within the first year of life are associated with the greatest mortality risk compared to later defects, and these results demonstrate that early developmental stress during critical periods has the most durable impact on later mortality risk.

Cultural Buffering. Although environmental cues become embodied during developmental plasticity, cultural factors experienced throughout the life course can work to exacerbate or mitigate the adverse effects of developmental stress (Krieger 2001, 2005; Temple 2019). Research in modern populations has focused primarily on how early experiences of negative cultural factors, like racial, social, economic, and environmental marginalization, are associated with later obesity and cardiometabolic disease (Kuzawa and Sweet 2009; McDade et al. 2017), mental health disorders (Thayer et al. 2017), and increased mortality risk (Collins and David 2009; Forastiere et al. 2007).

Bioarchaeological studies on early development and later mortality often utilize assemblages from populations experiencing upheavals and significant stress. Rose, Armelagos,

and Lallo (1978) published some of the earliest work linking enamel deformations to earlier age at death in groups undergoing a transition from horticulture to intensive agriculture. Additional studies have examined the developmental origins of health and disease in the context of colonial contact and oppression (Dent 2017; Garland 2020), warfare and conflict (Holder, Miliauskiené, Jankauskas, and Dupras 2020; Wilson 2014), economic and political transitions (Newman and Gowland 2017; Stojanowski and Carver 2011), and periods of significant scarcity (DeWitte and Wood 2008). These environments likely exacerbated developmental stress through the accumulation of psychosocial, nutritional, and immune insults throughout the life course.

Although archaeological evidence and Spanish ethnohistorical accounts indicate a similarly challenging Pre-Columbian environment of continuous conflict, rigid social hierarchy, and extreme inequality, recent work (Hoopes 2005; Smith-Guzmán and Cooke 2018) has demonstrated the need to reevaluate these assumptions. Instead, several aspects of Pre-Columbian lifeways in the Greater Coclé region may have worked to buffer the negative effects of developmental stress. Significant archaeobotanical (Dickau 2010; Piperno 2011), zooarchaeological (Cooke and Ranere 1999; Martínez-Polanco, Ranere, and Cooke 2020), and stable isotope (Huard 2013; Norr 1991, 1995; Sharpe et al. 2021) data points to a diverse marine and terrestrial diet for the inhabitants of Cerro Mangote and Sitio Sierra (Cooke 2005). Adequate diets may have prevented energetic tradeoffs during development or mitigated the effects of subsequent impaired physiological function.

The mortuary contexts at both sites contain few indications of hierarchical status. Individuals at Cerro Mangote were rarely buried with funerary items, and juveniles were the primary focus of special mortuary treatment (Briggs 1989; Cooke 1979, 1984; Huard 2013; Ranere 1980). Recent biodistance analyses also indicate the presence of kinship systems that

integrated community members through various biological and social relationships to meet potential economic, political, and religious needs (Berger, Smith-Guzmán, and Cooke, in prep). The lack of formalized inequality and the prominence of biocultural kinship systems suggest that inhabitants of Cerro Mangote and Sitio Sierra had adequate access to resources and social support throughout the life course.

These communities faced environmental challenges that likely contributed to physiological stress, such as population growth and the adoption of new lifeways. Although most individuals (n=66/85, 77%) exhibited at least one osteological marker of developmental stress, only a small percentage of the sample (n=4/85, 4.7%) exhibited three or more markers of developmental stress. The low prevalence of individuals in Class 3 with higher levels of development stress suggests that subsistence practices and social systems may have limited the number of adverse environmental exposures during development or buffered against the negative consequences of developmental insults throughout the life course.

6.7 Conclusions

This study presents data on the relationship between development and later life mortality risk from the understudied Isthmo-Colombian Area. A latent class analysis identified three classes with distinct profiles of developmental stress, and developmental stress was positively associated with frailty and increased mortality risk at two Pre-Colombian sites in the Greater Coclé region of Panama. Individuals with greater developmental stress died at younger ages compared to individuals with less developmental stress; while these differences in mortality risk were not significant, the effect size was large.

Further research will expand this analysis with a greater sample size and incorporate osteological markers that better capture early critical developmental periods. The use of continuous measures, such as number of enamel hypoplasias, would also provide better insight into the effects of developmental stress frequency on mortality risk. In addition to improving our understanding of developmental plasticity in diverse chronological and geographic contexts, this study highlights the role of both biological and cultural factors in shaping the effects of adverse development on later morbidity and mortality risk.

Table 6.1 Demographic Profile

	Cerro Mangote (n= 50)		Sitio Sierra (n=35)	
	n	%	n	%
Sex				
Male	16	27.5	13	26.5
Female	13	31.4	19	44.1
Undetermined	21	41.2	3	8.8
Age				
<20	19	37.3	4	11.8
20-34	24	41.2	12	29.4
35-49	4	5.9	4	8.8
50-65	1	1.9	1	2.9
65+	1	1.9	10	29.4
NA	1	11.8	4	17.6
Burial Type				
Single	10	19.6	17	50
Multiple	40	80.4	18	50

Note: The five individuals without point age estimates (NA) were included in the LCA but were excluded from the hazards analysis.

Table 6.2 Latent Class Model Goodness of Fit Measures

	Adjusted BIC	Loglikelihood	Entropy
2 Class Model	341.95	**-163.89**	0.80
3 Class Model	**322.38**	-150.25	**0.96**
4 Class Model	327.53	-148.95	0.70

Note: When comparing model fit measures: the lower the Adjusted BIC and loglikelihood, the better the model fit; the greater the entropy, the better the delineation between latent classes. The best values for each statistic have been highlighted in bold type.

Table 6.3 Three Class Latent Model Results

	Coefficient	Standard Error	Probability
Class 1 (n=15)			
LEH presence	-11.14	0.87	0%
High FA	-2.64	1.04	6.7%
Below average anterior root size	-15.00	0.00	0%
Below average posterior root size	-2.64	1.04	6.7%
Below average VNC size	-15.00	0.00	0%
Class 2 (n=66)			
LEH presence	15.00	0.00	100%
High FA	-0.87	0.27	29.4%
Below average anterior root size	-2.34	0.69	8.8%
Below average posterior root size	-2.65	0.52	6.6%
Below average VNC size	-1.85	0.66	13.6%
Class 3 (n=4)			
LEH presence	15.00	0.00	100%
High FA	0.51	1.56	62.5%
Below average anterior root size	15.00	0.00	100%
Below average posterior root size	0.55	2.10	63.3%
Below average VNC size	15.00	0.00	100%

Table 6.4 Chi-square Test Results

	Class 1 (n=15)	**Classes 2 & 3** (n=70)	x^2
Sex			
Female	6	26	
Male	5	24	0.03
* Undetermined	4	20	
Site			
Cerro Mangote	9	42	0.08
Sitio Sierra	6	28	
Burial Type			
Single	3	24	1.35
Multiple	12	46	

Note: None of the x^2 values were statistically significant (p> 0.05).
*Individuals with undetermined sex (predominantly juveniles) are listed in the contingency table but were not included in the Chi-square test.

Table 6.5 Parametric Hazards Model with Gompertz Distribution

	Coefficient	**95% CI**	**Standard Error**	**Hazards Ratio**	**P value**
Total Class 1= 14 Classes 2 & 3= 66	0.47	-0.16- 1.10	0.32	1.60	0.14
Cerro Mangote Class 1= 9 Classes 2 & 3= 41	0.61	-0.26- 1.48	0.45	1.84	0.17
Sitio Sierra Class 1= 5 Classes 2 & 3= 25	0.51	-0.47- 1.48	0.49	1.67	0.31

Note: None of the p values were statistically significant (p> 0.05).

CHAPTER 7: PRE-COLUMBIAN IMMUNE PHENOTYPES: THE ROLE OF INFLAMMATION IN THE DEVELOPMENTAL ORIGINS OF MORTALITY RISK IN THE GREATER COCLÉ REGION, PANAMA

7.1 Introduction

The Developmental Origins of Health and Disease (DOHaD) framework provides a model for exploring how early life experiences shape health and disease outcomes later in life (Barker 1995; Gluckman, Hanson, Cooper, and Thornburg 2008). Interdisciplinary research, such as Franz Boas' (1912) study of anthropometric variation among the children of new European immigrants, has long indicated a relationship between childhood environments and phenotypic variation, and the underlying principle that connects early life and later outcomes is developmental plasticity. Developmental plasticity enables the modification of individual phenotypes through various processes, such as organ development and epigenetic changes to gene expression. These modifications are made in response to environmental cues in order to enhance evolutionary fitness (Bateson et al. 2004; West-Eberhard 2003). The subsequent phenotypic variation, however, is not inherently adaptive. Adverse environmental cues during development have been implicated in the etiology of numerous chronic diseases, such as cardiovascular disease (Barker et al. 1989; Galobardes, Smith, and Lynch 2006; Wadsworth, Cripps, Midwinter, and Colley 1985), obesity and diabetes (Li et al. 2010; Monasta et al. 2010), and mental health disorders (Heim and Binder 2012) that account for the deaths of over 30 million people a year (WHO 2015).

To address the global burden of chronic disease, considerable research has investigated the specific physiological mechanisms that increase morbidity and mortality risk following

adverse developmental experiences (Ozanne and Constância 2007; Wadhwa, Buss, Entringer, and Swanson 2009; Wells 2010). Immune function, central to somatic maintenance and organism survival, has emerged as a critical pathway contributing to poor health outcomes. The field of ecological immunology has demonstrated that immune system development is highly dependent on environmental cues for appropriate calibration to local nutritional availability, pathogen exposure, and extrinsic mortality risk (McDade 2003, 2005; McDade, Georgiev, and Kuzawa 2016). Adverse environmental cues and physiological stress in early life can therefore compromise immune development and lead to dysregulated immune function, such as pro-inflammatory signaling, with negative long term consequences (McDade 2003, 2012).

The role of immune activity in shaping health and disease is largely explored in human biology research with modern populations (Adai et al. 2011; Blackwell, Snodgrass, Madimenos, and Sugiyama 2010; McDade, Beck, Kuzawa, and Adair 2001; Urlacher et al. 2018). The recent development of the osteoimmunology field, however, has highlighted the intimate interaction between the skeletal and immune systems. Molecular signaling and transcription parallels connect the systems through numerous pathways and enable mutually dependent skeletal and immune regulation (Arron and Choi 2000; Tsukasaki and Takayanagi 2019; Walsh et al. 2018). Bone remodeling, as a result, reflects skeletal and immune interaction and can serve as a useful indicator of immune activity (Berger, Griffin, and Dent 2020; Crespo 2019).

Following these observations, bioarchaeologists have made methodological strides towards exploring immune activity as an important physiological pathway underlying patterns of health and disease in past populations (Crespo, Klaes, Switala, and DeWitte 2017; Klaus 2014). DeWitte and Beckvalac (2011) explored the effect of comorbidities on immune competence by examining associations between periodontal disease and periosteal lesions in a medieval English

population. The positive, age-independent associations between the pathological conditions demonstrated that osteological markers could reflect changes in immune phenotypes. Recent methodological and theoretical contributions have highlighted how skeletal phenotypes can result from and further exacerbate inflammation and immune dysregulation (Dent, Berger, and Griffin 2020) and the utility and feasibility of calculating skeletal inflammatory indexes (Crespo 2019).

In an effort to incorporate osteoimmunology into bioarchaeological analysis, this study reconstructs immune phenotypes through a combination of osteological proxy markers and extends current research aims by focusing on immune activity within a life history framework. By comparing both developmental (Berger, Smith-Guzmán, and Hutchinson, n.d) and immune phenotypes, this study explores the role of pro-inflammatory immune activity as a critical physiological pathway connecting adverse early developmental experience to later life mortality risk in understudied Early and Late Ceramic Period (2480 BCE- CE 1158) skeletal assemblages from the Greater Coclé region of Panama. A similar longitudinal study in living populations would present considerable logistical and ethical challenges, so this study also provides a useful model for exploring the developmental origins of immune activity and the effect on later life health and disease outcomes in past populations.

7.2 The Immune System

The immune system plays a critical role in cellular renewal and repair, and it is the body's key defense against microbial invasion and uncontrolled cellular replication. While these functions are essential to somatic maintenance, the development of the immune system requires considerable energetic resources and must be balanced between competing demands, such as

growth and reproducton (Lochmiller and Deerenberg 2000; McDade 2005; Urlacher et al. 2018). For example, the thymus, an immune organ crucial for the development of T cells, produces 20-25% of its total 10^{11} T cells through cell division each day in infancy (Ritter and Crispe 1992). Over 95% of these cells die in the thymus, however, as a result of stringent T cell selection mechanisms, making thymus development an energetically expensive process (Egerton, Scollay, and Shortman 1990; George and Ritter 1996). Given the high energetic costs of the immune system, adverse early environmental cues, including malnutrition and psychosocial stress, can lead to developmental tradeoffs that constrain immune system development and result in permanent changes to immune function.

Human biology research in modern populations has demonstrated that impaired immune development greatly impacts later health outcomes (Chen et al. 2016; Fagundes, Glaser, and Kiecolt-Glaser 2013; Monk, Lugo-Candelas, and Trumpff 2019). Many cohort studies have found associations between adverse development and future risk of inflammatory diseases, including coronary heart disease (Eriksson et al. 1999; Leon et al.1998), osteoporosis (Cooper et al. 2006; Holroyd, Harvey, Dennison, and Cooper 2012), and obesity (Armitage, Poston, and Taylor 2008; Thompson 2012). One possible route through which impaired immune development impacts later health is through increasing allostatic load, defined as the wear and tear that results from chronic overactivity or underactivity of allostatic systems (Danese and McEwen 2012; McEwen 1998a; Urlacher et al. 2018). Impaired immune development also directly affects physiological function through dysregulated inflammation (McDade 2012).

Adverse development results in long-lasting or even permanent changes, such as DNA methylation and histone modification, that promote a pro-inflammatory state which contributes to increased morbidity and mortality risk later in life (Champagne 2012; Fagundes and Way

2014; McDade 2012; McDade et al. 2017). For example, Miller and colleagues (2009) demonstrated that adults with low socioeconomic status in childhood had significant down-regulation of glucocorticoid signaling genes and increased production of cortisol and pro-inflammatory cytokines, resulting in a persistent pro-inflammatory state.

Studies in diverse global contexts have found similar positive associations between childhood psychosocial and environmental stress with dysregulated, pro-inflammatory phenotypes later in life (Coelho et al. 2014; Danese et al. 2007). Other indicators of adverse early environments, such as low birth weight, have also been found to predict elevated CRP (McDade et al. 2010; Raqib et al. 2007; Sattar et al. 2004) and other inflammatory marker concentrations (Pellenda et al. 2009). Overall, these findings demonstrate the importance of considering pro-inflammatory immune activity as a critical pathway underlying the developmental origins of health and disease.

7.3 Osteoimmunology: Immune and Skeletal Integration

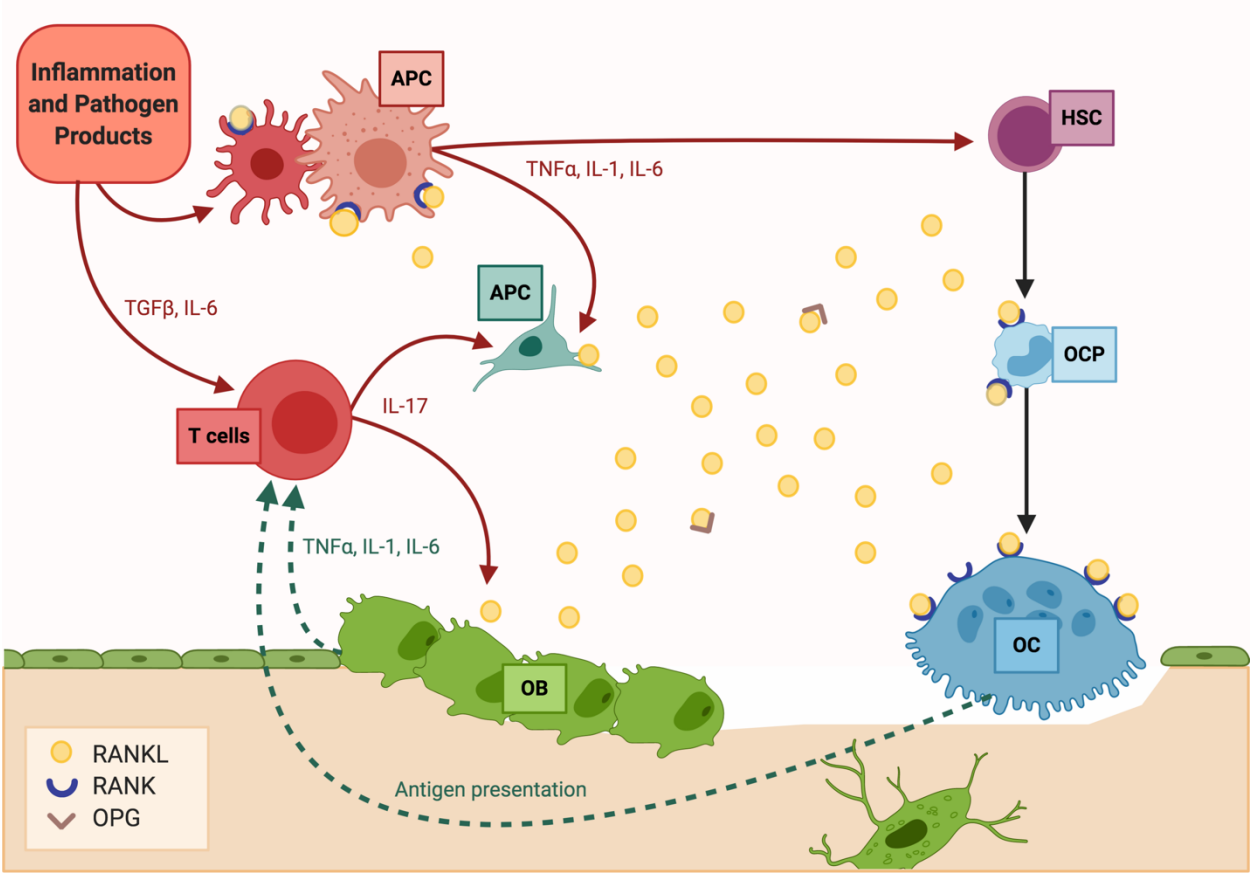
The field of osteoimmunology developed in response to several key discoveries regarding immune and skeletal interactions, and considerable research has demonstrated the intimate impact that immune function has on the skeletal system (Arron and Choi 2000; Tsukasaki and Takayanagi 2019; Walsh et al. 2018). Shared molecular parallels enable cross-talk between the skeletal and immune systems and provide a means of mutually dependent regulation (see Berger, Griffin, and Dent 2020 for summary). For example, RANKL (receptor activator of nuclear factor kappa-β ligand) is well known as the master regulator of bone resorption, but RANKL also plays a critical role in regulating dendritic cell survival, which is necessary for initiating adaptive T cell-mediated immunity (Josien et al. 2000; Takayanagi 2012). Similarly, TNF-α (tumor necrosis

factor alpha) cytokines play a critical role in the acute phase of the immune response by initiating dendritic cell differentiation, but TNF-α signaling also upregulates RANKL expression in osteoblasts to support osteoclast differentiation (Lam et al. 2000; Steeve, Marc, Sandrine, Dominique, & Yannick 2004; Walsh et al. 2006).

As a result of these shared signaling and transcriptional mechanisms, immune activation and inflammation directly impact bone homeostasis, and T lymphocyte and antigen presenting cell (APC) signaling pathways, in particular, link immune activity to skeletal remodeling (Figure 2) (Berger, Griffin, and Dent 2020; Walsh, Takegahara, Kim and Choi 2018). APC like macrophages and T helper 1 cells activate the cellular immune response through phagocytic activity and the production of cytokines.

The upregulation of pro-inflammatory cytokines by T_H1, T_H17, and APC, however, also stimulate osteoclastic activity. Pro-inflammatory cytokines including IL-17 (interleukin 17) and IL-6 (interleukin 6) initiate bone resorption by upregulating RANKL expression on numerous osteoclastogenic cells and promoting the expression of matrix-degrading enzymes (Sato & Takayanagi 2006; Takayanagi 2006), while other cytokines like TNF-α support osteoclastic activity by inhibiting bone formation (Heiland et al. 2010; Karmakar, Kay, and Gravallese 2010). These pro-inflammatory cytokines drive the patterns of bone resorption characteristic of chronic diseases such as osteoarthritis and osteoporosis (Ginaldi and De Martinis 2016; Karmakar, Kay, and Gravallese 2010).

Figure 7.1 Osteoimmunological Interactions in Uncoupled Bone Remodeling



Immune effects denoted by red arrows, and bone effects denoted by green dashed arrow. Inflammatory cytokine signaling through APCs and T cells leads to immune activation and the differentiation of T_H17 cells. T_H17 cell expression of IL-17 stimulates RANKL expression in osteoblasts and other stromal cells. RANKL and inflammatory signaling from additional immune cells further support osteoclast differentiation and activity, resulting in increased bone resorption. Osteoblasts and osteoclasts also perform immune functions by amplifying inflammatory signaling to boost the immune response and filling APC roles to improve immune clearance. Originally published in Berger, Dent, and Griffin (2020).

Importantly, bone cells further amplify inflammation in response to immune activity and pathogenic stimuli. Through TLR (toll-like receptors), osteoblasts can bind to pathogenic products and directly increase bone resorption through the expression of RANKL, as well as indirectly increasing bone resorption by expressing pro-inflammatory cytokines, chemokines, and prostaglandins that initiate an inflammatory immune response (Itoh et al. 2003; Kikuchi et

al. 2001; Marriott, 2004). The immune and bone cell interactions create a positive feedback loop that drives inflammation, which inhibits osteoblastic activity and bone formation.

These processes decouple normal physiological bone remodeling and result in periods of local and systemic bone resorption, potentially followed by bone formation after the resolution of inflammation (Ginaldi and De Martinis 2016; Matzelle et al. 2012; Walsh, Takegahara, Kim, and Choi 2018). Given the intimate ties between the immune and skeletal system demonstrated through considerable osteoimmunological research, skeletal phenotypes can serve as useful proxy indicators of pro-inflammatory immune activity.

7.4 Study Sample

Archaeological Context: Pre-Columbian Panama

The first archaeological evidence of human activity in the Greater Coclé region appears during the Holocene (Cooke et al. 2013). The climactic changes attendant to the Holocene benefited new subsistence strategies, and inhabitants practiced slash-and-burn agriculture in the Early Preceramic Period (9500-6000 BCE) with an increasingly diverse range of cultivars, including maize and manioc (Clary et al. 1984; Cooke and Ranere 1999; Dickau 2010; Piperno and Pearsall 1998; Piperno et al. 2000). Late Preceramic populations also foraged terrestrial and coastal resources from rich ecosystems as sea levels rose and marine habitats changed (Clary et al. 1984; Cooke and Ranere 1999). These environmental and subsistence shifts led to increased population and increased settlement size and density throughout the region.

Coastal progradation and soil depletion, exacerbated by population growth and cultivation, led to a regional settlement shift during the Ceramic Period (2500 BCE-CE 1520) (Piperno and Pearsall 1998; Ranere 1980). Populations developed new means of clearing land

and began occupying river valleys (Cooke 2005; Isaza 2007). The fertile alluvial bottomlands supported intensified cultivation, and by 2470 BCE, permanent villages become archaeologically visible and highlight the increasingly sedentary lifeways reliant on agriculture and regional trade (Cooke 1995; Isaza 2007).

The preparation and storage of food with new ceramic technology supported population growth, and archaeological evidence of workshops suggest that craft specialization and new forms of social hierarchy developed in the Middle Ceramic Period (Martín-Rincón and Sánchez Herrara 2007; Martín et al. 2016). The remains of monumental architecture, elaborate burials, and pottery from the Later Ceramic Period (CE 700- 1520) provide evidence of burgeoning inequality, and new elite sites may have served as meeting places for feasting, rituals, and mortuary practices that reinforced increasingly complex regional social ties (Cooke, 2005; Hoopes, 2005; Ichon, 1980; Mayo Torné and Mayo, 2013).

Skeletal Sample

This study explored the relationship between development and immune phenotypes and the moderating effect of immune phenotypes on the developmental origins of mortality risk at two Greater Coclé Region sites: Cerro Mangote and Sitio Sierra (Figure 2). These skeletal assemblages represent a broad temporal range, from 2480 BCE– CE 561 and CE 978-1158, that includes important Ceramic Period cultural transformations and provides insight into the range of Pre-Columbian immune phenotypes and mortality outcomes.

Cerro Mangote (CO-40). A shell midden on a large hill on the north bank of the Santa Maria River, Cerro Mangote is one of the oldest sites containing human remains in the Greater Coclé Region (McGimsey, 1956; Ranere 1980). Preceramic populations utilized the site as early as 6018 BCE, and archaeological evidence suggests that Early Ceramic inhabitants exploited

marine resources from nearby habitats and still consumed cultivars like maize (Huard, 2013; Norr, 1991, 1995).

Cerro Mangote was initially considered a dry season site for mobile foragers (Griggs 2005, Norr 1995), but evidence suggests that seasonal hunting strategies and extensive trade networks linking inland and coastal sites may have supported permanent habitation (Carvajal and Hansell, 2008; Martínez-Polanco, Ranere, and Cooke 2020). A total 110 individuals were recovered from the cemetery context, and the complex burial practices, such as large co-interments, evident at Cerro Mangote represent widespread mortuary traditions maintained throughout the Pre-Columbian period (Briggs, 1989; Smith-Guzmán and Cooke, 2018). Early studies of the skeletal remains noted a moderate to high prevalence of nutrient deficiencies, infection, and skeletal stress markers (Huard 2013; Norr 1991).

Figure 7.2 Map of Panama and Archaeological Sites

Locations of study sample sites, Cerro Mangote (CO-40) and Sitio Sierra (Ag3), are noted in bold. Adapted from Wikimedia Commons map - Alexrk2 (Bathymetry: NGDC ETOPO2v2 (public domain); Topography: NASA Shuttle Radar Topography Mission (SRTM30 v.2) (public domain); Shoreline and additional data: VMap-0 (public domain).

Sitio Sierra (*AG-3).* Situated approximately six kilometers upstream from Cerro Mangote, Sitio Sierra is a floodplain site characteristic of Middle Ceramic Period settlements (Cooke, 1972). The fertile alluvial soil supported two sequences of nucleated settlement and the intensive agriculture of numerous cultivars, including three different maize varieties (Cooke, 1984; Cooke, 1995). Although terrestrial and marine protein, acquired through hunting and trading, still contributed to the diet, archaeological and archaeobotanical evidence emphasizes the strong sedentary, agricultural focus of the community (Cooke, 1984; Norr, 1995).

Both sequences of occupation contain cemetery contexts (39 BCE- CE 561 and CE 978-1158), and the mortuary traditions demonstrate both cultural continuity and innovation as single primary interments supplant forms of multiple burial (Berger and Smith-Guzmán, n.d; Cooke 1984). A total of 39 individuals were recovered from the site (Cooke 1972, 1977, 1979). Norr (1991) noted minimal evidence of infection and malnutrition but attributed a greater frequency of osteological stress markers to changes in subsistence, settlement strategies, and population size.

7.5 Methods

The demographic profiles for each site are presented in Table 1. Standard bioarchaeological methods were utilized to estimate sex from pelvic and cranial morphology (Buikstra and Ubelaker, 1994; Klales et al., 2012).

Mortality

Individual age at death was used to quantify mortality risk. Adult age at death was estimated using transition analysis (TA), which produces point age estimates through maximum likelihood estimation. Following Boldsen and colleagues (2002), cranial suture closure, pubic

symphyseal changes, and iliac auricular surface changes were scored for each individual. Anthropological Database, Odense University (ADBOU) Age Estimation software combines these scores to generate age at death estimates. The program utilizes 17th century Danish rural parish records to estimate a prior distribution of age at death and then calculates the conditional probability of age indicators given known age at death with data collected from the Smithsonian Institution's Terry Collection. TA represents an important methodological improvement over traditional age estimation techniques, which require a high degree of skeletal preservation and result in broad age categories and the under-enumeration of older individuals (Boldsen, Milner, Konigsberg, and Wood, 2002; Milner and Boldsen, 2012).

Dental development, diaphyseal length, and epiphyseal fusion were used to estimate juvenile age (AlQahtani, Hector, and Liversidge, 2010; Buikstra and Ubelaker, 1994). All individuals younger than 20 years of age were excluded from further analysis.

Immune Phenotype

This study expands on previous work to explore the developmental origins of health and disease in Pre-Columbian Panama. Berger, Smith-Guzmán, and Hutchinson (n.d) utilized standard osteological markers to explore growth disruption and developmental stress. A latent class analysis (LCA) combined multiple skeletal and dental indicators to determine the range of developmental experiences within the Pre-Columbian sample, and the phenotype classes identified will be used in this analysis as a measure of developmental stress (see Berger, Smith-Guzmán, and Hutchinson for a full description of materials and methods).

We employ a similar technique here to estimate immune phenotypes in order to examine the potential role of pro-inflammatory immune activity in linking developmental stress to

increased mortality risk later in life. The following osteological proxy markers reflect different levels of inflammation and pro-inflammatory immune activity throughout the skeletal system.

Periosteal Lesions. Non-specific periosteal lesions are a commonly observed pathological lesion that cannot be attributed to a specific skeletal syndrome. A wide range of etiological factors, including trauma to the periosteum or infectious agents, elicit changes in bone remodeling homeostasis by triggering an inflammatory response (Berger, Griffin, and Dent 2020; Klaus 2014). Pro-inflammatory cytokine signaling increases osteoclast maturation and activity, which results in greater bone resorption during the initial phase of inflammation (Matzelle et al. 2012; Takayanagi 2007). As inflammation resolves and anti-inflammatory cytokines downregulate RANKL signaling, corresponding changes in the expression of the Wnt/ß-catenin pathway promotes osteoblast activity (O'Gradaigh and Compston 2003; Matzelle et al. 2012). Although bone formation can still occur during active inflammatory signaling (McQueen et al. 2011; Ragsdale and Lehmer 2012), periosteal new bone largely represents the resolution of inflammation (Matzelle et al. 2012).

Following differential diagnosis (Ragsdale and Lehmer 2012), non-specific periosteal lesions were scored to reflect the inflammatory activity represented by the sequence of lesion formation. Each individual received one of the following scores: 0 for lesion absence; 1 for late phase periosteal lesions characterized by "healed" or remodeled proliferative bone; and 2 for early phase periosteal lesions characterized by active resorption OR mixed lesions with multiple phases of bone resorption and formation that reflect chronic pro-inflammatory signaling. Lesion location was also recorded (0 for no or local lesions affecting one bone, 1 for systemic lesions affecting two or more bones). Systemic lesions increase the circulation of pro-inflammatory cytokines and pathogenic products, and the energetic cost and immunopathological damage of

subsequent systemic inflammation greatly increases allostatic load (Li and Verma 2002; Rahman and Adcock 2006).

Oral Inflammation. The oral cavity is home to a diverse community of commensal organisms that perform a variety of functions, such as aiding in digestion (Wade 2013). However, changes in oral environments, caused by factors ranging from oral pH, nutrient availability, and psychosocial stress, can enable anaerobic, pathogenic species to colonize the oral microbiome (Hajishengallis 2015; Kumar 2013). A dysbiotic oral microbiome has numerous consequences for both oral and systemic health (Dent, Berger, and Griffin 2020).

Periodontal disease (PD) is a chronic infection caused by a proliferation of pathogenic bacteria, such as *Porphyromonas gingivalis*, in dental plaque (Graves 2008; Hajishengallis 2014a). In a feedforward loop, the host inflammatory response to the bacteria causes periodontal tissue destruction, which benefits the pathogenic bacteria and further perpetuates inflammation-induced damage and breakdown of host tissues (Hajishengallis 2014b; Van Dyke 2020). The dysbiotic environment created by periodontal pathogens supports inflammation in the oral cavity, and the ulceration of connective tissues enables the translocation of pathogenic bacteria and local inflammatory mediators to the rest of the body, and this process has been linked to the etiology of atherosclerosis and neurodegeneration (Hajishengallis 2015; Han and Wang 2013).

PD, as a result, is an important driver of chronic local and systemic inflammation, and the immunopathological and energetic cost of PD-induced immune activation represents an important source of allostatic load (Crespo, Klaes, Switala, and DeWitte 2017; Loos 2005). PD results in the gradual destruction of periodontal tissues, and advanced PD can be identified in skeletal remains by the resorption of alveolar bone (DeWitte and Bekvalac 2011; Irfan, Dawson, and Bissada 2001; Larsen 1997). For this analysis, PD was scored as present (1) if alveolar crest

reduction exposed more than 50% of a single tooth root (Listi 2011; Lukacs 1989) and/or subgingival calculus, a plaque formed in the periodontal pocket in response to gingival inflammatory exudate, was observed on a single tooth (Lieverse 1999; Warriner, Speller, and Collins 2015). Damaged alveolar bone and teeth were not assessed.

A dysbiotic oral microbiome also results in dental caries, a chronic polymicrobial infection (Larsen 2018). Pathogenic bacteria produce lactic acid, which breaks down enamel during the digestion of dietary fermentable carbohydrates (Kane 2017), and the progression of carious lesions contributes to inflammation through two pathways. Odontoblasts drive local inflammation by producing pro-inflammatory cytokines in response to pathogens (Horst, Horst, Samudrala, and Dale 2011; Staquet et al. 2008). As carious lesions progress and expose the nerves, blood vessels, and connective tissues that maintain the tooth, the accumulation of inflammatory and bacterial products can translocate through the body and contribute to systemic inflammation (Hillson 2018; Love and Jenkinson 2002; Love, McMillan, Park, and Jenkinson 2000). Following standards (Buikstra and Ubelaker 1994; Hillson 2018), individuals with at least one carious lesion present on any tooth were scored as 1, and when a clear cavity penetrated the pulp chamber, pulp exposure was also scored as present (1).

The progression of both PD and dental caries may result in antemortem tooth loss (AMTL). The chronic inflammation of PD disrupts the periodontal ligament that binds the roots into their sockets, and once the connection with the root is broken, the tooth falls out (Coventry, Griffiths, Scully, and Tonetti 2000; Hajishengallis 2014). With caries, the most heavily affected teeth are primarily lost through extraction in order to reduce the pain from periapical inflammation and the risk of infection (Hillson 2018). AMTL caused by PD and caries therefore represent the resolution of chronic periods of immune and pathogenic activity that likely

contributed to inflammation. AMTL was marked as present (1) if one or more teeth were absent and showed evidence of alveolar bone remodeling (Hillson 2018). Although the exact cause of AMTL cannot be determined, possible AMTL representing non-inflammatory etiologies were reduced by excluding individuals with dental trauma, congenital absence of teeth, and impacted teeth.

Joint Inflammation. Largely attributed to joint wear and tear, osteoarthritis (OA) was considered a biomechanical consequence of senescence. Recent clinical and experimental studies show, however, that local inflammation in the synovium is a critical feature of OA (Berenbaum 2013). Synovial macrophages have recently been proposed as the primary trigger of the OA process, as synovial macrophage expression of pro-inflammatory cytokines triggers synovial and chrondrocyte production of matrix metalloproteinases that degrade cartilage (Blom et al. 2007; Hussein et al. 2008). The result is a feedforward loop where synovial inflammation perpetuates cartilage destruction. Importantly, this local joint inflammation has systemic impacts. Studies have demonstrated that individuals with OA have higher levels of circulating inflammatory mediators and differential peripheral blood leukocyte gene expression that increases the production of pro-inflammatory cytokine IL-1ß (Attur et al. 2011; Attur et al. 2012).

All observable joints were assessed for OA following standards for differential diagnosis (Waldron 2009), and OA was recorded as present if one or more non-vertebral joints were affected. To capture the cumulative immune and allostatic effect of multiple sources of inflammation, the number of joints affected by OA was estimated for each individual. The spinal joints, including all costal, transverse, and articular facets, were compiled into one site to minimize preservation biases. Due to data non-normality, a univariate sectioning point was used

to dichotomize scores as average (1= 6-10 affected joints) or above average (2= 11+ affected joints). Individuals without OA were scored as 0 for both variables.

Latent Class Analysis

Latent class models are a form of structural equation modeling introduced by Lazarsfeld (1950) and serve a similar function to cluster and factor techniques. Latent class models are particularly useful for data exploration and classification of unobservable constructs such as frailty or immune phenotype (Hagenaars and McCutcheon 2002; Moustaki and Papageorgiou 2004). Latent class analysis (LCA) uses the covariation between observed variables to create a parametric model that estimates classes of the latent or unobservable variable, and the latent variable classes in turn explain the relationship among the observed variables (McCutcheon 1987; Moustaki and Papageorgiou 2004; Vermunt and Magidson 2004). Conditional posterior probabilities generated by the model can be used to further estimate latent class membership for each individual sample (McCutcheon 1987).

Following the methodology described in Berger, Smith-Guzmán, and Hutchinson (n.d), the latent variable of immune phenotype was assessed with the osteological proxy markers detailed above. Three competing models were estimated with Mplus, a comprehensive mixture modeling program (Muthén and Muthén 2019). Goodness of fit measures were compared to determine the most appropriate model (McCutcheon 1987; Muthén and Muthén 2019). Sample size adjusted BIC is a criterion that penalizes overfitting to show true model fit, and loglikelihood provides a measure of model fit by measuring the likelihood of model estimates. Entropy values approaching 1 indicate clear delineation of classes (Celeux and Soromenho

1996). Posterior probabilities were used to assign immune phenotype class membership to each individual (Celeux and Soromenho 1996; Nylund 2007).

Statistical Analysis

The relationship between development, as well as additional biocultural factors, and immune class was explored with a multinomial logistic regression which models the log odds of the categorical outcome variables as a linear combination of the predictor variables. Multiple regression, which models the relationship between predictor and outcomes variables by fitting a linear equation to the observed data, was used to model the direct relationship between immune class and mortality. Immune class was added as an interaction term to a multiple regression of developmental phenotype and mortality to explore the modification effect of immune class. All analyses were completed in RStudio, an integrated environment for the R programming language to facilitate statistical computing and graphics (RStudio Team 2020).

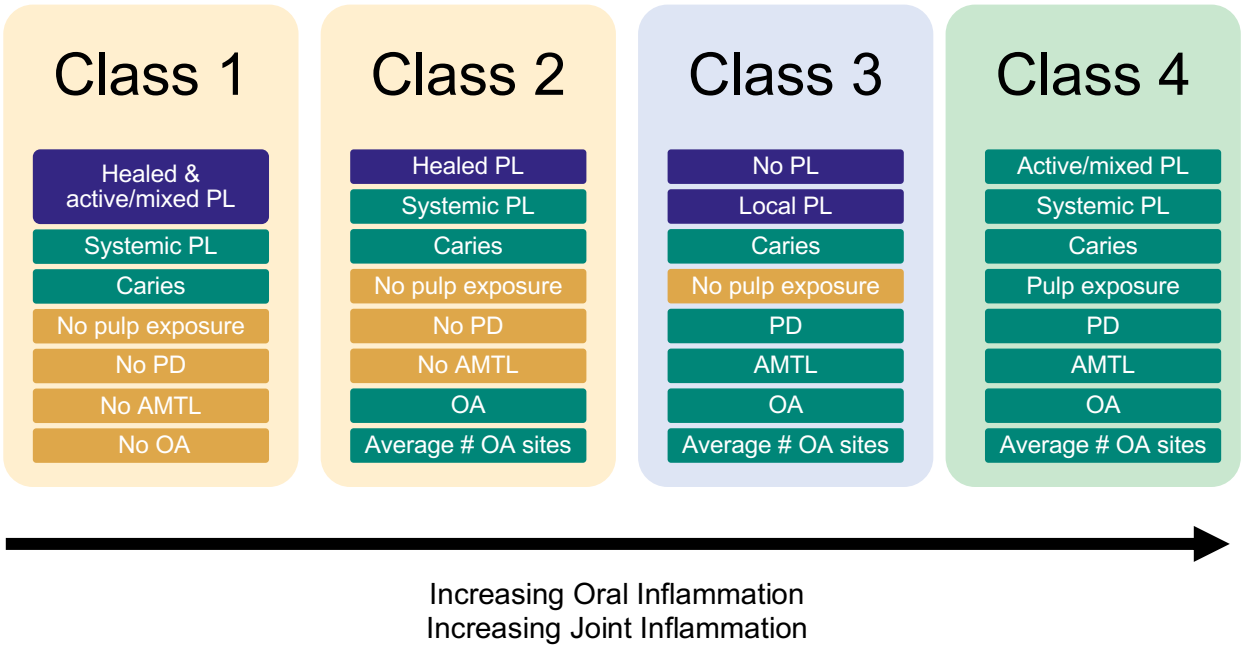
7.6 Results

Latent Class Analysis

Based on the goodness of fit measures, the four class model of immune phenotypes is the best model for the data (Table 2). The low sample size Adjusted BIC and high entropy values indicate better model fit and class delineation relative to competing models (Celeux and Soromenho 1996; Nylund 2007). The goodness of fitness measures and model parameters support the statistical and practical appropriateness of the four class model, but the results should be interpreted cautiously given the small sample size.

The results of the four class immune phenotype model are displayed in Table 3. The first two classes of immune phenotypes are categorized by minimal osteological markers of oral inflammation. In contrast, Classes 3 and 4 encompass individuals with increasing oral inflammation, joint inflammation and greater overall pro-inflammatory activity (Figure 2). Although the latent class model distinguishes between the degree of pro-inflammatory immune activity represented by Classes 1 & 2, these classes were combined for further statistical analysis to prevent issues of unevenness due to the small sample size of Class 1 (n=4). This decision was justified by the performance of the three class model as the second best fitting model, and the overlap in the posterior probabilities for Classes 1 & 2. Developmental phenotype classes, identified in Berger, Smith-Guzmán, and Hutchinson (n.d), were used to explore the effect of developmental plasticity on immune phenotypes.

Figure 7.3 Immune Phenotype Latent Classes



Multinomial Logistic Regression

The frequencies of immune class by developmental phenotype class, sex, site, and burial type are shown in Figure 4. The results of the multinomial logistic regression analysis are presented in Table 4. There were no significant associations between immune class and developmental phenotype, sex, or burial type. However, a significant association was found with the shift from the reference Immune Classes 1 & 2 to Class 3 and Class 4 and the Sitio Sierra site (Class 3 p=0.01, Class 4 p=0.01). Individuals at Sitio Sierra were significantly more likely to have pro-inflammatory Class 3 or Class 4 phenotypes compared to individuals at Cerro Mangote. This result is largely a function of the different age distributions between the sites (Cerro Mangote mean age= 23.4, Sitio Sierra mean age= 41.7), and the association between immune class and age is further discussed below.

Multiple Regression

The mean age at death for each immune class is shown in Table 5. Class 3 has the highest mean age at death, followed by Class 4. Classes 1 & 2 have the lowest mean age at death at 31.63 years. The results of the multiple regression model are shown in Table 6 and reveal a significant association between Class 4 and increasing age at death.

When added as an interaction, immune phenotype class modifies the effect of developmental phenotype on age at death (Figure 5). Although these relationships were not significant, the model demonstrates that immune Classes 3 and 4 are both associated with an earlier age at death for developmentally stressed individuals compared to individuals with an "average" developmental phenotype. The opposite relationship is apparent for immune Classes 1 & 2. The R^2 value, however, indicates that only 9% of the variation in age at death is explained

by immune and developmental phenotype classes, and the model is not significant at 0.05 ($p < 0.1$).

Figure 7.4 Immune Class Distribution

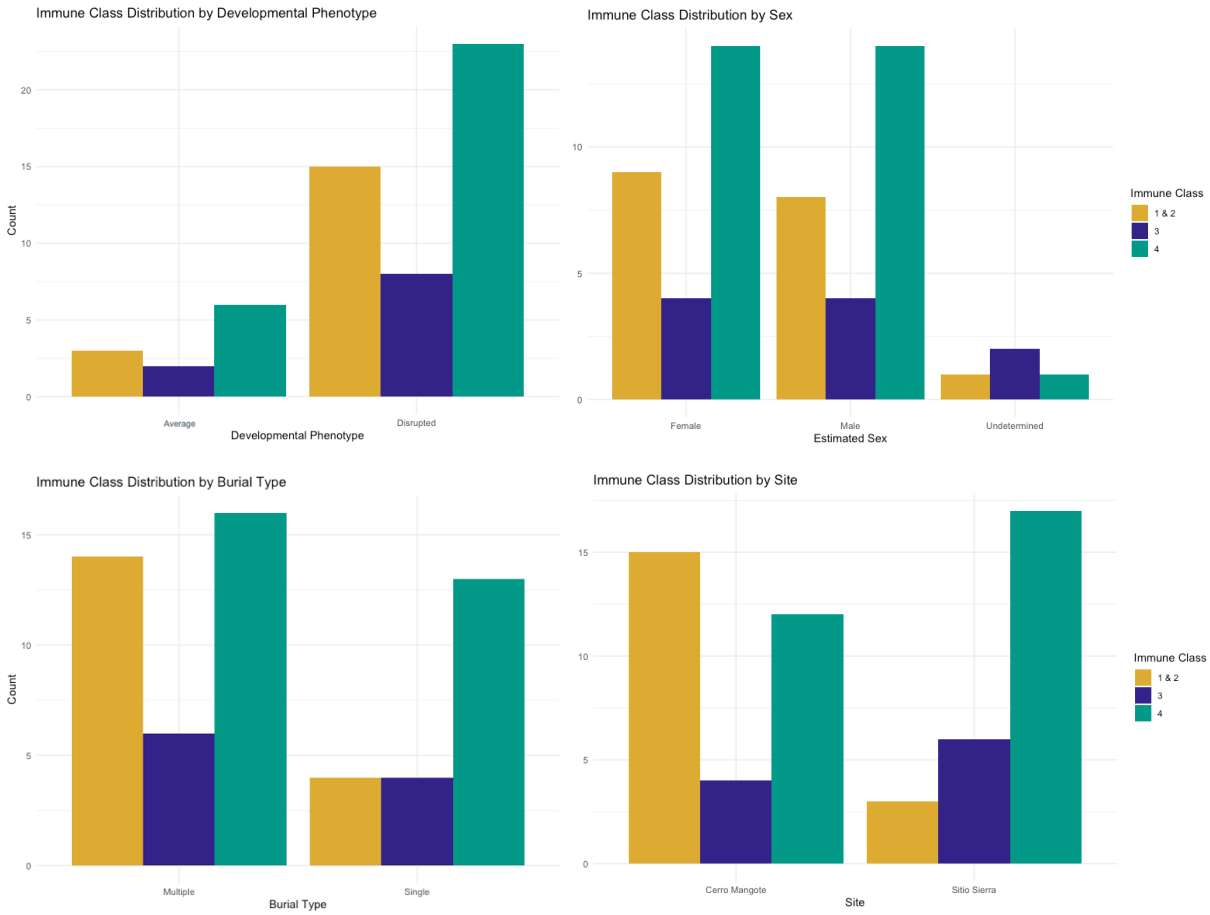
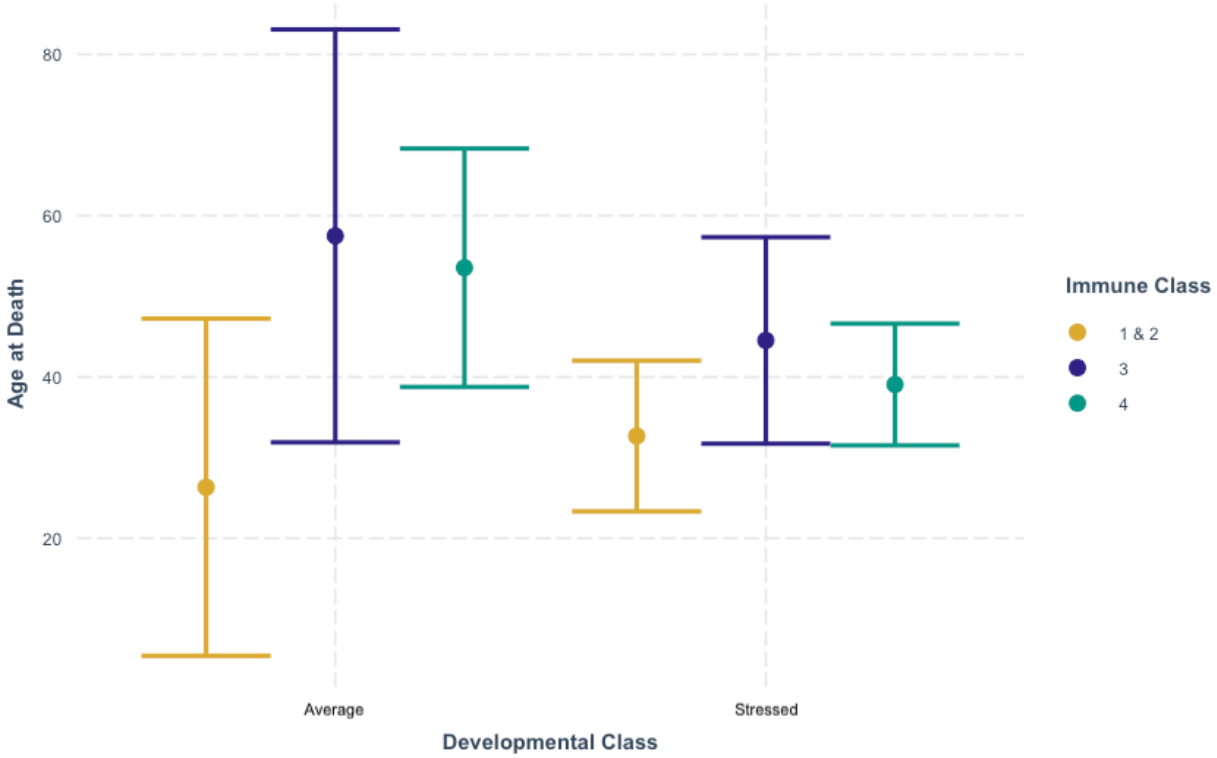


Figure 7.5 Modification Effect of Immune Phenotype on Developmental Origins of Mortality Risk



7.7 Discussion

Early environments determine later life outcomes by shaping the development and subsequent function of critical physiological systems such as the immune system (Kuzawa and Quinn 2009; Worthman and Kuzara 2005). While the mediating effect of the immune system on the developmental origins of health and disease is continually examined in modern populations, bioarchaeologists are developing new theoretical and methodological frameworks to explore these relationships in skeletal assemblages (Crespo 2019). This study contributes to this scholarship by reconstructing immune phenotypes through a latent class analysis of osteological markers of inflammation in a novel context. To gain further insight and a deep time perspective on the role of pro-inflammatory activity in linking developmental stress and mortality, this study

assessed interactions between developmental and immune phenotypes in Pre-Columbian Panama (2480 BCE- CE 1158).

Immune Phenotypes

Through the LCA, we identified four unique immune classes that represent different positions on a spectrum of pro-inflammatory activity (Figure 3). In regards to oral inflammation, Classes 1 & 2 represent low levels of pro-inflammatory activity with minimal likelihood of periodontal disease presence, antemortem tooth loss, and pulp exposure. In contrast, individuals in Classes 3 and 4 have greater than 75% likelihood of having periodontal disease and antemortem tooth loss. Interestingly, individuals in all 4 classes were more than 50% likely to have caries, which suggests that caries presence may not be useful in characterizing differences in inflammation in past populations with cariogenic diets and minimal oral hygiene. The presence of joint inflammation was generally associated with markers of oral inflammation, although individuals in Class 2 had a 100% likelihood of having osteoarthritis despite a lack of oral inflammation.

The differences in periosteal lesion activity and location between the phenotype classes are less straightforward. Despite minimal oral inflammation, individuals in Classes 1 & 2 are 100% likely to have a mix of healed and active periosteal lesions and more than 75% likely to have systemic periosteal lesions. In contrast, Class 3 is characterized by a 62.5% likelihood of having no periosteal lesions; when lesions are present, they are healed and local, indicative of inflammation resolution. The energetic and physiological burden represented by the Class 1 & 2 periosteal lesion state is considerably greater than Class 3. Finally, Class 4 represents the greatest degree of pro-inflammatory activity, with individuals more than 90% likely to have systemic,

active and/or mixed lesions indicative of chronic inflammatory signaling throughout the body. Overall, Classes 1 & 2 are defined by markers of systemic, pro-inflammatory bone remodeling but low to moderate levels of oral and joint inflammation. Classes 3 represents a greater degree of oral inflammation but lower to more moderate amounts of local, pro-inflammatory bone remodeling and joint inflammation in comparison to Classes 1, 2, and 4. Class 4 is characterized by a high level of all bone remodeling, oral, and joint pro-inflammatory markers.

Further analysis of immune class phenotypes and other biocultural factors revealed a significant association between immune class and age. The mean age at death in Classes 1 & 2 was 31.6 years of age, whereas the mean age at death for individuals in Classes 3 and 4 was 47.1 and 41, respectively. The positive relationship between immune class and mortality, where increasingly pro-inflammatory Classes 3 and 4 were associated with an older age at death, can be better understood within the osteological paradox and inflammaging frameworks.

In their seminal article on heterogeneous frailty and selective mortality, Wood and colleagues (1992) argued that skeletal lesions of physiological stress cannot be interpreted as straightforward indicators of poor health. Instead, some skeletal lesions may "paradoxically" indicate health because the individual survived the physiological disruption long enough for visible bone remodeling to occur and result in a new lesion (Ortner 1991; Wood et al. 1992). Individuals without lesions may have succumbed to illness, trauma or other stressors too quickly for bone remodeling to occur (DeWitte and Stojanowski 2015).

The potential "paradoxical" nature of skeletal lesions is particularly relevant in this study, given that many of the markers of inflammation are age related. While periodontal disease can begin early in life, the damage from chronic inflammation accumulates gradually, and the characteristic evidence of periodontal destruction is therefore most visible in older adults

(Albandar, Brunelle, and Kingman 1999; Hillson 2018). Similarly, osteoarthritis is classified as an age-related chronic disorder, as age is the greatest risk factor for osteoarthritis development (Anderson and Loeser 2010).

Franceschi and colleagues (2000) first coined the term "inflammaging" to refer to the progressive increase of chronic, low-grade inflammation characteristic of senescence. Various physiological mechanisms contribute to inflammaging, such as the accumulation of DNA, cellular, and tissue damage from reactive oxygen species that trigger an inflammatory response (Dall'Olio et al. 2013; Franceschi et al. 2000) and the increase of senescent cells that secrete pro-inflammatory cytokines (Campisi & Di Fagagna 2007). The net result is increasingly hyperactive innate immune activity and inflammation, and considerable studies have linked inflammaging to the pathogenesis of chronic conditions like osteoarthritis (Grant and Dixit 2013; Franceschi and Campisi 2014; McElhaney and Effros 2009; Shaw, Joshi, Greenwood, Panda, and Lord 2010).

Although Classes 3 and 4 represent increasingly pro-inflammatory states in the Pre-Columbian assemblage, the high degree of oral and joint inflammation "paradoxically" indicates improved survivorship because individuals lived long enough for these disease states to cause visible skeletal lesions. Similarly, these individuals lived long enough to experience senescence and age-related increases in inflammation that contributed to more pro-inflammatory immune states.

Despite the strong association with age, the immune phenotype classes still reveal important differences in mortality risks. The active or chronic inflammatory systemic periosteal lesions characteristic of Classes 1 & 2 were associated with increased mortality risk, and the results demonstrate that periosteal bone remodeling may serve as a useful indicator of immune activity in younger cohorts. When comparing older age cohorts, the Class 4 phenotype with

systemic, chronic inflammation is associated with an increased mortality risk compared to the Class 3 phenotype characterized by resolved local inflammation. These results suggest that age-related increases in inflammation may be unavoidable during the Pre-Columbian period, but individuals who resolved inflammation faster and limited chronic immune activation and pro-inflammatory signaling experienced a lower mortality risk. Senescence and inflammaging is primarily studied in modern populations, so these results greatly extend our understanding of inflammaging in the past and model the effects of pro-inflammatory states on mortality risk throughout human history.

Developmental and Immune Phenotype Interactions

This study examined the relationship between developmental and immune phenotypes to explore the effect of developmental stress on later immune activity. Although we expected developmental stress to correlate with more pro-inflammatory phenotypes, the multinomial regression revealed no significant association between developmental or immune phenotype classes. Given the sample size difference between individuals with the "average" developmental phenotype (n=12) compared to the developmentally stressed phenotype (n= 50), we argue these results may be an artifact of small sample size and should be retested with a larger sample.

The potential mediating role of immune function in the developmental origins of later mortality risk was also explored, and the results of the linear regression analysis provide greater insight into developmental and immune interactions. Although the associations were not statistically significant, the interaction plot (Figure 4) demonstrates how the consequences of developmental stress and potentially impaired immune development occurred later in life. For

individuals in Classes 1 & 2, who died at earlier ages, there is no increased mortality risk following developmental stress.

Amongst individuals in older age cohorts with pro-inflammatory immune phenotypes, however, developmental stress is associated with considerably greater mortality risk. These results suggest that adverse early environments do impair immune development, and subsequent dysregulation of immune activity serves as an important pathway contributing to increased mortality risk in the Pre-Columbian sample. The impact of dysregulated immune activity, however, appears to occur later in the life course of individuals who experienced developmental stress.

Interpreted within the framework of allostatic load, these results support the theory that adverse organ and system development can lead to impaired functioning, and the energetic and physiological costs of impaired function accumulates over time to greatly increase allostatic load and mortality risk later in life (Gluckman, Hanson, Cooper, and Thornburg 2008; McEwen 1998b). The allostatic burden caused by impaired immune function may also work synergistically with the processes of inflammaging. Individuals with adverse development may experience earlier or more intense dysregulation of immune activity, which results in a positive feedforward loop of inflammation caused by immunopathology and pro-inflammatory signaling as immune cells become senescent and hyperactive (Franceschi and Campisi 2014; Franceschi, Garagnani, Vitale, Capri, and Salvioli 2017).

The Greater Coclé region is a tropical climate, and evidence from Pre-Columbian skeletal remains suggest that the disease ecology resulted in a relatively high infectious pathogen load (Rojas-Sepúlveda, Rivera-Sandoval, and Martín-Rincón 2011; Smith-Guzmán and Berger, n.d). Similar to modern non-industrial populations living in tropical environments, Greater Coclé

inhabitants likely experienced frequent immune activation and physiological stress in response to pathogens (Hotez, Bottazzi, Franceo-Paredes, Ault, and Periago 2008; Pan, Erlien, and Bilsborrow 2010). This persistent exposure throughout the life course may have imposed an additional burden on individuals with impaired immune function, further contributing to heterogeneous mortality risk. Although additional studies with larger samples are needed to verify these patterns, these results provide new bioarchaeological evidence of the role of pro-inflammatory immune activity as critical pathways linking early environments with later mortality outcomes.

7.8 Conclusion

This study presents data on the relationship between development and subsequent immune phenotypes and later life mortality risk from the understudied Greater Coclé region of Panama. A latent class analysis identified four immune phenotype classes with distinct profiles of inflammation. The significant association between pro-inflammatory phenotypes and older age at death highlighted the role of inflammaging in creating "paradoxical" lesions that simultaneously represent increased survivorship and increased frailty later in the life course. When assessing older age cohorts specifically, chronic systemic inflammation was associated with an increased mortality risk. Importantly, this study demonstrates the role of pro-inflammatory immune activity in contributing to the developmental origins of mortality risk and provides a new model for exploring these relationships in skeletal assemblages.

Further research will expand this analysis to better capture the range of immune phenotypes in the Greater Coclé region during the Pre-Columbian period. In addition to improving our understanding of pro-inflammatory immune activity in diverse chronological and

geographic contexts, this study highlights the importance of inflammation as a critical pathway connecting adverse development with later morbidity and mortality risk.

Table 7.1 Demographic Profile

	Cerro Mangote (n= 62)		Sitio Sierra (n=39)	
	n	%	n	%
Sex				
Male	21	33.8	14	26.5
Female	19	30.6	21	44.1
Undetermined	22	35.5	4	8.8
Age				
0-19	19	30.6	5	12.8
20-29	16	25.8	8	20.5
30-39	19	30.6	3	7.7
40-59	4	6.5	10	25.6
60-69	1	1.6	2	5.1
70+	1	1.6	9	23.1
NA	2	3.2	2	5.1
Burial Type				
Single	11	17.7	18	46.2
Multiple	51	82.3	21	53.8

Note: Mean age at Cerro Mangote is 23.4 (n=52); Mean age at Sitio Sierra is 41.7 (n= 31)

Table 7.2 Latent Class Model Goodness of Fit Measures

	Adjusted BIC	Loglikelihood	Entropy
2 Class Model	601.31	**-288.15**	0.83
3 Class Model	578.22	-270.05	0.89
4 Class Model	**567.29**	-258.03	**0.92**

Note: When comparing model fit measures: the lower the Adjusted BIC and loglikelihood, the better the model fit; the greater the entropy, the better the delineation between latent classes. The best values for each statistic have been highlighted in bold type.

Table 7.3 Four Class Latent Model Probability Results

	Class 1 (n=4)	**Class 2 (n=20)**	**Class 3 (n=10)**	**Class 4 (n=43)**
Periosteal Lesion				
Absence	0%	0%	62.5%	0%
Healed	48.3%	61.5%	37.5%	6.9%
Active or mixed	51.7%	38.5%	0%	**93.1%**
Periosteal Lesion Location				
Local	22.5%	10.7%	100%	7.9%
Systemic	77.5%	**89.3%**	0%	**92.1%**
Caries				
Absence	0%	36.5%	12.9%	10.7%
Presence	100%	63.5%	87.1%	**89.3%**
Pulp Exposure				
Absence	100%	79.9%	50.3%	37.8%
Presence	0%	**20.1%**	49.7%	62.2%
Periodontal Disease				
Absence	100%	100%	23.5%	5.8%
Presence	0%	0%	76.5%	**94.2%**
AMTL				
Absence	66.7%	100%	23.8%	2.9%
Presence	33.3%	0%	76.2%	**97.1%**
Osteoarthritis				
Absence	100%	0%	13.1%	0%
Presence	0%	100%	86.9%	100%
Number of OA Sites				
Absence (0)	100%	0%	13.1%	0%
Average (6-10)	0%	74.9%	63.9%	71.7%
Above average (11+)	0%	**25.1%**	23%	**28.3%**

Note: Significant probabilities ($p < 0.05$), based on two-tailed p values of model estimates for each variable, are highlighted in bold font.

Table 7.4 Multinomial Regression Model

	Coefficient	Standard Error	Odds Ratio	P value
Developmental Phenotype				
Immune Class 3 x Disrupted Development	1.14	1.14	0.65	0.71
Immune Class 4 x Disrupted Development	0.88	0.88	0.56	0.51
Sex				
Immune Class 3 x Male	0.09	0.96	1.10	0.92
Immune Class 4 x Male	0.01	0.72	1.01	0.98
Site				
Immune Class 3 x Sitio Sierra	2.59	1.05	13.38	**0.01**
Immune Class 4 x Sitio Sierra	1.91	0.76	6.72	**0.01**
Burial Type				
Immune Class 3 x Single	0.81	0.98	2.24	0.41
Immune Class 4 x Single	0.98	0.79	2.68	0.21

Note: AIC= 123.90. Significant p values ($p < 0.05$) are highlighted in bold font. Immune Classes 1 & 2 serve as the references for the model.

Table 7.5 Immune Classes and Mortality

	n	Mean Age	SD
Classes 1 & 2	18	31.63	13.04
Class 3	10	47.13	24.70
Class 4	31	41.04	18.30

Table 7.6 Multiple Regression Model

	Coefficient	Standard Error	T value	P value
Immune Class 3	31.17	16.46	1.89	0.06
Immune Class 4	27.22	12.75	2.13	**0.04**
Interaction				
Disrupted Development x Immune Class 3	-19.32	18.26	-1.06	0.30
Disrupted Development x Immune Class 4	-20.84	14.09	-1.48	0.15

Note: F=2.01, df= 5 and 51, p=0.09, Adjusted R^2=0.09. Significant p values ($p < 0.05$) are highlighted in bold font. Immune Classes 1 & 2 and "average" developmental class serve as the references for the model. Sample size is reduced (n=59) as point ages could not be estimated for all individuals included in the LCA.

CHAPTER 8: PRE-COLUMBIAN HEALTH AND LIFEWAYS IN THE GREATER COCLÉ REGION OF PANAMA: CONCLUSIONS AND IMPLICATIONS

8.1 Summary and Significance of Research Findings

This project investigates the role of developmental stress in shaping later life mortality outcomes throughout the Pre-Columbian Period in Panama, and biocultural kinship systems in the Greater Coclé region were reconstructed to explore the potential role of kinship as a social determinant of health amidst emerging inequality. In particular, this research utilizes osteological markers to incorporate the understudied role of pro-inflammatory immune activity in mediating the effect of developmental stress on later life morbidity and mortality risk in bioarchaeological studies. Since immune function and inflammation have been increasingly associated with communicable and chronic disorders and mortality risk (Cohen et al. 2012; Hunter 2012; McDade 2012), exploring the physiological pathways that contribute to the developmental origins of health and disease in skeletal assemblages provides an important evolutionary perspective on these relationships in novel contexts throughout human history. The results of each specific aim are below.

This project's first aim, to reconstruct kinship groups through a biological distance analysis and assess the importance of biological and social relationships in structuring social identities during the Ceramic Period in the Greater Coclé region, was addressed in Chapter 5. Although scholars have long thought that kinship and ancestry were important aspects of social identity and shaped the complex mortuary rituals in Pre-Columbian Panama, these hypothesizes have not been widely tested with skeletal remains. In this chapter, we performed a biological

distance analysis of dental odontometric and morphological characteristics in order to reconstruct Greater Coclé biocultural kinship systems and postmarital residence practices.

We tested the following hypotheses: 1) that site cemeteries would be organized around biological relationships, with kin members grouped together in "multiple burials" (burials containing secondary burial, burial re-use, and/or burial co-interment) and broader cemetery sections; and 2) patrilineal and patrilocal kinship systems will result in greater female postmarital mobility. At the Early and Middle Ceramic site Cerro Mangote (2476 BCE- 231 CE), we found that social relationships likely shaped broader cemetery organization and inclusion in multiple burials, but biological relationships dictated where individuals were positioned within multiple burials. At Middle Ceramic site Sitio Sierra (39 BCE- 561 CE, 978-1158 CE), little evidence of close biological relationships was identified, and social relationships dictated burial inclusion and cemetery organization. Interestingly, inhabitants at both sites appear to follow similar bilocal or unilocal postmarital residence practices, and considerable gene flow connects the Greater Coclé communities. These results add to our understanding of the complexity of regional interactions, as well as driving new questions regarding the function of Cerro Mangote as a special regional cemetery site and the relationship between the early and later occupations at Sitio Sierra. Overall, this study provides new evidence of biological relationships within the Greater Coclé region and the existence of flexible biocultural kinship systems. Although ancient DNA analysis is becoming more common in the region, this study also provides an important model for future biodistance analyses in Panama that can overcome issues with soil acidity and preservation.

This project's second aim, to reconstruct developmental phenotypes from osteological markers of developmental stress and assess how developmental exposures impact later life

mortality outcomes in the Greater Coclé region, is addressed in Chapter 6. Previous research in modern populations has shown that adverse environmental cues during development is associated with increased morbidity and mortality risk later in life, but the developmental origins of health and disease in past populations, which represent numerous novel temporal and geographical contexts, is less understood. In this chapter, we first assessed standard osteological markers of age-specific growth disruptions and general developmental stress and then employed latent class analysis to quantify the range of developmental phenotypes within the sample.

Following a life history approach, we used development phenotypes as an exposure to test the effect of development stress on mortality risk at two Greater Coclé sites in Panama during the Early and Middle Ceramic periods (2480 BCE- CE 1200). We identified three developmental phenotype classes within the sample, and we hypothesized that the classes characterized by greater levels of developmental stress (Classes 2 & 3) would be associated with an increased mortality hazards and earlier age at death. Through our analysis, we confirmed that greater developmental stress was associated with earlier age at death in the combined Greater Coclé sample and at the individual sites during the Early and Middle Ceramic Periods, but these relationships were not statistically significant.

While our findings were likely affected by methodological limitations, such as small sample size, we argue that these results may be indicative of cultural buffering systems like robust biocultural kinship networks and diverse subsistence systems, that ensured adequate access to resources and support and helped mitigate the negative effects of environmental stressors. By assessing the effects of developmental stress on mortality risk in a novel historical and geographic context, this study adds a critical component to the literature on the developmental origins of health and disease and expands our understanding of the variability of

these relationships throughout human history. Further, this study is one of the few to combine multiple skeletal and dental markers to more fully investigate developmental stress throughout childhood and adolescence and provides a model for future bioarchaeological studies on the developmental origins of health and disease.

This project's third aim, to assess the range of pro-inflammatory immune activity in Pre-Columbian Panama and explore the role of inflammation as an important physiological pathway linking developmental stress to later life mortality outcomes, was assessed in Chapter 7. As a central but expensive component of maintenance and survival, immune system development is sensitive to environmental cues. Developmental stress can lead to impaired immune function later in life, such as increased activity and inflammation, that serves as an important pathway linking development to later health and disease outcomes. While these relationships are explored in modern assemblages, little is known about pro-inflammatory immune activity in skeletal assemblages despite robust osteoimmunological interactions. Using latent class analysis, we reconstructed the range of inflammatory phenotypes in the Greater Coclé, and multinomial logistic regression was used to assess the effects of biocultural factors, particularly developmental stress, on immune phenotype class.

Multiple regression models were also used to assess the effect of immune phenotype class on mortality risk and the modifying effect of immune phenotype class on the relationship between developmental stress and mortality risk. Our results show that great pro-inflammatory immune phenotypes were generally associated with older age at death due to the "paradoxical" nature of some osteological markers, but among older age cohorts, individuals with chronic, systemic inflammatory lesions had greater mortality risk than those with healed, local lesions. Although developmental stress was not associated with greater pro-inflammatory phenotypes,

individuals who experienced greater developmental stress and later developed pro-inflammatory phenotypes experienced died at earlier ages compared to individuals with less developmental stress. Our results support the inflammaging and allostatic load theories and demonstrate how early life physiological stress can increase wear and tear throughout the life course, as well as initiate or amplify age-related immune system decline and pro-inflammatory signaling. This study provides a bioarchaeological model for utilizing human biology and epidemiology frameworks to interpret "paradoxical" osteological lesions and bridges the gap between modern and skeletal population studies. Importantly, this study adds a critical deep time component to the literature on inflammaging and illustrates the effects of pro-inflammatory pathways on the developmental origins of health and disease in a novel context.

8.2 Overall Strengths and Limitations

This research utilizes a robust osteological approach to assess markers of developmental stress and pro-inflammatory immune activity to reconstruct phenotypes of interest and incorporate under-studied concepts, like developmental plasticity and inflammaging, into traditional bioarchaeological studies of past populations. This research also employed a biological distance analysis of dental odontometric and morphological characteristics to examine patterns of underlying genetic affiliation during the Early and Middle Ceramic Periods at two Greater Coclé sites in Panama. Further, this work uses interdisciplinary theories and frameworks to situate these results within anthropology and evolutionary medicine.

One key strength of this research is the multi-faceted approach used to interrogate the developmental origins of mortality risk in Pre-Columbian Panama. In Chapter 6, developmental phenotypes are reconstructed from several osteological markers of growth disruption and general

developmental stress. The use of varied markers provides a more holistic understanding of developmental stress and helps to refine bioarchaeological methodologies by comparing the effectiveness of commonly used markers in delineating different developmental phenotypes.

In Chapter 7, developmental stress is further explored through an investigation of a pro-inflammatory activity as a possible physiological pathway connecting developmental experiences to later life outcomes. Examining the developmental origins of health and disease in this way grounds the investigation in human biology and improves our understanding of the mechanisms that underlie associations between skeletal phenotypes, such as periosteal bone lesions, and morbidity risk. This approach also leverages skeletal biology to better understand hidden sources of heterogeneity, like immune function, that complicate bioarchaeological interpretations of skeletal remains and enables a more nuanced understanding of frailty as it changes throughout the life course of an individual.

This study's statistical approach is another key strength. Latent class analysis enabled the reconstruction of developmental and immune phenotypes, unobservable constructs, from a combination of observable osteological markers in order to explore the range of phenotypes within the Pre-Columbian assemblage (Chapters 6 and 7). The use of transition analysis ensured statistically rigorous age estimation even for older individuals typically under-enumerated in skeletal assemblages, and importantly, the point age estimates served as a continuous variable to enable regression modeling (Chapters 6 and 7).

While survival and hazards analyses have become more common in bioarchaeological studies, we incorporated a parametric hazards analysis with a Gompertz distribution that better reflects human mortality risk (Chapter 6). In the biological distance analysis in Chapter 5, the use of Gower similarity coefficients enabled us to utilized both odontometric and morphological

variables to gain a more complete understanding of genetic affiliation within the sample.

Last, while the majority of studies developmental origins of health and disease have been conducted with modern Caucasian populations in high-income countries (particularly the United States and Europe), this is study is part of an emerging effort to assess developmental plasticity in skeletal assemblages. Bioarchaeological studies of skeletal assemblages enable us to explore these relationships in various contexts and provide an important deep time perspective to the relationship between developmental stress and later life outcomes.

Importantly, this is the first study to assess these relationships in Pre-Columbian Panama. Past societies to the north and south of the Isthmo-Colombian region have received a great deal of scholarly attention, but bioarchaeological studies in Panama have been quite limited until recently. This study greatly broadens our understanding of health in the Greater Coclé region of Panama during the Early and Middle Ceramic Periods and provides new insight into the range of developmental stress and its effects on pro-inflammatory immune activity and mortality within communities. Bioarchaeological studies that incorporate DOHaD frameworks often focus on populations experiencing significant turmoil in the context of colonization, famine, and violent conflict. This study provides a better understanding of developmental plasticity and long term effects in Greater Coclé communities experiencing less upheaval.

Chapter 5 also presents the second ever dental biological distance analysis of Greater Coclé communities using dental characteristics, which tests key assumptions about the roles of kinship and ancestry in shaping Pre-Columbian mortuary ritual. These findings challenge our expectations and greatly expands our understanding of community interaction and social structure in the Greater Coclé region during the Early and Middle Ceramic Periods.

Despite these strengths, this research is not without limitations. First and foremost, the

2020 COVID-19 pandemic greatly impacted this research in a number of ways. The initial field collection trip was cut short by three months, and return travel was prevented due to travel bans and the closure of the Smithsonian Tropical Research Institute. As a result, the planned sample of 400 individuals across three Greater Coclé sites was reduced to 101 individuals at two Greater Coclé sites, limiting both the study's statistical power and the generalizability of the results. Although many aspects of the original research design were successfully adapted, the third study aim had to be reconfigured as the sample size was not large enough to compare developmental and immune phenotypes and morbidity and mortality outcomes between identified kinship groups.

The small sample size also impacted the latent class analysis of both developmental and immune phenotypes, as this analysis is recommended for samples with over 200 observations. The parameters of the latent model were reduced through the use of binary and category variables to dampen the negative effects of small sample size, but the models should still be interpreted with caution. Similarly, the regression models were also impacted by small sample sizes and unevenness between categories and should be interpreted with caution. While our studies detect clear relationships that meet theoretical expectations, we recognize that these studies serve as foundational exploratory research for larger projects needed to perform further testing with larger sample sizes in order to draw stronger conclusions.

8.3 Directions for Future Research

Future research should examine these pathways on a larger scale, both in Pre-Columbian Panama and in past societies more broadly. In regard to developmental stress, future research should work to incorporate more osteological measures of stress during critical developmental

periods, as well as more continuous measures of stress to capture repeated episodes of growth disruption. This research would provide better insight into how the timing and the frequency of developmental stress episodes affects developmental phenotypes and shapes later life mortality risk throughout human history. It would also help bioarchaeologists further refine their methodologies and identify osteological markers that best reflect developmental stress with the greatest impact on later life outcomes.

Further research on pro-inflammatory immune activity in skeletal assemblages should incorporate additional measures as well. Crespo (2019) developed an immune index that includes molecular markers of inflammation, such as infectious pathogen identification, which would provide a more robust understanding of pro-inflammatory activity. This research could inform our understanding of inflammaging by adding an important evolutionary component (Franceschi et al. 2007).

Inflammation is largely studied in the context of chronic disease risk in modern high-income populations, but bioarchaeological studies can provide insight into how development stress affects immune activity and interacts with senescence-related inflammation to shape morbidity and mortality risks in non-industrial contexts characterized by high pathogen burdens. This work would be useful for understanding the range of inflammatory phenotypes and subsequent morbidity and mortality risks in modern low income and non-industrial populations experiencing high infectious disease burdens. Skeletal assemblages also provide longitudinal data that can be difficult or expensive to collect in modern populations and therefore can fill important gaps in our understanding of impaired immune function and pro-inflammatory activity throughout the life course.

The biodistance analysis also provides a foundation for considerable future research projects. Biological distance should be assessed at other Greater Coclé sites to identify regional variation in biocultural kinship systems and postmarital residence practices. Data from additional sites would also be helpful in teasing apart regional interactions and the potential function of sites as special cemeteries. Ideally, this research would occur alongside additional carbon dating to refine site chronologies and mortuary analyses to assess how biological and social relationships may be reflected in material remains.

Sharpe and colleagues' (2021) recently published preliminary isotope also demonstrates the utility of dietary and mobility isotopes in reconstructing interactions and interpreting relationships between individuals at Greater Coclé sites. These studies have the potential to greatly improve our understanding of the Greater Coclé cultural trajectory and should be used to test hypotheses related to Pre-Columbian social stratification and inequality. Cerro Juan Diaz has a significantly larger cemetery assemblage, and a comparison of phenotypes, morbidity, and mortality outcomes within and between burial groups has the potential to reveal a great deal about th embodiment of inequality and health disparities.

More broadly, future research investigating the developmental origins of health and disease in past populations should consider additional physiological pathways that link early exposures to later life outcomes. For Panamanian skeletal assemblages, one potential avenue for future exploration is nutrition. Osteological markers, such as cribra orbitalia and hyperostosis, can be combined with stable isotope analysis of dental and skeletal samples to assess dietary intake throughout the life course. Recent research by Dent and colleagues (2020) shows that inflammatory processes underlying skeletal phenotypes like periodontal disease can cause nutrient deficiencies that further exacerbate infection and inflammation, so it is important to

consider these interactions. Osteological markers and stable isotope data would also allow us to better understand potential causal factors contributing to early life tradeoffs and model nutritional intake in order to test hypotheses related to ecological immunity in novel historical contexts (McDade et al. 2016).

8.4 Conclusions

This dissertation aimed to better understand the health and lifeways of Pre-Columbian communities in the Greater Coclé region of Panama. This work employed both biocultural and evolutionary frameworks to investigate the developmental origins of health and disease in a novel setting. By focusing on inflammation, this research incorporated under-studied concepts in bioarchaeology and created a model for exploring the physiological pathways that connect early life experiences with later life outcomes. Given the complexity of the mortuary record and lack of burial goods in non-elite cemeteries in Panama, the biodistance analysis and identification of kinship structures provides a first step towards identifying social determinants of health that can be explored in conjunction with biological data.

The results contribute novel findings and expand our understanding of the developmental origins of health and disease. First, in biological distance analysis, we identified cultural continuity and innovation in the biocultural kinship systems, postmarital residence practices, and mortuary practices at both Greater Coclé sites. Biological relationships and multiple burials were more common at Cerro Mangote, whereas the predominant single, primary burials at Sitio Sierra may represent increasing emphasis on social relationships. Both communities appear to practice bilocal or unilocal postmarital residence patterns, although the low genetic distance within samples may point to endogamous marriage practices. These results also indicate a high degree

of gene flow between the communities, which is expected given their proximity. Additional research is needed, however, to tease apart this relationship and determine if descent populations occupied Sitio Sierra and/or Cerro Mangote was continually used as special cemetery site throughout the Early and Middle Ceramic Period.

We found that low to moderate levels of developmental stress were common at the sample Greater Coclé sites (2480 BCE- 1200 CE), and only a few individuals experienced significant dental and skeletal growth disruptions. Greater developmental stress was associated with increased mortality hazards and earlier age at death. These results support the DOHaD framework and provide evidence of the costs of developmental stress in a novel context, but the lack of significant association between developmental stress and mortality risk suggests that other factors, such as cultural buffering systems, may be impacting this relationship. Second, we identified a range of pro-inflammatory activity, measured through osteological proxy markers of inflammation, in the Pre-Columbian samples. The immune phenotypes with greater pro-inflammatory activity were associated with later age at death in the entire sample, but among the older age cohorts, chronic and system inflammation increased mortality risk compared to local, healed inflammation.

These results fit in with both inflammaging and osteological paradox frameworks; osteological markers of age-related inflammatory disorders simultaneously indicate survivorship to older age but also increased frailty as senescence-related immune dysfunction fuels increasing pro-inflammatory activity. Further, we found that greater pro-inflammatory phenotypes were associated with an increased mortality risk among individuals who had experienced developmental stress. Evolutionarily, these results fit in with life history and allostatic load frameworks, whereby early life course tradeoffs and physiological wear accumulate throughout

the life course and increase mortality risk. This is the first study to examine this physiological pathway and demonstrate interactions between developmental experiences and later life pro-inflammatory immune activity in a skeletal assemblage.

Overall, these findings contribute novel insights into the developmental origins of health and disease in an under-studied past population and reinforces the importance of utilizing interdisciplinary frameworks to focus on the physiological pathways that connect early life exposures with later life outcomes. These results can be combined with intersecting social identities to explore social determinants of health and health disparities in past populations. Results from this work can be used to inform future bioarchaeological studies to better contribute to interdisciplinary research on the developmental origins of health and disease and expand our evolutionary understanding of key biological processes in past populations.

APPENDIX A: TRANSITION ANALYSIS SCORING SHEET

Symphyseal Relief

1. Sharp billowing: Sharply crested ridges of bone cover at least half of the surface. Deep furrows that extend completely across the symphyseal face separate distinct ridges. The deepest furrows cut into the ventral and dorsal margins of the symphyseal face, interrupting the edge of the bone and giving it a jagged appearance. The distance between the high and low points of adjacent ridges and furrows can often be 3 mm or more. Occasionally, round instead of sharp crests occur on the high ridges in specimens that otherwise have deep furrows exceeding 3 mm. Such specimens are also considered examples of Sharp Billowing. Sharp Billowing has only been seen in teenagers.

2. Soft, deep billowing: Softly crested to low billows separated by deep furrows extend across at least half of the surface, typically the dorsal demiface. The furrows do not appear as if they have been filled in with bone. The distance between high and low points of adjacent ridges and furrows is 3 mm or less.

3. Soft, shallow billowing: Low but clearly visible and discrete billows separated by shallow furrows are present on at least half of the dorsal demiface. The remnants of an earlier ridge and furrow system dominate the dorsal demiface, and the furrows look as if they were partially filled with bone. Billows extend most or all of the way across the dorsal demiface, and in some individuals they reach the ventral margin.

LOCATION

The terms for this feature generally follow those of McKern and Stewart (1957). Although the entire face should be considered, the billowing is typically most clearly seen in the dorsal half of the symphyseal face (Figure 2.1). In fact, low ridges of bone, the billowing, can be entirely absent from the ventral symphyseal face, beginning as early as the ventral beveling stage.

4. Residual billowing: Billows are barely elevated above the symphyseal face, and they blend into one another to form low and indistinct raised areas that lack clearly defined furrows between them. The slightly raised areas, however, are still an important element of the surface, and they almost invariably occur on the inferior half of the face, often in the dorsal demiface. Individual billows usually cross only part of the symphyseal face, typically less than one-half its width. There must be two or more adjacent raised areas corresponding to billows to qualify as Residual Billowing. A single isolated bony elevation is not sufficient to be classified as Residual Billowing; instead, such specimens are considered Flat.

5. Flat: More than one-half of the symphyseal face within well-defined margins is flat or slightly recessed, especially if surrounded by a well-developed Rim (see below). Occasionally small, flat, pillows of bone give the surface a pebbly appearance. The remainder of the symphyseal face does not conform to Residual Billowing (i.e., there is no more than one discrete low raised area). Sometimes there is a gap where the ventral rampart has failed to extend along the entire ventral edge of the pubis (see below); when that occurs, the surface within the gap does not receive a score.

6. *Irregular:* Pitting, which can be deep, covers more than one-half of the symphyseal face, giving it an irregular and disfigured appearance. The pits can be accompanied by small, sharp exostoses scattered across the face. Occasionally, in old people an otherwise flat face is thickly covered by rounded sharp exostoses of bone. Pitting in such specimens might be minor, but the bone is still classified as Irregular. Similar to the Flat category, the scored part of the symphyseal face does not include the ventral gap, if present. In Irregular specimens, the margins of the symphyseal face are typically defined by the Rim and Breakdown stages of the Ventral and Dorsal Margin components.

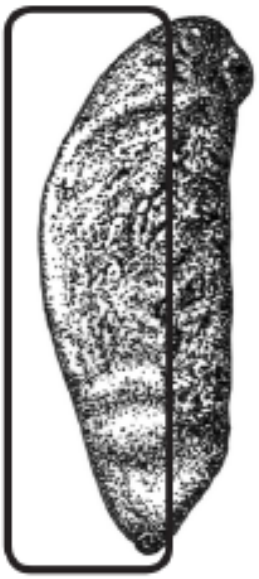
Dorsal Symphyseal Texture

1. Smooth (fine grained): Smooth to fine-grained bone extends across most, or all, of the dorsal demiface.

2. Coarse grained (little net): Coarse-textured bone covers over one-third of the dorsal demiface. The surface looks like packed fine sand, similar to fine-grained sandpaper. Americans will perhaps recognize it best as the surface of a sugar cube.

3. Microporosity: Porous bone covers over one-third of the dorsal demiface. It looks as if the surface was pierced by closely packed pin pricks.

4. Macroporosity: Deep pits cover over one-third of the dorsal demiface, giving it an irregular appearance. The pits are at least 0.5 mm in diameter, and are generally spaced close together. Sometimes the symphyseal surface is so irregular from pitting that it resembles the edge of a sponge. The surface looks as if it was pierced by closely packed pin heads (the bulbous end of the same shirt or blouse pin in the previous description).



LOCATION

The dorsal portion of the surface is examined (Figure 2.8).

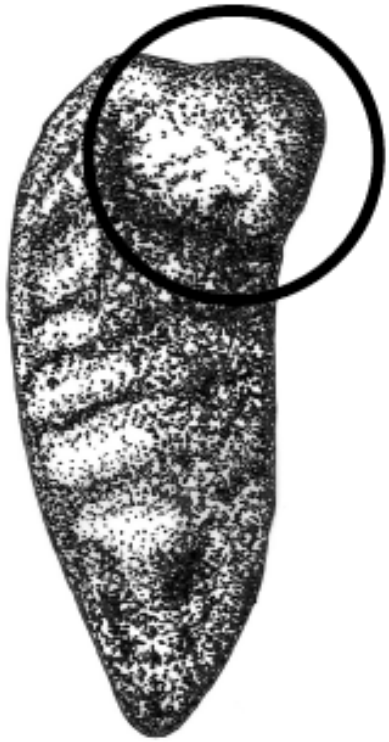
Superior Protuberance

1. No protuberance: Deep to shallow billowing is present in the superior part of the symphyseal face. There are no signs of a bony protuberance. In young individuals, this part of the symphyseal face can be poorly differentiated from the non-articular portion of the pubis immediately lateral to the joint. This stage is on a symphyseal face characterized by a youthful ridge-and-valley surface.

2. Early protuberance: A distinct bony knob of variable dimensions with well-defined margins is visible in the superior part of the symphyseal face. It projects above the plane(s) defined by the immediately adjacent symphyseal face (i.e., the superior portions of the dorsal and ventral demifaces, where the latter can be characterized by ventral beveling). The surface of the bony protuberance is typically smooth to fine grained. The bony knob often reminds one of a split pea stuck on the bone

3. Late protuberance: The superior part of the symphyseal face is raised somewhat above the rest of the articulation surface. The elevated area is typically located on the ventral side of the midline.2 The margins of the slightly raised area tend to be poorly defined. Thus the Late Protuberance is more completely integrated with the rest of the symphyseal face than the distinctly knob-like Early Protuberance. Integration is partly a result of ventral rampart formation. Late Protuberance should not be confused with a narrow raised rim that can border the cranial end of the symphyseal face in many specimens. For a Late Protuberance to be scored as present, the slightly raised area must extend onto the symphyseal face; that is, it is not restricted to the margin that can feature a pronounced rim. Occasionally, the superior part of the symphyseal face can be isolated by marked pitting of the middle symphyseal surface, but these specimens should not be considered as a Late Protuberance stage. For Late Protuberance to be present, the slightly raised area must be visible on a rather smooth symphyseal face

4. Integrated: The symphyseal face's superior end displays no signs of a low bony elevation. The area where the protuberance was formerly present is fully integrated with the rest of the symphyseal face. That is, the smooth to irregular (usually pitted) symphyseal face is essentially flat. This stage, the absence of a raised area, is distinguishable from Stage 1, No Protuberance, because the superior portion of the symphyseal face is flat, not the ridge-and-valley surface typical of the initial No Protuberance stage. The Integrated stage also frequently has a narrow elevated rim demarcating the superior symphyseal surface. In addition, do not confuse that narrow rim with the previous Late Protuberance stage if the elevated portion is confined to a narrow rim that borders an otherwise flat face



LOCATION

The superior part of the symphyseal face is examined for a distinct knob of bone or, later, an elevated area (Figure 2.13).

Ventral Symphyseal Margin

1. Serrated: Ridges and furrows typical of Sharp or Soft Deep Billowing extend uninterrupted across the ventral part of the symphyseal face, producing a serrated or jagged ventral margin.

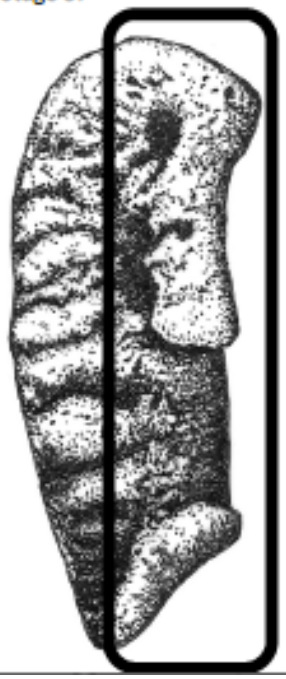
2. Beveling: Billows are flattened in the ventral demiface, a process that generally starts at the superior end. The flattening, or beveling, must extend along at least one-third of the ventral margin to be scored as present. There is generally a well-defined margin where the ventral surface of the pubis (the beveled part) meets the articular surface located immediately posterior to it.

3. Rampart incomplete: An incomplete rampart frequently extends inferiorly from the bony protuberance defining the cranial end of the face, sometimes forming a bony elevation that resembles a comma, with the rampart being the tail. A rampart can also extend superiorly from the inferior end of the symphysis. Bony extensions from the superior and inferior ends of the symphysis, if both are present, typically leave a gap in the middle one-third of the ventral margin. An early Rampart Incomplete stage can consist of one or more bony knobs, commonly located in the middle one-third of the ventral margin. The knobs can occur with, or without, the formation of a bony rampart extending from the superior and inferior ends of the symphysis. If the rampart is more than two-thirds complete but there is a gap in the superior part of it, you should consider the possibility that the specimen is in the Rampart Complete I or II stages. Occasionally a rampart never completely forms along the ventral margin (see below).

4. Rampart complete with anterior sulcus: Here the ventral rampart is complete, but there is a shallow sulcus extending along much of the length of the ventral pubis immediately lateral to the symphysis (often more pronounced inferiorly). The groove is a residual feature related to rampart formation along the ventral margin. A reasonably flat symphyseal surface extends uninterrupted from the dorsal to ventral margins, so the face is unlike the somewhat furrowed appearance of many Rampart Incomplete specimens where there is a shallow groove just dorsal to an incomplete ventral rampart. Occasionally a gap exists in the ventral margin, usually in its superior half; the ventral rampart is otherwise completely formed.4 This stage is only occasionally found in most skeletal samples that have been examined

5. Rampart complete without sulcus: Here the ventral rampart is complete, and there is no shallow sulcus. A reasonably flat symphyseal surface extends uninterrupted from its dorsal to ventral margins, so the face is unlike the somewhat furrowed appearance of many Rampart Incomplete specimens where there is also a shallow groove just dorsal to the incomplete ventral rampart. Occasionally there is a gap in the superior half of the ventral margin, but the ventral rampart is otherwise complete. These specimens should be classified as Rampart Complete. With regard to Rampart Complete With and Without Anterior Sulcus (Stages 4 and 5), most specimens are in the later Stage 5.

6. Rim: A narrow, bony rim defining the ventral margin of the symphysis, perched on top of the ventral rampart, demarcates a usually flat or irregular face. The rim does not have to be complete, but it must be at least 1 cm long and readily visible as a raised ridge adjacent to a slightly recessed symphyseal face. The rim can be either a continuous ridge of bone or several segments, as long as 1 cm of an elevated border is present. The rim's crest can be low and rounded, or narrow and sharp. A ventral rim is always formed on top of a ventral rampart. Odd rim-like bone formations on gaps in a rampart or formed with no rampart at all are *not* scored as a ventral rim.



7. Breakdown: The ventral margin of the symphyseal face has begun to break down, as indicated by pitting and an erosion of the Rim. The breakdown of the ventral margin must exceed 1 cm (either in one spot, or when two or more areas of erosion are combined) to be scored as present. Care must be taken to distinguish antemortem degeneration – that is, true Breakdown – from postmortem damage. The latter, of course, can render the bone unscorable if it is extensive enough.

Dorsal Symphyseal Margin

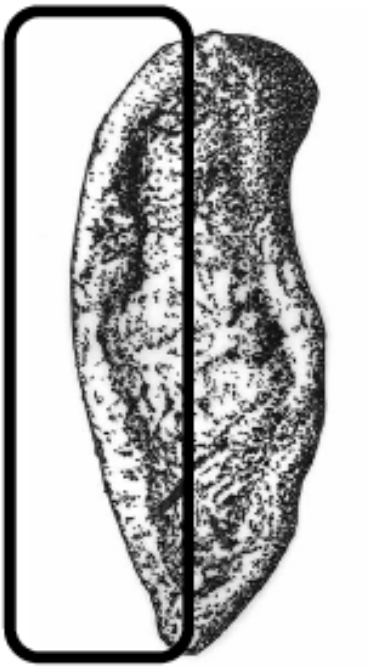
1. Serrated: The dorsal margin of the symphyseal face is irregular because ridges and furrows typical of pronounced billowing extend uninterrupted to the edge of the bone.

2. Flattening incomplete: A well-defined flattened area at least 1 cm long is present where the symphyseal face meets the dorsal margin. Flattening usually starts in the superior part of the dorsal demiface. Billowing is also present on the dorsal demiface, and it typically produces an undulating edge to the pubic symphysis, although it is not as extreme as what is found in Serrated specimens. The undulating edge usually occurs in the inferior part of the symphyseal face.

3. Flattening complete: There is a rather obvious area of flattening that completely (or almost entirely) covers the symphyseal face where it meets the dorsal margin. This flattening seemingly occurs partly through a coalescence of billows. A small area at the inferior end of the dorsal margin occasionally retains an undulating appearance.

4. Rim: An elevated bony rim demarcates a flat or, infrequently, an irregular face. The rim projects slightly above the symphyseal face, and its crest can be blunt or sharp. The rim does not have to extend along the entire dorsal margin to be scored as present, but it must be at least 1 cm long. The 1 cm rule pertains to either a continuous rim or discontinuous segments that together sum to that length. A rim typically develops first along the superior part of the dorsal margin. It can, however, occur anywhere along the dorsal margin.

5. Breakdown: The dorsal margin where the Rim is located shows evidence of breakdown, specifically a pitting and erosion of the edge of the pubic symphysis. The breakdown must exceed 1 cm in length either in one spot or when two or more areas of erosion are combined. Care must be taken to differentiate antemortem degeneration of the margin from postmortem damage, which is of no concern. Antemortem destruction attributable to large parity pits in females that can undercut the dorsal margin is not considered breakdown in the sense of the term as used here. It might not be possible to score those specimens; when that occurs, the component is simply missing data.



LOCATION

The dorsal part of the pubic symphysis is scored separately from the ventral margin (Figure 2.24). In females, dorsally located characteristics can be partly or entirely obscured by large postpartum, or parity, pits. Occasionally, such specimens cannot be scored properly.

Superior and Inferior Demiface Topography

1. Undulating: The surface is undulating, particularly in a superior to inferior direction. There is no centrally located and linear area of elevated bone (Median Elevation). When the entire articulation surface is viewed in aggregate, the overall effect is of two or three low waves proceeding lengthwise along the joint.

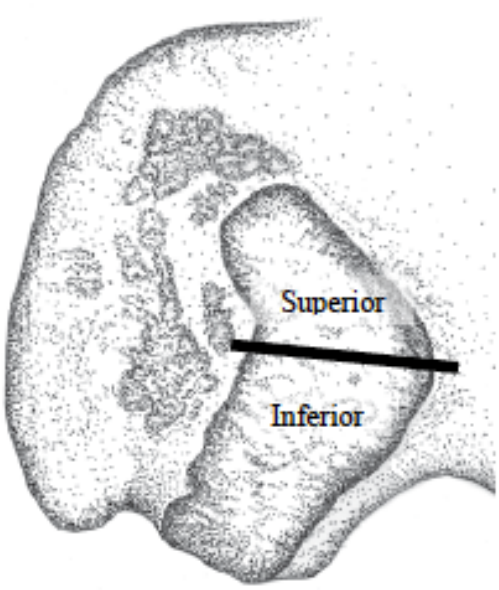
2. Median elevation: In the middle to posterior part of the demiface there is a broad raised area where the joint surface is elevated slightly above the rest of the joint. The elevation is flanked anteriorly, posteriorly, or both by one or two long low areas. The elevated area takes the form of an elongated ridge with the long axis paralleling the main orientation of the demiface. Occasionally the elevated area is restricted to a noticeable raised area, especially in the inferior portion of the superior demiface. The elevated area occupies as much as one-third of the joint surface.

3. Flat to irregular: The surface is essentially flat or recessed, a result of marginal lipping, or it is irregular from degeneration of the joint or the formation of low pillow-like exostoses

Scoring Tips

These comments pertain equally to both Superior and Inferior Demiface Topography. The first thing to do is to place your thumb such that it masks the superior demiface, and then score the inferior part of the joint. The characteristics of the inferior part are generally easier to see, and to score reliably, than the superior part, especially with regard to the Median Elevation. Then move your thumb so it masks much of the inferior part of the joint so you just look at the superior end. The purpose of this exercise is to prevent being unduly influenced by what is happening in the part of the joint that is not being scored.

When deciding which of the stages is represented, it is easiest to first determine if a Median Elevation is present. It will generally be more pronounced on the inferior demiface. If it is not present, typically the surface is in the Flat to Irregular stage. The Undulating variant is almost always found on the ilia of young people in their teens or twenties. The Superior Demiface Undulating and Median Elevation features are typically more subdued than those of the Inferior Demiface; that is, they are harder to distinguish



LOCATION

The superior demiface is examined. The two demifaces (superior and inferior) are divided by a line extending posteriorly from the most anterior point of the apex to the posterior joint margin.

LOCATION

The inferior demiface is examined. The superior and inferior demifaces are divided by a line extending posteriorly from the most anterior point of the apex to the posterior border of the joint (see Figure 2.30).

Superior, Middle, and Inferior Surface Characteristics

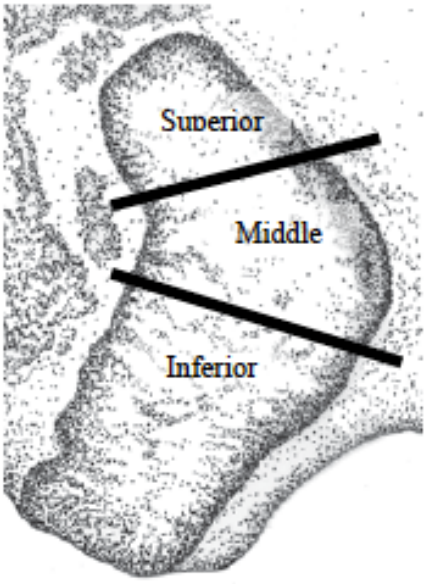
1. Billows cover >2/3 of the surface: Low rounded ridges separated by furrows, which have distinctly rounded bases, are clearly identifiable. The ridge surfaces are curved from the depths of the furrows completely across their crests. Most or all of the billowing is oriented roughly anterior to posterior, and furrows can run across much of the face. Billowing covers most (>2/3) of the joint surface (i.e., it is a dominant element of the surface). *2. Billows cover 1/3-2/3 of the surface*: About one-half of the surface is covered by billows.

3. Billows cover <1/3 of the surface: Billows are a noticeable, but minor, component of the joint surface. The rest of the surface is flat or bumpy.

4. Flat (no billows): The joint surface is flat.

5. Bumps: Most, or all, of the joint surface is covered by low, rounded bony exostoses, much like little irregular pillows. Part of the surface may be flat, but over one-half of it is bumpy. One is often reminded of lentils squished onto the joint surface. The bumps can be discrete low elevations or confluent, in which case the raised areas have irregular margins.

Unscorable: If defects in the joint surface are so extensive they obscure much of the face, this characteristic is considered unscorable. The defects often take one of two forms. Irregular and large pits can be present that are for the most part either separate or confluent with one another. The pits can be found anywhere on the joint face. Alternatively, the defects are linear grooves that can occur in isolation or as multiple nearby grooves. In either case, they can be up to a centimeter long, and they generally extend more or less in a transverse direction. For the linear defects in particular, it frequently appears as if the smooth bone of the joint surface laps over into the defect for a short distance. The grooves are more commonly found in the middle part of the joint surface than toward the superior or inferior ends. They are not to be considered as some form of anomalously deep billows. Occasionally the surface defects are sufficient to obscure the Superior and Inferior Demiface Topography, although they more often interfere with the proper scoring of the Superior, Middle, and Inferior Surface Character.



These comments pertain to the Superior, Middle, and Inferior Surface Characteristics. The best way to proceed is to first bracket the part of the auricular surface that includes the apex with your two thumbs. That defines the middle, or apical, portion. Then move one thumb to define the inferior margin of the superior portion. Finally, use a thumb to define the superior margin of the inferior surface. This simple masking procedure will help counteract the tendency to be influenced by what is present on adjacent parts of the auricular surface.

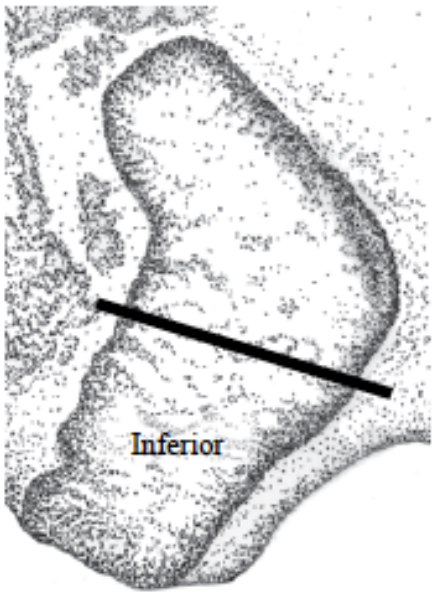
When scoring the surface characteristics, it is generally easiest to first look for billows. If present, then determine how abundant they are: there are only a few of them, they cover roughly one-half of the surface, or they spread out across much of the surface. If billows are not present, then look for bumps. The low and irregular bumps do not display the generally transverse organization of the billows.

Inferior Surface Texture

1. Smooth: Most, or all, of the joint surface appears to be smooth to slightly granular.

2 Microporosity: At least one-half of the surface has a porous appearance with apertures less than 0.5 mm in diameter. The symphyseal face looks as if it is covered by many closely spaced pinpricks.

3. Macroporosity: At least one-half of the surface is porous, with most, or all, of the apertures exceeding 0.5 mm in diameter. Here the surface looks as if it was penetrated by multiple closely spaced pin heads (the large end of the same shirt pin mentioned above).



LOCATION

Only one part of the joint surface – the inferior area – is scored for texture (Figure 2.47). This part of the joint is 1 cm long, as measured in a superior to inferior direction. Occasionally there can be an elongated portion of the joint surface, often accompanied by marginal lipping, that extends well beyond the main body of the ilium. While it can occur in both sexes, it is more often seen in females. Do not score the auricular surface that extends beyond the margin of the ilium proper.

Superior & Inferior Posterior Iliac Exostoses

1. Smooth: The surface is often elevated in this area, but shows no evidence of discrete bony elevations. At most there are a few isolated small exostoses projecting from the bone surface.

2. Rounded exostosess: Definite raised areas of bone with rounded crests dominate the scoring area.

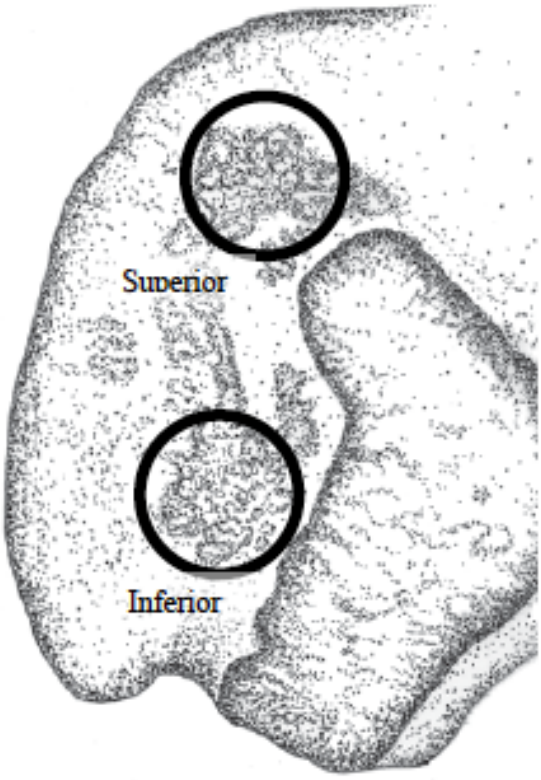
3. Pointed exostoses: Over one-half of the rough area where ligaments attach is dominated by sharply pointed, but short, elevations of bone.

4. Jagged exostoses: The raised areas of bone have a jagged appearance, and round or sharp exostoses dominate the rough area where ligaments attach in life. The exostoses are tall, extending several millimeters upward from the original bone surface.

5. Touching exostoses: There is a pronounced growth of bone with a relatively flat top, usually roughly oval, where exostoses touch the sacrum.

6. Fusion: The ilium and sacrum are fused by exostoses in this area.

These comments pertain equally to the Superior and Inferior Posterior Iliac Exostoses. The common adult condition is to have either Rounded or Pointed Exostoses. The Smooth variant is typically found on the ilia of only very young adults, although it rarely occurs on old ones. You will only occasionally see sharp Jagged Exostoses that jut upward several millimeters from the original bone surface. The ilium and sacrum can be fused in several places, generally on the anterior-superior margin of the auricular surface some distance from the posteriorly located Superior and Inferior Posterior Iliac Exostoses. If the ilium is fused to the sacrum, regardless of where that occurs, you are well advised to score the Superior and Inferior Exostoses as not observable, as the effect of fusion on these joint characteristics is unknown.



LOCATION

The inferior posterior iliac exostoses are scored. This area refers to the inferior part of the medial surface of the posterior ilium where ligaments attach (see Figure 2.51). It is located inferior to a line that passes from the anterior superior iliac spine, to the most superior point of the sacroiliac joint surface (the superior angle), and on through the posterior part of the ilium. This area is located immediately posterior to the middle of the sacroiliac joint; that is, it lies behind the most anteriorly projecting part of the posterior margin of the joint. In some individuals, the bone is distinctly raised in this area, so care must be taken to differentiate jagged (or high) exostoses from rounded or pointed ones perched on top of a raised elevation of bone.

LOCATION

Superior posterior iliac exostoses are scored. This area refers to the superior part of the medial surface of the posterior ilium where ligaments attach (Figure 2.51). It is located superior to the sacroiliac joint surface; that is, to a line that passes from the anterior superior iliac spine, to the most superior point of the joint surface (the superior angle), and on through the posterior part of the ilium. In some individuals, the bone is distinctly raised in this area, so care must be taken to differentiate jagged (or high) exostoses from rounded or pointed ones perched on top of a raised elevation of bone.

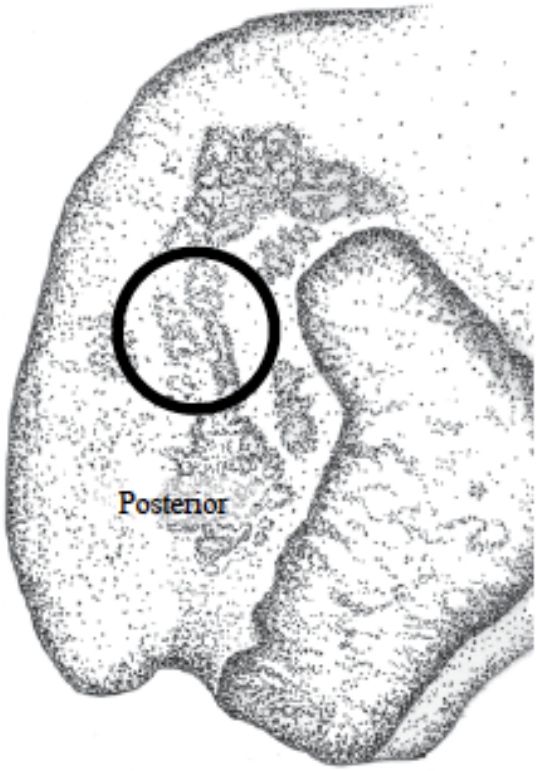
Posterior Exostoses

1. Smooth: The area posterior to the sacroiliac joint is smooth, except for the two areas scored separately as Superior and Inferior Posterior Iliac Exostoses.

2. Rounded exostoses: Low, rounded exostoses (or spicules) cover the *entire* bone surface posterior to the sacroiliac joint, except for a ca. 0.5 cm band of smooth bone immediately adjacent to the posterior edge of the joint. The exostoses are normally lower than the Superior and Inferior Posterior Iliac Exostoses. The low exostoses give the normally smooth iliac surface a rough appearance. It looks as if the surface is covered by coarse (construction) sand.

3. Pointed exostoses: Low, pointed exostoses (or spicules) cover the *entire* bone surface posterior to the sacroiliac joint, except for a ca. 0.5 cm band of smooth bone immediately adjacent to the posterior edge of the joint. The exostoses are normally lower than the Superior and Inferior Posterior Iliac Exostoses. The sharp exostoses give the normally smooth iliac surface a rough appearance. It looks as if the surface is covered by coarse (construction) sand.

By far the most common condition is Smooth, as it is defined here. Keep in mind that the Smooth category also includes surfaces with bony exostoses, which are typical of the 20s onward. Bones are only scored as the Rounded or Pointed categories if the entire surface is covered with exostoses; that is, the surface looks as if it is thickly covered by construction sand (little, if any, of the original smooth surface remains visible). Furthermore, the ilium and sacrum can be fused in several places. If that occurs, you are well advised to score Posterior Exostoses as not observable, as the effect of fusion on this joint characteristic is unknown.



LOCATION

The posterior iliac area between the **Superior and Inferior Posterior Iliac Exostoses** (defined above) is scored. These exostoses are found on the medial side of the ilium bordered posteriorly by the iliac crest, anteriorly by the sacroiliac joint surface, superiorly by a slightly raised area often surmounted by bony exostoses (**Superior Posterior Iliac Exostoses**), and inferiorly by a similar area (**Inferior Posterior Iliac Exostoses**) (Figure 2.60). Most individuals are *Smooth* as defined below. The feature is best considered an old-age trait.

APPENDIX B: BIODISTANCE VARIABLES

Non-metric and Metric Variable List

	Element Observed	Scoring
Non-Metric Variables		
Winging	UI1	0, 1-3
Labial Convexity	UI1	0, 1-4
Shoveling	UI1, UI2, LI1, LI2	0, 1-7
Double Shoveling	UI1, UI2	0, 1-6
Interruption Groove	UI1, UI2	0, 1
Tuberculum Dental	UI1, UI2, UC	0, 1-6 (I), 1-7
Canine Distal Accessory Ridge	UC, LC	(C)
Premolar Distal Accessory Ridge	UP3, UP4, LP3, LP4	0, 1-5
Accessory Cusps	UP3, UP4	0, 1-4
Premolar Lingual Cusps	LP3, LP4	1, 2-3
Metacone	UM1, UM2, UM3	0, 1-3
Hypocone	UM1, UM2, UM3	0, 1-5
Cusp 5	UM1, UM2, UM3	0, 1-6
Carabelli Cusp	UM1, UM2, UM3	0, 1-5
Parastyle	UM1, UM2, UM3	0, 1-7
Enamel Extension	UM1, UM2, UM3, LP3, LM1, LM2, LM3	0, 1-7
Root Number	UP3, UP4, UM1, UM2, UM3, LC, LP3, LM1, LM2, LM3	0, 1-2 1-3
Groove Pattern	LM1, LM2, LM3	
Cusp Number	LM1, LM2, LM3	1-3
Deflecting Wrinkle	LM1, LM2, LM3	0, 1-5
Mid-trigonid Crest	LM1, LM2, LM3	0, 1-3
Protostylid	LM1, LM2, LM3	0, 1
Cusp 6	LM1, LM2, LM3	0, 1-7
Cusp 7	LM1, LM2, LM3	0, 1-5
Peg Variant	UI2, UM3	0, 1-4
Odontome	UP3, UP4, LP3, LP4	0, 1-2
Congenital Absence	UI2, UP4, UM3, LI1, LP4, LM3	0, 1
Midline Diastema	UI1, maxillae	0, 1
Palatine Torus	Maxillae	0, 1
Mandibular Torus	Mandible	0, 1-4
Rocker Jaw	Mandible	0, 1-4 0, 1-2
Metric Variables		
Crown mesiodistal diameter (CMD)	UI1, UC, UP3, UM1, LI2, LC, LP3, LM1	
Crown buccolingual diameter (CBL)	UI1, UC, UP3, UM1, LI2, LC, LP3, LM1	
Root mesiodistal diameter (RMD)	UI1, UC, UP3, UM1, LI2, LC, LP3, LM1	
Root buccolingual diameter (RBL)	UI1, UC, UP3, UM1, LI2, LC, LP3, LM1	

Note: For non-metric scoring, 0 denotes trait absence and 1-x denotes range of trait expression. For traits with no absent form (Root Number and Groove Pattern), only the range of trait expression is included. Metric variable measurements were taken to the nearest 0.01 mm.

Final Variable List

	Metric Variables	**Non-metric Variables**
Cerro Mangote	UI1 RMD, UI1 RBL, UC RMD, UC RBL, UP3 RBL, UM1 RMD, UM1 RBL, LI2 RBL, LC RMD, LP3 RBL, LM1 RMD, LM1 RBL	UM2 Metacone, UM2 Hypocone, UM1 Cusp 5, UM2 Carabelli Cusp, UM2 Parastyle, UM2 Enamel Extension, LM1 Groove Pattern, LM2 Cusp Number, LM2 Protostylid, LM2 Cusp 6, LM2 Cusp 7, LM2 Enamel Extension, LM2 Mid-trigonid Crest
Sitio Sierra	UI1 RMD, UC RMD, UC RBL, UP3 RMD, UP3 RBL, UM1 RMD, UM1 RBL, LC RMD, LC RBL, LP3 RMD, LP3 RBL, LM1 RMD, LM1 RBL	UI1 Shovel, UI2 Shovel, UI1 Double Shovel, UI2 Double Shovel, UI2 Tuberculum Dentale, UI2 Interruption Groove, UM2 Carabelli Cusp, UM2 Enamel Extension, UM1 Cusp 5, LP3 Cusp Number, LP4 Cusp Number, LM1 Groove Pattern, LM2 Protostylid, LM2 Cusp 6, LM2 Cusp, LM2 Enamel Extension,
Combined	UI1 RMD, UC RMD, UC RBL, UP3 RBL, UM1 RMD, UM1 RBL, LC RMD, LP3 RBL, LM1 RMD, LM1 RBL	UM2 Carabelli Cusp, UM2 Enamel Extension, LM2 Protostylid, LM2 Cusp 6, LM2 Cusp 7, LM2 Enamel Extension

APPENDIX C: INTRAOBSERVER ERROR RESULTS

Results of ICC Error Test

	ICC	**ICC Interval**	**F**	**p**
Nonmetric	0.99	0.98-0.99	226	1.10 e -148
Metric	0.98	0.98-0.99	166	3.80 e -105

Raw Nonmetric Scores

Ag3- B2

	Test 1	Test 2	Test 3
LLM1	1	1	1
LLM1	5	4	5
LLM1	0	0	0
LLM1	0	0	0
LLM1	0	0	0
LLM1	0	0	0
LLM1	0	0	0
LLM1	2	2	2
LLM1	2	2	2
LLPM3	1	1	1
LLPM3	0	0	0
LLPM3	0	0	0
LLPM3	0	0	0
ULI1	0	0	0
ULI1	0	0	0
ULI1	5	5	5
ULI1	6	6	6
ULI1	0	0	0

Ag3- B4

	Test 1	Test 2	Test 3
ULM1	5	5	5
ULM1	4	4	4
ULM1	0	0	0
ULM1	1	1	0
ULM1	0	0	0
ULM1	1	1	1
ULM1	3	3	3
LRP4	2	2	2
LRP4	0	0	0
LRP4	0	1	0
URI1	0	0	0
URI1	0	0	0
URI1	4	4	4

URI1	4	4	4
URI1	0	0	0
URI1	0	0	0

Ag3- B6

	Test 1	Test 2	Test 3
LRM3	2	2	2
LRM3	0	0	0
LRM3	0	0	0
LRM3	0	0	0
LRM3	0	0	0
LRM3	0	0	0
LRM3	2	2	2
LRM3	2	2	2
LLP4	2	2	2
LLP4	0	0	0
LLP4	0	0	0
ULI1	0	0	0
ULI1	3	3	2
ULI1	4	4	4
ULI1	0	0	0
ULI1	1	0	0
LLC	1	1	1

Ag3- B11

	Test 1	Test 2	Test 3
ULM1	5	4	4
ULM1	4	4	4
ULM1	0	0	0
ULM1	1	1	1
ULM1	0	0	0
ULM1	1	1	1
ULM1	3	3	3
LLP4	2	2	2
LLP4	0	0	0
LLP4	0	0	0
LLP4	0	0	0
URI1	0	0	0
URI1	0	0	0
URI1	3	3	3
URI1	5	5	4
URI1	0	0	0
URI1	0	0	0
LLC	1	1	1

Ag3- B16

	Test 1	Test 2	Test 3
ULM1	4	5	5
ULM1	4	4	4
ULM1	0	0	0
ULM1	0	0	0
ULM1	0	0	0
ULM1	1	1	0
ULM1	3	3	3
LLP3	1	1	1
LLP3	0	0	0
LLP3	1	1	1
LLP3	0	0	0
ULI1	0	0	0
ULI1	0	0	0
ULI1	6	6	6
ULI1	4	5	4
ULI1	0	0	0
ULI1	0	0	0
LLC	0	1	0

Raw Metric Measurements

*C= crown, R= root

Ag3- B2

	Test 1	Test 2	Test 3
LLM1CMD	11.26	11.22	11.28
LLM1CBL	10.61	10.63	10.73
LLM1RMD	8.86	8.72	8.71
LLM1RBL	8.58	8.66	8.57
LLPM3CMD	6.7	6.7	6.7
LLPM3CBL	8.01	7.97	7.99
LLPM3RMD	4.76	4.82	4.75
LLPM3RBL	6.96	7.02	6.99
ULI1CMD	8.27	8.35	8.14
ULI1CBL	7.48	7.39	7.61
ULI1RMD	5.7	5.97	6.04
ULI1RBL	6.75	6.77	6.67
URCCMD	7.58	7.57	7.61
URCCBL	8.36	8.38	8.43
URCRMD	5.34	5.29	5.3
URCRBL	7.69	7.75	7.7

Ag3- B4

	Test 1	Test 2	Test 3
ULM1CMD	10.75	10.87	10.65
ULM1CBL	11.6	11.57	11.71

ULM1RMD	7.97	8.17	8.08
ULM1RBL	11.72	11.62	11.67
LRP4CBL	9.29	9.23	9.3
LRP4RMD	5.44	5.35	5.36
URI1CMD	7.98	8.03	8.03
URI1CBL	7.35	7.3	7.3
URI1RMD	6.48	6.48	6.47
URI1RBL	6.71	6.66	6.71
URCRMD	6.25	6.17	6.13
URCRBL	8.54	8.6	8.68

Ag3- B6

	Test 1	Test 2	Test 3
LRM1CBL	10.9	10.91	10.88
LRM1RMD	9.8	9.83	9.84
LRM1RBL	9.19	9.21	9.25
LLPM4CBL	8.78	8.91	8.95
LLPM4RMD	5.17	5.09	5.17
LLPM4RBL	6.84	6.93	6.99
ULI1CBL	7.2	7.2	7.19
ULI1RMD	7.04	7.17	7.04
ULI1RBL	6.48	6.44	6.45
LLCCBL	7.5	7.46	7.49
LLCRMD	5.57	5.6	5.56
LLCRBL	7.4	7.48	7.45

Ag3- B11

	Test 1	Test 2	Test 3
ULM1CMD	11.46	11.25	11.34
ULM1CBL	11.94	12.25	12.3
ULM1RMD	8.62	8.76	8.42
ULM1RBL	11.68	11.56	11.65
LLPM4CMD	7.77	7.73	7.71
LLPM4CBL	8.67	8.71	8.71
LLPM4RMD	5.16	8.76	5.13
LLPM4RBL	7.36	7.36	7.33
URI1CMD	8.6	8.52	8.56
URI1CBL	7.12	7.03	7.05
URI1RMD	5.61	5.63	5.62
LLCCMD	7.22	7.2	7.26
LLCCBL	7.56	7.92	7.91
LLCRMD	5.36	5.28	5.33
LLCRBL	7.76	7.84	7.87

Ag3- B16

	Test 1	Test 2	Test 3
ULM1CBL	12.32	12.46	12.41
ULM1RMD	8.02	7.74	7.79

ULM1RBL	12.29	12.46	12.42
LLPM4CBL	8.15	8.19	8.19
LLPM4RMD	4.98	5.18	5.23
LLPM4RBL	6.75	6.83	6.82
ULI1CMD	7.92	7.92	7.93
ULI1CBL	6.88	6.93	6.93
ULI1RMD	6.02	5.98	6.03
ULI1RBL	6.4	6.43	6.4
LLCCMD	6.8	6.8	6.77
LLCCBL	7.46	7.53	7.57
LLCRMD	5.22	5.19	5.23
LLCRBL	7.8	7.75	7.8

APPENDIX D: DEVELOPMENTAL LATENT CLASS ANALYSIS

Raw Scores- Mplus Program input
*AR= anterior root, PR= posterior root

ID	LEH	FA	ARsize	PRsize	VNC
2	0	1	1	1	0
3	0	1	0	1	1
4	0	1	1	1	1
5	0	0	1	1	1
6	0	1	1	1	1
7	1	1	1	0	1
8	0	1	1	1	1
9	0	1	1	1	1
10	0	0	1	1	
11	0	1	1	1	1
13	0	0	0	1	
14	0	0	1	1	1
15	1	1	1	1	1
16	0	1	0	1	0
17	0	0	1	1	
18	0	1	1	1	0
19	0	1	1	1	1
20	0	1	1	1	1
21	1	1	1	1	1
22	0	1	1	1	1
23	0	1	1	1	
24	0	0	1	1	1
25	1	1	1	1	1
26	0	1	1	1	1
27	1	1	1	1	
28	0	1	1	1	
29	0	1	1	1	
30	0	1	1	1	1
31	1	0	1	1	
32	0	1	0	1	
33	0	1	1	1	
35	0	1	1	1	
37	0	0	1	1	
38	0	0	1	1	
40	0	1	0	1	

43	0	1	1	1	1
44	1	1	1	1	
45	0	0	1	1	
46	0	1	1	1	
47	1	1	1	1	1
49	0	1	1	1	1
48	1	1	1	1	1
50	0	0	1	1	1
51	0	1	0	1	1
52	0	1	1	1	
53	0	0	0	0	
54	0	1	1	1	
55	0	1	1	1	
56	0	0	1	1	
57	0	0	0	1	
61	1	1	1	1	
62	0	1	1	1	
64	1	1	1	1	
65	0	1	1	1	
66	0	1	1	0	
67	0	0	0	0	
68	1	1	1	1	
69	0	1	1	1	1
70	0	1	1	1	
71	1	1	1	1	
73	0	1	1	1	
75	0	1	1	1	0
77	0	1	1	1	
78	0	1	1	0	
79	1	1	1	1	
80	0	1	1	1	
81	0	1	1	1	
82	0	1	1	1	1
83	0	0	1	1	
84	0	1	1	1	
85	0	0	1	1	1
88	1	1	1	1	
89	0	0	1	1	1
90	0	1	1	1	
91	0	1	0	0	

92	0	1	1	1
93	0	1	1	1
94	0	0	1	1
95	0	0	1	1
96	0	1	1	0
97	0	1	1	1
98	0	0	1	1
99	0	0	1	0
100	0	0	1	1
101	0	1	1	1

Mplus Posterior Probabilities (0-1) and Class Assignment

ID	Class 1	Class 2	Class 3	Final Class Assignment
2	0	1	0	2
3	0	1	0	2
4	0	1	0	2
5	0	1	0	2
6	0	1	0	2
7	1	0	0	1
8	0	1	0	2
9	0	1	0	2
10	0	1	0	2
11	0	1	0	2
13	0	0.623	0.377	2
14	0	1	0	2
15	1	0	0	1
16	0	0.473	0.527	3
17	0	1	0	2
18	0	1	0	2
19	0	1	0	2
20	0	1	0	2
21	1	0	0	1
22	0	1	0	2
23	0	1	0	2
24	0	1	0	2
25	1	0	0	1
26	0	1	0	2
27	1	0	0	1
28	0	1	0	2

29	0	1	0	2
30	0	1	0	2
31	1	0	0	1
32	0	0.868	0.132	2
33	0	1	0	2
35	0	1	0	2
37	0	1	0	2
38	0	1	0	2
40	0	0.868	0.132	2
43	0	1	0	2
44	1	0	0	1
45	0	1	0	2
46	0	1	0	2
47	1	0	0	1
49	0	1	0	2
48	1	0	0	1
50	0	1	0	2
51	0	1	0	2
52	0	1	0	2
53	0	0.063	0.937	3
54	0	1	0	2
55	0	1	0	2
56	0	1	0	2
57	0	0.623	0.377	2
61	1	0	0	1
62	0	1	0	2
64	1	0	0	1
65	0	1	0	2
66	0	1	0	2
67	0	0.063	0.937	3
68	1	0	0	1
69	0	1	0	2
70	0	1	0	2
71	1	0	0	1
73	0	1	0	2
75	0	1	0	2
77	0	1	0	2
78	0	1	0	2
79	1	0	0	1
80	0	1	0	2

81	0	1	0	2
82	0	1	0	2
83	0	1	0	2
84	0	1	0	2
85	0	1	0	2
88	1	0	0	1
89	0	1	0	2
90	0	1	0	2
91	0	0.213	0.787	3
92	0	1	0	2
93	0	1	0	2
94	0	1	0	2
95	0	1	0	2
96	0	1	0	2
97	0	1	0	2
98	0	1	0	2
99	0	1	0	2
100	0	1	0	2
101	0	1	0	2

APPENDIX E: IMMUNE LATENT CLASS ANALYSIS

Raw Scores- Mplus Program input
*Plesion= periosteal bone lesion, Ploc= periosteal lesion location

ID	Plesion	Ploc	Caries	Pulp	PD	AMTL	OA	OASites
1	2	1	1	0	1	0	1	1
2	2	1	1	1	1	1	1	1
4	2	1	1	1	1	1	1	2
5	1	1	1	1	1	1	1	1
6	2	0	1	1	1	1	1	1
7	2	1	1	1	1	1	1	2
8	1	1	1	0	0	0	1	2
10			1	1	1	1		
11	0	0	1	0	0	0	1	1
12	2	1			1	1	1	1
13	1	0	1	0	0	1	1	2
14	0	0	1	1	1	1	1	1
15	1	0	1	1	1	1	1	1
16	2	0	1	0	1	1	1	1
17	2	1	1	0	1	1	1	2
18	2	1	0	0			1	1
20	2	1	1	0	1	1	1	1
21	2	1	1	1	1	1	1	1
22	0	0	1	1	1	1	1	2
23	1	1	1	0	0	1	0	0
24	2	1					1	1
25	2	1	0	1	1	1	1	1
26	1	0					1	1
27	2	1	1	1	1	1	1	1
28	1	1	1	1	0	0	1	2
29	2	1	1	1	1	1	1	1
30	2	1	1	1	1	1	1	1
31	2	1	1	1	1	1	1	2
32	2	0	1	0	1	1	1	1
33	2	1	1	1	0	1	1	1
34	1	1					1	1
36	2	1					1	1
37	2	1	1	0	1	1	1	2
38	2	1	1	1	1	1	1	1
40	2	1	1	0	1	1	1	1

41	1	0					0	0
42	2	1			0	1	1	1
43	2	1	1	0	1	1	1	2
44	2	1	1	0	0	0	0	0
45	2	1	1	0	0	0	1	1
47	2	1	0	0	1	1	1	2
49	1	1	1	0	0	0	1	1
50	1	1			1	1	1	1
51	1	1	0	0	0	0	1	1
52	2	1	1	0	0	0	1	1
54	2	1	1	0	0	0	0	0
56	2	1	1	1	1	1	1	2
57	1	1	0	0	0	0	1	1
58	2	1			1	1	1	1
59	2	1			1	1	1	1
60	2	1					1	1
62	2	1	1	0	1	1	1	1
63	2	1			1	1	1	1
64	2	1	1	1	1	1	1	2
65	2	1	0	0	0	0	1	2
69	1	1					1	1
71	2	1	0	0	0	0	1	1
72	1	0	0	0	0	0	1	1
73	2	1	1	0	0	0	1	2
74	2	1					1	1
75	2	1	0	1			1	2
76	2	1					1	1
78	1	0			1	1	1	1
79	0	0	0	0	1	0	1	1
80	2	1					1	1
81	0	0	1	1	1	1	0	0
82	1	0			0	0	1	1
84	2	1	1	1	1	1	1	2
85	2	1	1	0	1	1	1	1
86	2	1					1	1
87	1	1					1	1
91	1	1	1	1	0	0	1	1
92	1	1	1	0	0	0	1	1
94	2	1	1	0	0	0	1	2
96	0	0	1	0	1	1		

97	1	1	1	1	0	0	1	1
100	2	1	1	1	1	1	1	2

Mplus Posterior Probabilities (0-1) and Class Assignment

ID	Class 1	Class 2	Class 3	Class 4	Final Class Assignment
1	1	0	0	0	1
2	1	0	0	0	1
4	1	0	0	0	1
5	1	0	0	0	1
6	1	0	0	0	1
7	1	0	0	0	1
8	0	1	0	0	2
10	0.899	0	0	0.101	1
11	0	0	0	1	4
12	1	0	0	0	1
13	0.022	0	0	0.978	4
14	0	0	0	1	4
15	0.144	0	0	0.856	4
16	1	0	0	0	1
17	1	0	0	0	1
18	0.41	0.59	0	0	2
20	1	0	0	0	1
21	1	0	0	0	1
22	0	0	0	1	4
23	0	0	1	0	3
24	0.833	0.167	0	0	1
25	1	0	0	0	1
26	0.053	0.316	0	0.632	4
27	1	0	0	0	1
28	0.002	0.998	0	0	2
29	1	0	0	0	1
30	1	0	0	0	1
31	1	0	0	0	1
32	1	0	0	0	1
33	1	0	0	0	1
34	0.188	0.812	0	0	2
36	0.833	0.167	0	0	1
37	1	0	0	0	1
38	1	0	0	0	1

40	1	0	0	0	1
41	0	0	0.87	0.13	3
42	1	0	0	0	1
43	1	0	0	0	1
44	0	0	1	0	3
45	0.006	0.994	0	0	2
47	1	0	0	0	1
49	0	1	0	0	2
50	1	0	0	0	1
51	0	1	0	0	2
52	0.006	0.994	0	0	2
54	0	0	1	0	3
56	1	0	0	0	1
57	0	1	0	0	2
58	1	0	0	0	1
59	1	0	0	0	1
60	0.833	0.167	0	0	1
62	1	0	0	0	1
63	1	0	0	0	1
64	1	0	0	0	1
65	0.001	0.999	0	0	2
69	0.188	0.812	0	0	2
71	0.001	0.999	0	0	2
72	0	0.976	0	0.024	2
73	0.007	0.993	0	0	2
74	0.833	0.167	0	0	1
75	0.843	0.157	0	0	1
76	0.833	0.167	0	0	1
78	0.116	0	0	0.884	4
79	0	0	0	1	4
80	0.833	0.167	0	0	1
81	0	0	0	1	4
82	0	0.899	0	0.1	2
84	1	0	0	0	1
85	1	0	0	0	1
86	0.833	0.167	0	0	1
87	0.188	0.812	0	0	2
91	0.002	0.998	0	0	2
92	0	1	0	0	2
94	0.007	0.993	0	0	2

96	0	0	0	1	4
97	0.002	0.998	0	0	2
100	1	0	0	0	1

REFERENCES

Aalen, O., Borgan, O., & Gjessing, H. (2008). *Survival and event history analysis: a process point of view*. New York: Springer Science & Business Media.

Abramson, S. B. (2011). Increased interleukin-1β gene expression in peripheral blood leukocytes is associated with increased pain and predicts risk for progression of symptomatic knee osteoarthritis. *Arthritis & Rheumatism*, *63*(7), 1908-1917.

Adair, L. S., Popkin, B. M., Akin, J. S., Guilkey, D. K., Gultiano, S., Borja, J., ... & Hindin, M. J. (2011). Cohort profile: the Cebu longitudinal health and nutrition survey. *International journal of epidemiology*, *40*(3), 619-625.

Adelson, N., 2005. The embodiment of inequity: Health disparities in Aboriginal Canada. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, S45-S61.

Agarwal, S. C. (2016). Bone morphologies and histories: Life course approaches in bioarchaeology. *AJPA*, *159*, 130-149.

Albandar, J. M., Brunelle, J. A., & Kingman, A. (1999). Destructive periodontal disease in adults 30 years of age and older in the United States, 1988-1994. *Journal of periodontology*, *70*(1), 13-29.

AlQahtani, S. J., Hector, M. P., & Liversidge, H. M. (2014). Accuracy of dental age estimation charts: Schour and Massler, Ubelaker and the London Atlas. *AJPA*, *154*(1), 70-78.

Alt, K. W., Pichler, S., Vach, W., Klíma, B., Vlček, E., & Sedlmeier, J. (1997). Twenty-five thousand-year-old triple burial from Dolní Věstonice: An ice-age family?. *AJPA*, *102*(1), 123-131.

Alt, K. W., & Vach, W. (1995). Odontologic kinship analysis in skeletal remains: concepts, methods, and results. *Forensic science international*, *74*(1-2), 99-113.

Alt, K. W., & Vach, W. (1998). Kinship studies in skeletal remains—Concepts and examples. In *Dental Anthropology* (pp. 537-554). Springer, Vienna.

Anderson, A. S., & Loeser, R. F. (2010). Why is osteoarthritis an age-related disease?. *Best practice & research Clinical rheumatology*, *24*(1), 15-26.

Antoine, D., Hillson, S., & Dean, M. C. (2009). The developmental clock of dental enamel: a test for the periodicity of prism cross-striations in modern humans and an evaluation of the most likely sources of error in histological studies of this kind. *Journal of anatomy*, *214*(1), 45-55.

Armelagos, G. J. (2003). Bioarchaeology as anthropology. *Archeological Papers of the American Anthropological Association*, *13*(1), 27-40.

Armelagos, G. J., Goodman, A. H., Harper, K. N., & Blakey, M. L. (2009). Enamel hypoplasia and early mortality: Bioarcheological support for the Barker hypothesis. *Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews*, *18*(6), 261-271.

Armitage, J. A., Poston, L., & Taylor, P. D. (2008). Developmental origins of obesity and the metabolic syndrome: the role of maternal obesity. *Obesity and metabolism*, *36*, 73-84.

Arron, J. R., & Choi, Y. (2000). Bone versus immune system. *Nature*, *408*(6812), 535-536.

Ash, P., Loutit, J. F., & Townsend, K. M. S. (1980). Osteoclasts derived from haematopoietic stem cells. *Nature*, *283*(5748), 669-670.

Ashworth, A. (1998). Effects of intrauterine growth retardation on mortality and morbidity in infants and young children. *European journal of clinical nutrition*, *52*, S34.

Attur, M., Belitskaya-Lévy, I., Oh, C., Krasnokutsky, S., Greenberg, J., Samuels, J., ... & Attur, M., Krasnokutsky-Samuels, S., Samuels, J., & Abramson, S. B. (2013). Prognostic biomarkers in osteoarthritis. *Current opinion in rheumatology*, *25*(1), 136.

Aubry, B. S. (2014). Cervical dimensions for in situ and loose teeth: A critique of the Hillson et al.(2005) method. *AJPA*, *154*(1), 159-164.

Barker, D. J. (1995). Fetal origins of coronary heart disease. *Bmj*, *311*(6998), 171-174.

Barker, D. J. (2004). The developmental origins of adult disease. *Journal of the American College of Nutrition*, *23*(sup6), 588S-595S.

Barker, D.J. (2012). Developmental origins of chronic disease. *Public health*, *126*(3), pp.185-189.

Barker, D. J., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ*, *298*(6673), 564-567.

Barrett, R., Kuzawa, C. W., McDade, T., & Armelagos, G. J. (1998).Emerging and reemerging infectious diseases: The third epidemio-logic transition.Annual Review of Anthropology,27, 247–271.

Bar-Shavit, Z. (2008). Taking a toll on the bones: regulation of bone metabolism by innate immune regulators. *Autoimmunity*, *41*(3), 195-203.

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'udine, B., Foley, R.A., Gluckman, P., Godfrey, K., Kirkwood, T., Lahr, M.M. and McNamara, J. (2004). Developmental plasticity and human health. *Nature,* 430(6998), 419.

Bauer, G.R., 2014. Incorporating intersectionality theory into population health research methodology: Challenges and the potential to advance health equity. *Social science & medicine*, *110*, pp.10-17.

Beijers, R., Buitelaar, J. K., & de Weerth, C. (2014). Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis. *European child & adolescent psychiatry*, *23*(10), 943-956.

Berenbaum, F. (2013). Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis and cartilage*, *21*(1), 16-21.

Berger, S. M., Griffin, J. S., & Dent, S. C. (2020). Phenotypes and pathways: Working toward an integrated skeletal biology in biological anthropology. *AJHB*, 33(2), 1-19.

Berger, S. M., Smith-Guzmán, N. E., Hutchinson, D. L. n.d. Reconstructing Developmental Phenotype and Mortality Risk in Pre-Columbian Panama.

Berger, S.M., Smith-Guzmán, N.E., Sharpe, A., and Cooke, R. In preparation. Pre-Columbian Kinship Systems and Complex Mortuary Tradition in the Greater Coclé Region, Panama.

Berrey, C.A., 2015. Inequality, demography, and variability among early complex societies in Central Pacific Panama. JAA, 40, 196-212.

Berrey, C.A., 2018. Making absolute population estimates in the intermediate area using the area and density of ceramic sherd scatters: An application of regression analysis. JAS, 97, 147-158.

Bishop, N. A., & Knüsel, C. J. (2005). A palaeodemographic investigation of warfare in prehistory. *Warfare, Violence and Slavery in Prehistory. BAR International Series*, *1374*, 201-216.

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., De Onis, M., Ezzati, M., ... & Maternal and Child Undernutrition Study Group. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *The lancet*, *371*(9608), 243-260.

Blom, A. B., van Lent, P. L., Libregts, S., Holthuysen, A. E., van der Kraan, P. M., van Rooijen, N., & van den Berg, W. B. (2007). Crucial role of macrophages in matrix metalloproteinase–mediated cartilage destruction during experimental osteoarthritis: involvement of matrix metalloproteinase 3. *Arthritis & Rheumatism*, *56*(1), 147-157.

Boas, F. (1912). Changes in the bodily form of descendants of immigrants. *American Anthropologist*, *14*(3), 530-562.

Boldsen, J. L. (2007). Early childhood stress and adult age mortality—a study of dental enamel hypoplasia in the medieval Danish village of Tirup. *AJPA*, *132*(1), 59-66.

Boldsen, J. L., Milner, G. R., Konigsberg, L. W., Wood, J. W., Hoppa, R. D., & Vaupel, J. W. (2002). Transition analysis: a new method for estimating age from skeletons. In *Paleodemography: age distributions from skeletal samples* (pp. 73-106). Cambridge University Press.

Bornstein, M.H. (1989). Sensitive periods in development: Structural characteristics and causal interpretations. *Psychological Bulletin*, 105. 179–197.

Bourdieu, P. (1977). *Outline of a Theory of Practice* (No. 16). Cambridge: Cambridge University Press.

Boyce, B. F., Yao, Z., & Xing, L. (2010). Functions of NF-κB in Bone. *Annals of the New York Academy of Sciences*, *1192*, 367.

Brennan, J., Kuhns, L.M., Johnson, A.K., Belzer, M., Wilson, E.C., Garofalo, R. and Adolescent Medicine Trials Network for HIV/AIDS Interventions, 2012. Syndemic theory and HIV-related risk among young transgender women: the role of multiple, co-occurring health problems and social marginalization. *American journal of public health*, *102*(9), pp.1751-1757.

Briggs, P.S. (1989). Art, death and social order: the mortuary arts of pre-Conquest Central Panamá (Vol. 550). *British Archaeological Reports Limited.*

Briggs, P.S (1993). Fatal attractions: Interpretation of Prehistoric Mortuary Remains from Lower Central America. In *Reinterpreting Prehistory of Central America*, ed. M M. Graham, University of Colorado Press, 141-168.

Buikstra, J. E., & Ubelaker, D. H. (1994). Standards for data collection from human skeletal remains. Arkansas archaeological survey. *Research Series*, (44).

Bush, M. B., Piperno, D. R., Colinvaux, P. A., De Oliveira, P. E., Krissek, L. A., Miller, M. C., & Rowe, W. E. (1992). A 14 300-yr paleoecological profile of a lowland tropical lake in Panama. *Ecological monographs*, *62*(2), 251-275.

Butler, P. M. (1939, May). Studies of the Mammalian Dentition–Differentiation of the Post-canine Dentition. *Proceedings of the Zoological Society of London*, 109(1), 1-36.

Campisi, J., & Di Fagagna, F. D. A. (2007). Cellular senescence: when bad things happen to good cells. *Nature reviews Molecular cell biology*, *8*(9), 729-740.

Capodiferro, M. R., Raveane, A., Rambaldi Migliore, N., Colombo, G., Ongaro, L., Rivera, J., ... & Achilli, A. (2020). Archaeogenomic Distinctiveness of the Isthmo-Colombian Area. *bioRxiv*, 1-50.

Carr, C. (1995). Mortuary practices: Their social, philosophical-religious, circumstantial, and physical determinants. *Journal of Archaeological Method and Theory*, *2*(2), 105-200.

Carsten, J. (Ed.). (2000). *Cultures of relatedness: New approaches to the study of kinship*. Cambridge University Press.

Carvajal-Contreras, D. R., Cooke, R.G., and Jiménez, M. (2008). Taphonomy at two contiguous coastal rockshelters in Panama: Preliminary observations focusing on fishing and curing fish. *Quaternary International*, 180(1), 90–106.

Carvajal, D.R., Diaz, C. P., Sánchez Herrera, L.A., and Cooke, R.G. (2006). ¿Fue Cerro Juan Díaz, una aldea precolombina en el valle del río La Villa, el pueblo de indios de Cubitá? *Proceedings of the Memorias del VI Congreso Centroamericano de Historia, Panamá, 22-26.7.2002*: 100–123.

Carvajal, D. R., and Hansell, P. (2008). Molluscs in Central Panama: A Review. *Archaeofauna*, 17: 157-174.

Celeux, G., & Soromenho, G. (1996). An entropy criterion for assessing the number of clusters in a mixture model. *Journal of classification*, *13*(2), 195-212.

Chapais, B., Allen, N. J., Alvard, M., Apostolou, M., Barnard, A., Dunbar, R. I. M., ... & Chapais, B. (2014). Complex kinship patterns as evolutionary constructions, and the origins of sociocultural universals. *Current Anthropology*, *55*(6), 000-000.

Champagne, F. A. (2012). Interplay between social experiences and the genome: epigenetic consequences for behavior. *Advances in genetics*, *77*, 33-57.

Chang, J., Wang, Z., Tang, E., Fan, Z., McCauley, L., Franceschi, R., ... & Wang, C. Y. (2009). Inhibition of osteoblastic bone formation by nuclear factor-κB. *Nature medicine*, *15*(6), 682.

Chen, T., Liu, H. X., Yan, H. Y., Wu, D. M., & Ping, J. (2016). Developmental origins of inflammatory and immune diseases. *Mhr: Basic science of reproductive medicine*, *22*(8), 858-865.

Clark, G. A., Hall, N. R., Armelagos, G. J., Borkan, G. A., Panjabi, M. M., & Wetzel, F. T. (1986). Poor growth prior to early childhood: Decreased health and life-span in the adult. *AJPA*, *70*(2), 145-160.

Clary, J., Hansell, P., Ranere, A.J., and Buggey, T. (1984). The Holocene geology of the western Parita Bay coastline of central Panama. In *Recent Developments in Isthmian Archaeology*, ed. F.W. Lange, pp. 55-83. *BAR Series 212*, Oxford.

Coelho, R., Viola, T. W., Walss-Bass, C., Brietzke, E., & Grassi-Oliveira, R. (2014). Childhood maltreatment and inflammatory markers: a systematic review. *Acta Psychiatrica Scandinavica*, *129*(3), 180-192.

Collier, J. F., & Yanagisako, S. J. (1987). *Gender and kinship: Essays toward a unified analysis*. Stanford University Press.

Collins Jr, J. W., & David, R. J. (2009). Racial disparity in low birth weight and infant mortality. *Clinics in perinatology*, *36*(1), 63-73.

Collins, L. M., Schafer, J. L., & Kam, C. M. (2001). A comparison of inclusive and restrictive strategies in modern missing data procedures. *Psychological methods*, *6*(4), 330.

Contreras, D. R. C., & Hansell, P. (2008). Molluscs in Central Panama: a review. *Archaeofauna*, *17*, 157-174.

Cook, R. A., & Aubry, B. S. (2014). Aggregation, interregional interaction, and postmarital residence patterning: A study of biological variation in the late prehistoric middle Ohio Valley. *American journal of physical anthropology*, *154*(2), 270-278.

Cook, D. C., & Buikstra, J. E. (1979). Health and differential survival in prehistoric populations: prenatal dental defects. *AJPA*, *51*(4), 649-664.

Cooke, R.G. (1972). The Archaeology of the Western Coclé Province of Panama. Doctoral dissertation, London: University of London.

Cooke, R.G. (1977). El Carpintero y El Hachero: Dos artesanoes del Panama prehistorico. *Revista Panameña de Antropologia,* 2, 48-77.

Cooke, R. (1979). Los Impactos de las comunidades agricolas precolombinas sobre los ambientes del tropico estacional: datos del Panama prehistorico. In *Actas del Symposium de Ecologia Tropical, 4*. Smithsonian Tropical Research Institute.

Cooke, R. G., 1995. Monagrillo, Panama's First Pottery (3800-1200 CAL BC): Summary of Research (1984-1993), with New Interpretations of Chronology, Subsistence, and Cultural Geography. In *The Emergence of Pottery*, ed. W.K Barnett and J.W. Hoopes, pp. 169-184. Washington, DC: Smithsonian Institution Press.

Cooke, R. G. (1998). Subsistencia y economía casera de los indígenas precolombinos de Panama. In *Antropología panameña: pueblos y culturas*, ed. A. Pastor, Editorial Universitaria, pp. 61-134.

Cooke, R. G. (2001). Cuidando a los ancestros: Rasgos mortuorios precolombinos en cerro Juan Díaz, Los Santos. In *Panamá: puente biológico*, ed. S. Heckadon-Moreno. Panama City: Smithsonian Tropical Research Institute, pp. 54-62.

Cooke, R.G. (2004). Rich, poor, shaman, child: Animals, rank, and status in the 'Gran Coclé'culture area of pre-Columbian Panama. In *Behaviour behind Bones: The Zooarchaeology of Ritual, Religion, Status and Identity*, pp.271-284.

Cooke, R. (2005). Prehistory of Native Americans on the Central American Land Bridge: Colonization, Dispersal, and Convergence. *JAR,* 13(2), 129-187.

Cooke, R. and Ranere, A.J., (1992). Prehistoric human adaptations to the seasonally dry forests of Panama. *World Archaeology*, *24*(1), pp.114-133.

Cooke, R.G., and Ranere, A.J. (1999). Precolumbian fishing on the pacific coast of Panama. In: Pacific Latin America in Prehistory: The Evolution of Archaic and Formative Cultures, ed. Blake, M.P, 103-134. Washington State University Press.

Cooke, R., Ranere, A., Pearson, G. and Dickau, R. (2013). Radiocarbon chronology of early human settlement on the Isthmus of Panama (13,000–7000 BP) with comments on cultural affinities, environments, subsistence, and technological change. *Quaternary international*, *301*, pp.3-22.

Cooke, R. G., and Sánchez, L.A.H. (2004). Arqueología en Panamá (1888-2002). *Panama: cien años de República*.

Cooke, R., Sanchez, L.A., Ilean, H., Isaza, I., and Aguilardo Perez, Y. (1998). Rasgos Mortuorios y Artefactos Inusitados de Cerro Juan Diaz: Una Aldea Precolombina del Gran Cocle. *La Antigua*, 53: 127-196.

Cooper, C., Westlake, S., Harvey, N., Javaid, K., Dennison, E., & Hanson, M. (2006). Developmental origins of osteoporotic fracture. *Osteoporosis International*, *17*(3), 337-347.

Corruccini, R. S. (1972). The biological relationships of some prehistoric and historic Pueblo populations. *AJPA*, *37*(3), 373-388.

Corruccini, R. S. (1973). Size and shape in similarity coefficients based on metric characters. *AJPA*, *38*(3), 743-753.

Corruccini, R. S., & Potter, R. H. Y. (1981). Developmental correlates of crown component asymmetry and occlusal discrepancy. *AJPA*, *55*(1), 21-31.

Courtois, G., & Gilmore, T. D. (2006). Mutations in the NF-κB signaling pathway: implications for human disease. *Oncogene*, *25*(51), 6831.

Coventry, J., Griffiths, G., Scully, C., & Tonetti, M. (2000). Periodontal disease. *Bmj*, *321*(7252), 36-39.

Cox, D. R. (1972). Regression models and life-tables (with discussion). J. R. Statist. Soc. B 34, 187-220.

Cox, M. and Mays, S. eds., 2000. Human osteology: in archaeology and forensic science. Cambridge University Press.

Creamer, W., & Haas, J. (1985). Tribe versus chiefdom in lower Central America. *American Antiquity*, 738-754.

Crespo, F. (2020). Reconstructing Immune Competence in Skeletal Samples: A theoretical and methodological approach. In *Theoretical Approaches in Bioarchaeology,* eds. C.M Cheverko, J. R. Prince-Buitenhuys, and M. Hubbe, Routledge, pp. 76-92.

Crespo, F. A., Klaes, C. K., Switala, A. E., & DeWitte, S. N. (2017). Do leprosy and tuberculosis generate a systemic inflammatory shift? Setting the ground for a new dialogue between experimental immunology and bioarchaeology. *AJPA*, *162*(1), 143-156.

Cunningham, C., Scheuer, L., & Black, S. (2016). *Developmental juvenile osteology*. New York: Academic press.

Daar, A. S., Singer, P. A., Persad, D. L., Pramming, S. K., Matthews, D. R., Beaglehole, R., ... & Bell, J. (2007). Grand challenges in chronic non-communicable diseases. *Nature*, *450*(7169), 494-496.

Dahlberg, A. A. (1945). The changing dentition of man. *The Journal of the American Dental Association*, *32*(11), 676-690.

Dahlberg, A. A. (1991). Historical perspective of dental anthropology. In M.A. Kelly and C.S. Larsen (eds), *Advances in Dental Anthropology.* New York: Wiley-Liss*,* 7-11.

Dall'Olio, F., Vanhooren, V., Chen, C. C., Slagboom, P. E., Wuhrer, M., & Franceschi, C. (2013). N-glycomic biomarkers of biological aging and longevity: a link with inflammaging. *Ageing research reviews*, *12*(2), 685-698.

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiology & behavior*, *106*(1), 29-39.

Danese, A., Pariante, C. M., Caspi, A., Taylor, A., & Poulton, R. (2007). Childhood maltreatment predicts adult inflammation in a life-course study. *PNAS*, *104*(4), 1319-1324.

DeLeon, V. B. (2007). Fluctuating asymmetry and stress in a medieval Nubian population. *AJPA: The Official Publication of the American Association of Physical Anthropologists*, *132*(4), 520-534.

de Wille, M. E. B. (1975). *Birth and death in the belief system of the Bribri Indians of Costa Rica.* (Doctoral dissertation, University of Georgia).

Dempsey, P. J., Townsend, G. C., Martin, N. G., & Neale, M. C. (1995). Genetic covariance structure of incisor crown size in twins. *Journal of dental research*, *74*(7), 1389-1398.

Dent, S. C. (2017). Interindividual differences in embodied marginalization: Osteological and stable isotope analyses of antebellum enslaved individuals. *AJHB*, *29*(4), e23021.

Dent, S. C., Berger, S. M., & Griffin, J. S. (2020). Biocultural pathways linking periodontal disease expression to food insecurity, immune dysregulation, and nutrition. *AJHB*, 33(2), e23549.

DeWitte, S. N. (2014). Differential survival among individuals with active and healed periosteal new bone formation. *International Journal of Paleopathology*, *7*, 38-44.

DeWitte, S. N., & Bekvalac, J. (2011). The association between periodontal disease and periosteal lesions in the St. Mary Graces cemetery, London, England AD 1350–1538. *AJPA*, *146*(4), 609-618.

DeWitte, S. N., & Hughes-Morey, G. (2012). Stature and frailty during the Black Death: the effect of stature on risks of epidemic mortality in London, AD 1348–1350. *Journal of Archaeological Science*, *39*(5), 1412-1419.

DeWitte, S. N., & Stojanowski, C. M. (2015). The osteological paradox 20 years later: past perspectives, future directions. *Journal of Archaeological Research*, *23*(4), 397-450.

DeWitte, S. N., & Wood, J. W. (2008). Selectivity of Black Death mortality with respect to preexisting health. *Proceedings of the National Academy of Sciences*, *105*(5), 1436-1441.

Díaz, C.P., 1999. Estudio bio-antropológico de rasgos mortuorios de la operación 4 del sitio arqueológico, Cerro Juan Díaz, Panamá Central. Licenciatura thesis, Department of Anthropology, Bogotá: Universidad de los Andes.

Dickau, R. (2010). Microbotanical and macrobotanical evidence of plant use and the transition to agriculture in Panama. In *Integrating Zooarchaeology and Paleoethnobotany*, pp. 99-134. New York: Springer.

Drake, A.J. and Liu, L., 2010. Intergenerational transmission of programmed effects: public health consequences. *Trends in Endocrinology & Metabolism*, 21(4), pp.206-213.

Drake, A. J., & Walker, B. R. (2004). The intergenerational effects of fetal programming: non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. *Journal of Endocrinology*, *180*(1), 1-16.

Egerton, M., Scollay, R., & Shortman, K. (1990). Kinetics of mature T-cell development in the thymus. *PNAS*, *87*(7), 2579-2582.

Ensor, B. E. (2013). *The archaeology of kinship: Advancing interpretation and contributions to theory*. Tucson: University of Arizona Press.

Ensor, B. E., Irish, J. D., Keegan, W. F., McConvell, P., Peregrine, P. N., Schillaci, M. A., ... & Keegan, W. F. (2017). The bioarchaeology of kinship: Proposed revisions to assumptions guiding interpretation. *CA*, *58*(6), 000-000.

Eriksson, J. G., Forsen, T., Tuomilehto, J., Winter, P. D., Osmond, C., & Barker, D. J. (1999). Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *Bmj*, *318*(7181), 427-431.

Espinosa, G. (1913) Relación del Proceso quell Licenciado Gaspar de Espinosa, alcalde mayor hizoenviado por el muy magnifico señor Pedrarias Dávila desde esta ciudad a las provincias de Nata e Parisy alas otras provincias Comarcanas. El descubri-miento del Océano Pacifico: Vasco Núñez de Balboa,Fernando de Magallanes y sus Compañeros. Editorial Universitaria, Santiago de Chile.

Espinosa, G. (1994). Relación de lo hecho por el licenciado Gaspar de Espinosa…que hiciese y cumpliese en el viaje a las provincias de Paris y Natá y Cherú y a las otras comarcanas. In *Indios y negros en Panamá en los siglos XVI y XVII: Selecciones de los documentos del archivo general de indias*, ed. by C.F. Jopling, Centro de Investigaciones Regionales de Mesoamérica, pp. 61–74.

Evans-Pritchard, E. E. (1940). *The Nuer: A description of the modes of livelihood and political institutions of a Nilotic people.* Oxford: Oxford University Press.

Evans-Pritchard, E. E. (1951). *Kinship and Marriage among the Nuer.* Oxford: Clarendon Press.

Everson, S.A., Maty, S.C., Lynch, J.W. and Kaplan, G.A., 2002. Epidemiologic evidence for the relation between socioeconomic status and depression, obesity, and diabetes. *Journal of psychosomatic research*, *53*(4), pp.891-895.

Fagundes, C. P., Glaser, R., & Kiecolt-Glaser, J. K. (2013). Stressful early life experiences and immune dysregulation across the lifespan. *Brain, behavior, and immunity*, *27*, 8-12.

Fagundes, C. P., & Way, B. (2014). Early-life stress and adult inflammation. *Current Directions in Psychological Science*, *23*(4), 277-283.

Forastiere, F., Stafoggia, M., Tasco, C., Picciotto, S., Agabiti, N., Cesaroni, G., & Perucci, C. A. (2007). Socioeconomic status, particulate air pollution, and daily mortality: differential exposure or differential susceptibility. *American journal of industrial medicine*, *50*(3), 208-216.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De Benedictis, G. (2000). Inflamm-aging: an evolutionary perspective on immunosenescence. *Annals of the new York Academy of Sciences*, *908*(1), 244-254.

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *69*(Suppl_1), S4-S9.

Franceschi, C., Garagnani, P., Vitale, G., Capri, M., & Salvioli, S. (2017). Inflammaging and 'Garb-aging'. *Trends in Endocrinology & Metabolism*, *28*(3), 199-212.

Galobardes, B., Smith, G. D., & Lynch, J. W. (2006). Systematic review of the influence of childhood socioeconomic circumstances on risk for cardiovascular disease in adulthood. *Annals of epidemiology*, *16*(2), 91-104.

Gamba, C., Jones, E. R., Teasdale, M. D., McLaughlin, R. L., Gonzalez-Fortes, G., Mattiangeli, V., ... & Pinhasi, R. (2014). Genome flux and stasis in a five millennium transect of European prehistory. *Nature communications*, *5*(1), 1-9.

Garland, C. J. (2020). Implications of accumulative stress burdens during critical periods of early postnatal life for mortality risk among Guale interred in a colonial era cemetery in Spanish Florida (ca. AD 1605–1680). *AJPA*.

Garland, C. J., Reitsema, L. J., Larsen, C. S., & Thomas, D. H. (2018). Early Life Stress at Mission Santa Catalina de Guale: An Integrative Analysis of Enamel Defects and Dentin Incremental Isotope Variation in Malnutrition. *Bioarchaeology International*, *2*(2), 75-94.

Garn, S. M., Lewis, A. B., & Blizzard, R. M. (1965a). Endocrine factors in dental development. *Journal of Dental Research*, *44*(1), 243-258.

Garn, S. M., Lewis, A. B., & Kerewsky, R. S. (1965b). Genetic, nutritional, and maturational correlates of dental development. *Journal of dental research*, *44*(1), 228-242.

Garn, S. M., Lewis, A. B., & Kerewsky, R. S. (1966). The meaning of bilateral asymmetry in the permanent dentition. *The Angle Orthodontist*, *36*(1), 55-62.

Garn, S. M., Lewis, A. B., & Kerewsky, R. S. (1967). Buccolingual size asymmetry and its developmental meaning. *The Angle Orthodontist*, *37*(3), 186-193.

George, A. J., & Ritter, M. A. (1996). Thymic involution with ageing: obsolescence or good housekeeping?. *Immunology today*, *17*(6), 267-272.

Gillespie, S. D. (2001). Personhood, agency, and mortuary ritual: a case study from the ancient Maya. *JAA*, *20*(1), 73-112.

Ginaldi, L., & De Martinis, M. (2016). Osteoimmunology and beyond. *Current medicinal chemistry*, *23*(33), 3754-3774.

Gluckman, P.D, Hanson, M.A, and Beedle, S. (2007). Early life events and their consequences for later disease: a life history and evolutionary perspective. AJHB. 2007 Jan-Feb;19(1):1-19.

Gluckman, P. D., Hanson, M. A., & Buklijas, T. (2010). A conceptual framework for the developmental origins of health and disease. *Journal of Developmental Origins of Health and Disease*, *1*(1), 6-18.

Gluckman, P. D., Hanson, M. A., Cooper, C., & Thornburg, K. L. (2008). Effect of in utero and early-life conditions on adult health and disease. *NEJM*, *359*(1), 61-73.

Gluckman, P. D., Hanson, M. A., & Pinal, C. (2005). The developmental origins of adult disease. *Maternal & child nutrition*, *1*(3), 130-141.

Godde, K., Pasillas, V., & Sanchez, A. (2020). Survival analysis of the Black Death: Social inequality of women and the perils of life and death in Medieval London. *AJPA*, e24081.

Godfrey, K. M., Gluckman, P. D., & Hanson, M. A. (2010). Developmental origins of metabolic disease: life course and intergenerational perspectives. *Trends in Endocrinology & Metabolism*, *21*(4), 199-205.

Goldstein, L. (1980). *Mississippian mortuary practices: A case study of two cemeteries n the lower Illinois valley.* (Doctoral dissertaton, Northwestern University.

Goldstein, L. (1981). One-dimensional archaeology and multi-dimensional people: spatial organization and mortuary analysis. *The archaeology of death*, 5349.

Gompertz, B. (1824). On the nature of the function expressive of the law of human mortality and on the new mode of determining the value of life contingencies. Phil.Trans. Royal Soc. A., 115, 513-580.

Goodman, A. H., Armelagos, G. J., & Rose, J. C. (1980). Enamel hypoplasias as indicators of stress in three prehistoric populations from Illinois. *Human biology*, 515-528.

Goodman, A. H., & Rose, J. C. (1990). Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures. *AJPA*, *33*(S11), 59-110.

Gower, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika*, *53*(3-4), 325-338.

Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, 857-871.

Gowland, R. L. (2015). Entangled lives: Implications of the developmental origins of health and disease hypothesis for bioarchaeology and the life course. *AJPA*, *158*(4), 530-540.

Grant, R. W., & Dixit, V. D. (2013). Mechanisms of disease: inflammasome activation and the development of type 2 diabetes. *Frontiers in immunology*, *4*, 50.

Graves D. 2008. Cytokines that promote periodontal tissue destruction. J Periodontol 79(8S):1585-1591.

Graves, D., Li, J., & Cochran, D. L. (2011). Inflammation and uncoupling as mechanisms of periodontal bone loss. *Journal of dental research*, *90*(2), 143-153.

Gravlee, C. C. 2009. How race becomes biology: embodiment of social inequality. *American Journal of Physical Anthropology*, *139*(1), 47-57.

Griggs, J. C. (2005). *The archaeology of central Caribbean Panama*. (Doctoral dissertation, University of Texas at Austin).

Hagenaars, J. A., & McCutcheon, A. L. (2002). *Applied latent class analysis*. Cambridge University Press.

Hajishengallis G. (2014b). The inflammophilic character of the periodontitis-associated microbiota. Mol Oral Microbiol 29(6):248-257.

Hajishengallis, G. (2014a). Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends in immunology*, *35*(1), 3-11.

Hajishengallis, G. (2015). Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology*, *15*(1), 30-44.

Hall, J.M., 1999. Marginalization revisited: Critical, postmodern, and liberation perspectives. *Advances in Nursing Science*, *22*(2), pp.88-102.

Haller, M. J. (2004). *The emergence and development of chiefly societies in the Río Parita Valley, Panama* (Doctoral dissertation, University of Pittsburgh).

Haller, M. J. (2008). *Asiento Viejo and the development of the Río Parita chiefdom, Panama* (No. 19). Center for Comparative Arch.

Ham, A. C., Temple, D. H., Klaus, H. D., & Hunt, D. R. (2020). Evaluating life history trade-offs through the presence of linear enamel hypoplasia at Pueblo Bonito and Hawikku: A biocultural study of early life stress and survival in the Ancestral Pueblo Southwest. *AJHB*, 33(2), e23506.

Han, Y.W., and Wang, X. (2013). Mobile Microbiome: Oral Bacteria in Extra-oral Infections and Inflammation. J Dent Res 92(6):485-491.

Hanson, M. A., & Gluckman, P. D. (2014). Early developmental conditioning of later health and disease: physiology or pathophysiology?. *Physiological reviews*.

Hansson, G.K., 2005. Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine*, *352*(16), pp.1685-1695.

Harikumar, S., & Surya, P. V. (2015). K-medoid clustering for heterogeneous datasets. *Procedia Computer Science*, *70*, 226-237.

Harris, E. F., & Nweeia, M. T. (1980). Dental asymmetry as a measure of environmental stress in the Ticuna Indians of Colombia. *AJPA*, *53*(1), 133-142.

Heijmans, B. T., Tobi, E. W., Stein, A. D., Putter, H., Blauw, G. J., Susser, E. S., ... & Lumey, L. H. (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences*, *105*(44), 17046-17049.

Heiland, G. R., Zwerina, K., Baum, W., Kireva, T., Distler, J. H., Grisanti, M., ... & Schett, G. (2010). Neutralisation of Dkk-1 protects from systemic bone loss during inflammation and reduces sclerostin expression. *Annals of the rheumatic diseases*, *69*(12), 2152-2159.

Heim, C., & Binder, E. B. (2012). Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene–environment interactions, and epigenetics. *Experimental neurology*, *233*(1), 102-111.

Herrera, F. (1972). Aspectos del desarrollo económico y social de los indios Kuna de San Blas. *América Indígena*, *32*(1), 113-138.

Hill, K., & Kaplan, H. (1999). Life history traits in humans: Theory and empirical studies. *Annual review of anthropology*, *28*(1), 397-430.

Hillson, S. (2014). *Tooth development in human evolution and bioarchaeology*. Cambridge University Press.

Hillson, S. 2018. Dental pathology. In *Biological anthropology of the human skeleton,* ed. M.A. Katzenberg and A.L. Grauer, Hoboken: John Wiley & Sons, 295-333.

Hillson, S., FitzGerald, C., & Flinn, H. (2005). Alternative dental measurements: proposals and relationships with other measurements. *AJPA, 126*(4), 413-426.

Hillson, S.W., Larsen, C.S., Boz, B., Pilloud, M.A., Sadvari, J.W., Agarwal, S.C., Glencross, B., Beauchesne, P., Pearson, J., Ruff, C.B. and Garofalo, E.M., 2000. The human remains I: interpreting community structure, health and diet in Neolithic Çatalhöyük. Humans and Landscapes of Çatalhöyük: Reports from the, 2008, 339-96.

Hinck, V.C., Clark, W.M. and Hopkins, C.E. (1966). Normal interpediculate distances (minimum and maximum) in children and adults. *American Journal of Roentgenology*, *97*(1), pp.141-153.

Hirota, K., Yoshitomi, H., Hashimoto, M., Maeda, S., Teradaira, S., Sugimoto, N., ... & Sakaguchi, N. (2007). Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *Journal of Experimental Medicine*, *204*(12), 2803-2812.

Hoffman, D. J., Reynolds, R. M., & Hardy, D. B. (2017). Developmental origins of health and disease: current knowledge and potential mechanisms. *Nutrition reviews*, *75*(12), 951-970.

Holder, S., Miliauskienė, Ž., Jankauskas, R., & Dupras, T. (2020). An integrative approach to studying plasticity in growth disruption and outcomes: A bioarchaeological case study of Napoleonic soldiers. *AJHB*, e23457.

Holloman, R. (1976). Cuna Household Types and the Domestic Cycle. In *Frontier Adaptation in Lower South America*, eds. M. Helms and F. Loveland, Institute for the Study of Human Issues, pp. 131-149.

Holmes, W.H., (1919). *Handbook of aboriginal American antiquities* (No. 60). US Government Printing Office.

Holroyd, C., Harvey, N., Dennison, E., & Cooper, C. (2012). Epigenetic influences in the developmental origins of osteoporosis. *Osteoporosis International*, *23*(2), 401-410.

Hoopes, J.W. (2005). The emergence of social complexity in the Chibchan world of southern Central America and northern Colombia, AD 300–600. *Journal of Archaeological Research*, *13*(1), pp.1-47.

Hoopes, J. W., & Fonseca, O. M. (2003). Goldwork and Chibchan identity: Endogenous change and diffuse unity in the Isthmo-Colombian area. *Gold and power in ancient Costa Rica, Panama, and Colombia*, 49-89.

Horst, O. V., Horst, J. A., Samudrala, R., & Dale, B. A. (2011). Caries induced cytokine network in the odontoblast layer of human teeth. *BMC immunology*, *12*(1), 1-13.

Hotez, P. J., Bottazzi, M. E., Franco-Paredes, C., Ault, S. K., & Periago, M. R. (2008). The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. *PLoS Negl Trop Dis*, *2*(9), e300.

Howell, T. L., & Kintigh, K. W. (1996). Archaeological identification of kin groups using mortuary and biological data: an example from the American Southwest. *AA*, 537-554.

Huard, A.E. 2013. Cerro Mangote: Interpretations of space based on mortuary analysis. Doctoral dissertation, Binghamton: Binghamton University State University of New York.

Huffman, M. (2014). *Biological variation in South American populations using dental non-metric traits: Assessment of isolation by time and distance* (Doctoral dissertation, The Ohio State University).

Hussein, M. R., Fathi, N. A., El-Din, A. M. E., Hassan, H. I., Abdullah, F., Eman, A. H., & Backer, E. A. (2008). Alterations of the CD4+, CD8+ T cell subsets, interleukins-1β, IL-10, IL-17, tumor necrosis factor-α and soluble intercellular adhesion molecule-1 in rheumatoid arthritis and osteoarthritis: preliminary observations. *Pathology & Oncology Research*, *14*(3), 321-328.

Ibarra Rojas, E. (2012). Exploring warfare and prisoner capture in indigenous southern Central America. *Revista de Arqueología Americana*, 105-131.

Ichon, A. (1980). Archéologie du sud de la péninsule d'Azuero, Panama. *Etudes Mésoaméricaines/Estudios Mesoamericanos. Série II Mexico*, (3), pp.1-521.

Irfan, U. M., Dawson, D. V., & Bissada, N. F. (2001). Epidemiology of periodontal disease: a review and clinical perspectives. *Journal of the International Academy of Periodontology*, *3*(1), 14-21.

Isaza Aizpurúa, I. I. (2007). *The Ancestors of Parita: Pre-Columbian Settlement Patterns in the Lower La Villa River Valley, Azuero Peninsula, Panama.* (Doctoral dissertation, Boston University).

Itoh, K., Udagawa, N., Kobayashi, K., Suda, K., Li, X., Takami, M., ... & Takahashi, N. (2003). Lipopolysaccharide promotes the survival of osteoclasts via Toll-like receptor 4, but cytokine production of osteoclasts in response to lipopolysaccharide is different from that of macrophages. *The Journal of Immunology*, *170*(7), 3688-3695.

Jackson, C. H. (2016). flexsurv: a platform for parametric survival modeling in R. *Journal of statistical software*, *70*.

Jacobi, K. P. (1997). Dental genetic structuring of a colonial Maya cemetery, Tipu, Belize. In *Bones of the Maya*, eds. S.L Whittington and D.M. Reed, Smithsonian Institution Press, pp. 138–153.

Johnson, K. M., & Paul, K. S. (2016). Bioarchaeology and kinship: integrating theory, social relatedness, and biology in ancient family research. *JAR*, *24*(1), 75-123.

Jopling, C. F. (1994). *Indios y negros en Panamá en los siglos XVI y XVII: selecciones de los documentos del Archivo General de Indias* (Vol. 7). Centro de Investigaciones Regionales de Mesoamérica.

Josien, R., Li, H. L., Ingulli, E., Sarma, S., Wong, B. R., Vologodskaia, M., ... & Choi, Y. (2000). TRANCE, a tumor necrosis factor family member, enhances the longevity and

adjuvant properties of dendritic cells in vivo. *Journal of Experimental Medicine*, *191*(3), 495-502.

Joyce, R. A. (2001). Burying the dead at Tlatilco: Social memory and social identities. *Archeological Papers of the American Anthropological Association*, *10*(1), 12-26.

Kamath, A. T., Henri, S., Battye, F., Tough, D. F., & Shortman, K. (2002). Developmental kinetics and lifespan of dendritic cells in mouse lymphoid organs. *Blood*, *100*(5), 1734-1741.

Kaplan, H., Hill, K., Lancaster, J., & Hurtado, A. M. (2000). A theory of human life history evolution: Diet, intelligence, and longevity. *Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews*, *9*(4), 156-185.

Karin, M., & Greten, F. R. (2005). NF-κB: linking inflammation and immunity to cancer development and progression. *Nature reviews immunology*, *5*(10), 749.

Karmakar, S., Kay, J., & Gravallese, E. M. (2010). Bone damage in rheumatoid arthritis: mechanistic insights and approaches to prevention. *Rheumatic Disease Clinics*, *36*(2), 385-404.

Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nature immunology*, *11*(5), 373.

Kermack, W. O., McKendrick, A. G., & McKinlay, P. L. (1934). Death-Rates in Great Britain and Sweden. Some General Regularities and their Significance. *Lancet*, 698-703.

Kikuchi, T., Matsuguchi, T., Tsuboi, N., Mitani, A., Tanaka, S., Matsuoka, M., ... & Yoshikai, Y. (2001). Gene expression of osteoclast differentiation factor is induced by lipopolysaccharide in mouse osteoblasts via Toll-like receptors. *The Journal of Immunology*, *166*(5), 3574-3579.

King, T., Humphrey, L. T., & Hillson, S. (2005). Linear enamel hypoplasias as indicators of systemic physiological stress: Evidence from two known age-at-death and sex populations from postmedieval London. *AJPA: The Official Publication of the American Association of Physical Anthropologists*, *128*(3), 547-559.

Klales, A. R., Ousley, S. D., & Vollner, J. M. (2012). A revised method of sexing the human innominate using Phenice's nonmetric traits and statistical methods. *AJPA*, *149*(1), 104-114.

Klapisch-Zuber, C. (1991). The genesis of the family tree. *I Tatti Studies in the Italian Renaissance*, *4*, 105-129.

Klaus, H.D., (2010). Bioarchaeology of structural violence: Theoretical model and case study. In *The Bioarchaeology of Violence*, eds. D. L. Martin and R. P. Harrod, pp. 144-144. Hoboken: John Wiley & Sons, Inc.

Klaus, H. D. (2014). Frontiers in the bioarchaeology of stress and disease: Cross-disciplinary perspectives from pathophysiology, human biology, and epidemiology. *AJPA*, *155*(2), 294-308.

Komatsu, N., Okamoto, K., Sawa, S., Nakashima, T., Oh-hora, M., Kodama, T., ... & Takayanagi, H. (2014). Pathogenic conversion of Foxp3+ T cells into T H 17 cells in autoimmune arthritis. *Nature medicine*, *20*(1), 62.

Konigsberg, L. W. (1987). *Population genetic models for interpreting prehistoric intra-cemetery biological variation*. (Doctoral dissertation, Northwestern University).

Konigsberg, L. W. (1988). Migration models of prehistoric postmarital residence. *AJPA*, *77*(4), 471-482.

Konigsberg, L. W., & Frankenberg, S. R. (2016). Postmarital residence analysis. In *Biological Distance Analysis*, Academic Press, pp. 335-347.

Koo, T. K., & Li, M. Y. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *Journal of chiropractic medicine*, *15*(2), 155-163.

Krieger, N. (2001). Theories for social epidemiology in the 21st century: an ecosocial perspective. *International journal of epidemiology*, *30*(4), 668-677.

Krieger, N. (2005). Embodiment: a conceptual glossary for epidemiology. *Journal of Epidemiology & Community Health*, *59*(5), 350-355.

Kumar PS. 2017. From focal sepsis to periodontal medicine: A century of exploring the role of the oral microbiome in systemic disease. J Physiol 595(2):465-476.

Kuzawa, C. W. (2005). Fetal origins of developmental plasticity: Are fetal cues reliable predictors of future nutritional environments? AJHB, 17, 5–21.

Kuzawa, C. W. (2008). The developmental origins of adult health: intergenerational inertia in adaptation and disease. *Evolution and health*, 325-349.

Kuzawa, C. W., & Sweet, E. (2009). Epigenetics and the embodiment of race: developmental origins of US racial disparities in cardiovascular health. *AJHB: The Official Journal of the Human Biology Association*, *21*(1), 2-15.

Kuzawa, C. W., & Quinn, E. A. (2009). Developmental origins of adult function and health: evolutionary hypotheses. *Annual Review of Anthropology*, *38*, 131-147.

Ladd, J. (1964). Archeological investigations in the Parita and Santa Maria zones of Panama. *Bureau of American Ethnology Bulletin*.

Lam, J., Takeshita, S., Barker, J. E., Kanagawa, O., Ross, F. P., & Teitelbaum, S. L. (2000). TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *The Journal of clinical investigation*, *106*(12), 1481-1488.

Lane, R. A. (1977). *The Allegany Seneca: a test of the genetic reliability of nonmetric osteological traits for intrapopulation analysis*. (Doctoral dissertation, University of Texas).

Larsen, C. S. (2015). *Bioarchaeology: interpreting behavior from the human skeleton* (Vol. 69). Cambridge University Press.

Larsen, C. S. (2018). The bioarchaeology of health crisis: Infectious disease in the past. *Annual Review of Anthropology*.

Larsen, C.S. (2006). Foreword Emerging frontiers in the bioarchaeology of Southeast Asia. In Bioarchaeology of Southeast Asia (Vol. 43), eds. M. Oxenham and N. Tayles, xi-xiii. Cambridge: Cambridge University Press.

Lazarsfeld, P. F. (1950). The logical and mathematicalfoundation of latent structure analysis. In Studies in social psychology in World War II Vol. IV: Measurement and prediction, eds. S. A. Star, ,J. A. Stouffer, L. Guttman, E. A. Suchman,P. F. Lazarsfeld, S. A. Star, & J. A. Clausen, Princeton: Princeton University Press, 362–412.

Leary, R. F., & Allendorf, F. W. (1989). Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution*, *4*(7), 214-217.

Leon, D. A., Lithell, H. O., Vågerö, D., Koupilová, I., Mohsen, R., Berglund, L., ... & McKeigue, P. M. (1998). Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. *Bmj*, *317*(7153), 241-245.

Lévi-Strauss, C. (1969). *The elementary structures of kinship* (No. 340). Beacon Press.

Li, Y., He, Y., Qi, L., Jaddoe, V. W., Feskens, E. J., Yang, X., ... & Hu, F. B. (2010). Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. *Diabetes*, *59*(10), 2400-2406.

Li, Y., Li, A., Strait, K., Zhang, H., Nanes, M. S., & Weitzmann, M. N. (2007). Endogenous TNFα lowers maximum peak bone mass and inhibits osteoblastic Smad activation through NF-κB. *Journal of Bone and Mineral Research*, *22*(5), 646-655.

Li, Q., & Verma, I. M. (2002). NF-κB regulation in the immune system. *Nature Reviews Immunology*, *2*(10), 725-734.

Lieverse, A. R. (1999). Diet and the aetiology of dental calculus. *IJO*, *9*(4), 219-232.

Linares, O. F. (1968). *Cultural chronology of the Gulf of Chiriqui, Panama* (No. 8). Smithsonian Institution Press.

Linares, O. F. (1977). Ecology and the arts in ancient Panama: on the development of social rank and symbolism in the central provinces. *Studies in Pre-Columbian art and archaeology*, (17), 1-86.

Listi, G. A. (2011). Bioarchaeological analysis of diet during the Coles Creek period in the southern Lower Mississippi Valley. *AJPA*, *144*(1), 30-40.

Locascio, W. A. (2010). *Communal tradition and the nature of social inequality among the prehispanic households of El Hatillo (HE-4), Panamá* (Doctoral dissertation, University of Pittsburgh).

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity?. *Oikos*, *88*(1), 87-98.

Loos, B. G. (2005). Systemic markers of inflammation in periodontitis. *Journal of periodontology*, *76*, 2106-2115.

Lothrop, S. (1937). Coclé: An archaeological study of Central Panama, vol. 1. *Cambridge: Memoirs of the Peabody Museum of American Archaeology and Ethnology*.

Lothrop, S.K., 1954. Suicide, sacrifice and mutilations in burials at Venado Beach, Panama. AA, 19(3), 226-234.

Love, R. M., & Jenkinson, H. F. (2002). Invasion of dentinal tubules by oral bacteria. *Critical reviews in oral biology & medicine*, *13*(2), 171-183.

Love, R. M., McMillan, M. D., Park, Y., & Jenkinson, H. F. (2000). Coinvasion of Dentinal Tubules by Porphyromonas gingivalis and Streptococcus gordonii Depends upon Binding Specificity of Streptococcal Antigen I/II Adhesin. *Infection and immunity*, *68*(3), 1359-1365.

Lüdecke, D. (2018). sjmisc: Data and Variable Transformation Functions. *Journal of Open Source Software*, **3**(26), 754.

Lukacs, J. R. (1989). Dental paleopathology: methods for reconstructing dietary patterns. In *Reconstruction of Life from the Skeleton*, eds. M. Y. Íscan and K. A. Kennedy, Wiley-Liss, pp. 261-286.

Lynam, M.J. and Cowley, S., 2007. Understanding marginalization as a social determinant of health. *Critical Public Health*, *17*(2), pp.137-149.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., Hornik, K.(2019). *cluster: Cluster Analysis Basics and Extensions.* R package version 2.1.0.

Maes, M. (2011). Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(3), 664-675.

Marklein, K. E., Leahy, R. E., & Crews, D. E. (2016). In sickness and in death: Assessing frailty in human skeletal remains. *AJPA*, *161*(2), 208-225.

Marmot, M., Friel, S., Bell, R., Houweling, T.A., Taylor, S. and Commission on Social Determinants of Health, 2008. Closing the gap in a generation: health equity through action on the social determinants of health. The Lancet, 372(9650), 1661-1669.

Marriott, I. (2004). Osteoblast responses to bacterial pathogens. *Immunologic research*, *30*(3), 291-308.

Martin, T. J., Romas, E., & Gillespie, M. T. (1998). Interleukins in the control of osteoclast differentiation. *Critical Review in Eukaryotic Gene Expression*, *8*(2).

Martínez-Polanco, M. F., & Cooke, R. G. (2019). Zooarchaeological and taphonomical study of the white-tailed deer (Cervidae: Odocoileus virginianus Zimmerman 1780) at Sitio Sierra, a pre-Columbian village in Pacific Coclé province, Panama, with an evaluation of its role in feasts. *Archaeological and Anthropological Sciences*, *11*(10), 5405-5422.

Martínez-Polanco, M. F., Ranere, A., & Cooke, R. G. (2020). Following white-tailed deer to the hilltop: A zooarchaeological and taphonomic analysis of deer hunting at Cerro Mangote, a Late Preceramic (7800-4600 cal yr BP) site in central Pacific Panama. *Quaternary International*.

Martín-Rincón, J.G., Cooke, R.G., Bustamante, F., Holst, I., Lara, A.,, and Redwood, S. (2016). Ocupaciones Prehispánicas En Isla Pedro González, Archipiélago de Las Perlas, Panamá: Aproximación a Una Cronología Con Comentarios Sobre Las Conexiones Externas. *LAA* 27 (3): 378–396.

Martín-Rincón, J.G. and Sánchez Herrera, L. A. (2007). El Istmo Mediterráneo: Intercambio, Simbolismo y Filiación Social En La Bahía de Panamá Durante El Período 500-1000 DC. *Arqueología Del Área Intermedia* 7: 113–122.

Martyr D'Anghiera, P. (1912). *De orbe novo: the eight decades of Peter Martyr D'Anghera* (Vol. 2). New York: GP Putnam's Sons.

Mason, J. A., & Oesher, P. H. (1942). New Excavations at the Sitio Conté, Coclé, Panama. In *Proceedings of the 8th American Scientific Congress* (Vol. 2, pp. 103-107).

Masterson, E. E., Fitzpatrick, A. L., Enquobahrie, D. A., Mancl, L. A., Eisenberg, D. T., Conde, E., & Hujoel, P. P. (2018). Dental enamel defects predict adolescent health indicators: a cohort study among the Tsimane'of Bolivia. *AJHB*, *30*(3), e23107.

Matzelle, M. M., Gallant, M. A., Condon, K. W., Walsh, N. C., Manning, C. A., Stein, G. S., ... & Gravallese, E. M. (2012). Resolution of inflammation induces osteoblast function and regulates the Wnt signaling pathway. *Arthritis & Rheumatism*, *64*(5), 1540-1550.

Mayhall, J. T. (2000). Dental morphology: techniques and strategies. In *Biological Anthropology of the Human Skeleton*, eds. Katzenberg, M.A. and Saunders, S.R., Wiley-Liss, pp. 103-134.

Mayo Torné, J., & Mayo, C. (2013). The discovery of an elite cemetery at El Caño: Traces of a mortuary pattern in Río Grande valley, Coclé, Panama. *Arqueología Iberoamericana*, (20), 3-27.

McCutcheon, A. L. (1987). *Latent class analysis* (No. 64). Sage.

McDade, T. W. (2003). Life history theory and the immune system: steps toward a human ecological immunology. *AJPA*, *122*(S37), 100-125.

McDade, T. W. (2005a). Life history, maintenance, and the early origins of immune function. *AJHB*, *17*(1), 81-94.

McDade, T. W. (2005b). The ecologies of human immune function. *Annu. Rev. Anthropol.*, *34*, 495-521.

McDade, T. W. (2012). Early environments and the ecology of inflammation. *PNAS*, *109*(Supplement 2), 17281-17288.

McDade, T. W., Beck, M. A., Kuzawa, C., & Adair, L. S. (2001a). Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. *The American journal of clinical nutrition*, *74*(4), 543-548.

McDade, T. W., Beck, M. A., Kuzawa, C. W., & Adair, L. S. (2001b). Prenatal undernutrition and postnatal growth are associated with adolescent thymic function. *The Journal of nutrition*, *131*(4), 1225-1231.

McDade, T. W., Georgiev, A. V., & Kuzawa, C. W. (2016). Trade-offs between acquired and innate immune defenses in humans. *Evolution, medicine, and public health*, *2016*(1), 1-16.

McDade, T. W., Rutherford, J., Adair, L., & Kuzawa, C. W. (2010). Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1684), 1129-1137.

McDade, T. W., Ryan, C., Jones, M. J., MacIsaac, J. L., Morin, A. M., Meyer, J. M., ... & Kuzawa, C. W. (2017). Social and physical environments early in development predict DNA methylation of inflammatory genes in young adulthood. *PNAS*, *114*(29), 7611-7616.

McElhaney, J. E., & Effros, R. B. (2009). Immunosenescence: what does it mean to health outcomes in older adults?. *Current opinion in immunology*, *21*(4), 418-424.

McEwen, B. S. (1998a). Protective and damaging effects of stress mediators. *New England journal of medicine*, *338*(3), 171-179.

McEwen, B. S. (1998b). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York academy of sciences*, *840*(1), 33-44.

McGimsey, C. R. (1956). Cerro Mangote: a preceramic site in Panama. *AA*, *22*(2), 151-161.

McGimsey, C.R., Collins, M.B., and McKern, T.W. (1987). Cerro Mangote and its population. *Journal of the Steward Anthropological Society*, 16: 125-157.

McQueen, P., Ghaffar, S., Guo, Y., Rubin, E. M., Zi, X., & Hoang, B. H. (2011). The Wnt signaling pathway: implications for therapy in osteosarcoma. *Expert review of anticancer therapy*, *11*(8), 1223-1232.

Meyer, C., Ganslmeier, R., Dresely, V., & Alt, K. W. (2012). New approaches to the reconstruction of kinship and social structure based on bioarchaeological analysis of Neolithic multiple and collective graves. *Theoretical and methodological considerations in Central European Neolithic archaeology*, 11-23.

Meyer, C., Kranzbühler, J., Drings, S., Bramanti, B., Nehlich, O., Richards, M. P. and Alt, K. W. (2008). Die menschlichen Skelettfunde aus der neolithischen Totenhütte von Benzingerode. Anthropologische Untersuchungen an den Bestattungen eines Kollektivgrabs der Bernburger Kultur. *Archäologie in Sachsen-Anhalt Sonderband*, 7: 107-151.

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological psychiatry*, *65*(9), 732-741.

Milner, G. R., & Boldsen, J. L. (2012). Transition analysis: A validation study with known-age modern American skeletons. *AJPA*, *148*(1), 98-110.

Møller, A. P., & Pomiankowski, A. (1993). Fluctuating asymmetry and sexual selection. *Genetica*, *89*(1-3), 267.

Monasta, L., Batty, G. D., Cattaneo, A., Lutje, V., Ronfani, L., Van Lenthe, F. J., & Brug, J. (2010). Early-life determinants of overweight and obesity: a review of systematic reviews. *Obesity reviews*, *11*(10), 695-708.

Monk, C., Lugo-Candelas, C., & Trumpff, C. (2019). Prenatal developmental origins of future psychopathology: mechanisms and pathways. *Annual review of clinical psychology*, *15*, 317-344.

Moore, S. E., Cole, T. J., Collinson, A. C., Poskitt, E. M., McGregor, I. A., & Prentice, A. M. (1999). Prenatal or early postnatal events predict infectious deaths in young adulthood in rural Africa. *International journal of epidemiology*, *28*(6), 1088-1095.

Moore, S. E., Collinson, A. C., N'Gom, P. T., Aspinall, R., & Prentice, A. M. (2006). Early immunological development and mortality from infectious disease in later life. *Proceedings of the Nutrition Society*, *65*(3), 311-318.

Moore, M. K., DiGangi, E. A., Ruíz, F. P. N., Davila, O. J. H., & Medina, C. S. (2016). Metric sex estimation from the postcranial skeleton for the Colombian population. *Forensic science international*, *262*, 286-e1.

Moore, S. E., Fulford, A. J., Darboe, M. K., Jobarteh, M. L., Jarjou, L. M., & Prentice, A. M. (2012). A randomized trial to investigate the effects of pre-natal and infant nutritional supplementation on infant immune development in rural Gambia: the ENID trial: Early Nutrition and Immune Development. *BMC pregnancy and childbirth*, *12*(1), 107.

Moorrees, C. F., Fanning, E. A., & Hunt Jr, E. E. (1963). Age variation of formation stages for ten permanent teeth. *Journal of dental research*, *42*(6), 1490-1502.

Morales Arce, A. Y. (2017). Ancient mitochondrial DNA in Mesoamerica and its Borderlands. The cases of Paquimé (AD 1200-1450), Greater Nicoya (AD 800-1250), and Central Mexico (AD 900-1519). (Doctoral dissertation, University of Calgary).

Morita, W., Yano, W., Nagaoka, T., Abe, M., Ohshima, H., & Nakatsukasa, M. (2014). Patterns of morphological variation in enamel–dentin junction and outer enamel surface of human molars. *Journal of anatomy*, *224*(6), 669-680.

Moustaki, I., & Papageorgiou, I. (2005). Latent class models for mixed variables with applications in Archaeometry. *Computational statistics & data analysis*, *48*(3), 659-675.

Muthén, B. (2004). Latent variable analysis. *The Sage handbook of quantitative methodology for the social sciences*, *345*(368), 106-109.

Muthén, L. K., & Muthén, B. (2019). Mplus. *The comprehensive modelling program for applied researchers: user's guide*, *5*.

National Center for Health Statistics. (2019). Health, United States, 2018. National Center for Health Statistics.

Newman, S. L., & Gowland, R. L. (2017). Dedicated followers of fashion? Bioarchaeological perspectives on socio-economic status, inequality, and health in urban children from the industrial revolution (18th–19th C), England. *International Journal of Osteoarchaeology*, *27*(2), 217-229.

Norr, L.C. (1991). Nutritional consequences of prehistoric subsistence strategies in lower Central America. Doctoral dissertation, Urbana: University of Illinois at Urbana-Champaign.

Norr, L.C. (1995). Interpreting dietary maize from bone stable isotopes in the American tropics: the state of the art. *Archaeology in the lowland American tropics: Current analytical methods and recent applications*, pp.198-223.

Nylund, K. L. (2007). *Latent transition analysis: Modeling extensions and an application to peer victimization*. Doctoral dissertation, University of California, Los Angeles.

Nylund, K. L., Asparouhov, T., & Muthén, B. O. (2007). Deciding on the number of classes in latent class analysis and growth mixture modeling: A Monte Carlo simulation study. *Structural equation modeling: A multidisciplinary Journal*, *14*(4), 535-569.

O'Byrne, K.J. and Dalgleish, A.G., 2001. Chronic immune activation and inflammation as the cause of malignancy. *British journal of cancer*, *85*(4), p.473.

O'Donnell, L., & Moes, E. (2020). Increased dental fluctuating asymmetry is associated with active skeletal lesions, but not mortality hazards in the precontact Southwest United States. *AJPA*.

O'Gradaigh, D., & Compston, J. E. (2003). T-cell involvement in osteoclast biology: implications for rheumatoid bone erosion. *Rheumatology*, *43*(2), 122-130.

Ortner, D.J. (1991). Theoretical and methodological issues in pale-opathology. In *Human paleopathology: current syntheses and future options*, eds. D. Ortner and A. Aufderheide, Smithsonian Institution Press, pp. 5–11.

Ortner, D. J. (2007). Differential diagnosis of skeletal lesions in infectious disease. *Advances in human palaeopathology*, 189-214.

Oviedo y Valdés, G. F. (1944). *Historia General y Natural de las Indias, Islas, y Tierra-firme del Mar Océano, 4 vols*. Banco de América, Managua.

Ozanne, S. E., & Constância, M. (2007). Mechanisms of disease: the developmental origins of disease and the role of the epigenotype. *Nature clinical practice Endocrinology & metabolism*, *3*(7), 539-546.

Palubeckaitė, Ž., Jankauskas, R., & Boldsen, J. (2002). Enamel hypoplasia in Danish and Lithuanian late medieval/early modern samples: a possible reflection of child morbidity and mortality patterns. *International Journal of Osteoarchaeology*, *12*(3), 189-201.

Pan, W. K. Y., Erlien, C., & Bilsborrow, R. E. (2010). Morbidity and mortality disparities among colonist and indigenous populations in the Ecuadorian Amazon. *Social science & medicine*, *70*(3), 401-411.

Parsons, P. A. (1990). Fluctuating asymmetry: an epigenetic measure of stress. *Biological Reviews of the Cambridge Philosophical Society*, *65*(2), 131-145.

Parsons, P. A. (1992). Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity*, *68*(4), 361-364.

Passalacqua, N. V. (2012). *Bioarchaeological Investigations of Health and Demography in Medieval Asturias, Spain*. Doctoral dissertation, Michigan State University.

Passalacqua, N. V., Zhang, Z., & Pierce, S. J. (2013). Sex determination of human skeletal populations using latent profile analysis. *AJPA*, *151*(4), 538-543.

Pellanda, L. C., Duncan, B. B., Vigo, A., Rose, K., Folsom, A. R., Erlinger, T. P., & ARIC investigators. (2009). Low birth weight and markers of inflammation and endothelial activation in adulthood: the ARIC study. *International journal of cardiology*, *134*(3), 371-377.

Petersen, H. C. (2000). On statistical methods for comparison of intrasample morphometric variability: Zalavár revisited. *AJPA*, *113*(1), 79-84.

Pilloud, M. A., & Kenyhercz, M. W. (2016). Dental metrics in biodistance analysis. In *Biological distance analysis*, ed. Pilloud, M. A., & Kenyhercz, M. W. Academic Press, 135-155.

Pilloud, M. A., & Larsen, C. S. (2011). "Official" and "practical" kin: Inferring social and community structure from dental phenotype at Neolithic Çatalhöyük, Turkey. *AJPA*, *145*(4), 519-530.

Piperno, D. R. (2011). The origins of plant cultivation and domestication in the New World tropics: patterns, process, and new developments. *Current anthropology*, *52*(S4), S453-S470.

Piperno, D. R., & Holst, I. (1998). The presence of starch grains on prehistoric stone tools from the humid neotropics: indications of early tuber use and agriculture in Panama. *Journal of Archaeological Science*, *25*(8), 765-776.

Piperno, D. R., Ranere, A. J., Holst, I., & Hansell, P. (2000). Starch grains reveal early root crop horticulture in the Panamanian tropical forest. *Nature*, *407*(6806), 894-897.

Preston, S., Heuveline, P., & Guillot, M. (2000). Demography: measuring and modeling population processes. *Malden, MA: Blackwell Publishers*.

Prevedorou, E., & Stojanowski, C. M. (2017). Biological kinship, postmarital residence and the emergence of cemetery formalisation at Prehistoric Marathon. *International Journal of Osteoarchaeology*, *27*(4), 580-597.

Radcliffe-Brown, A. R. (1930). The social organization of Australian tribes. *Oceania*, *1*(1), 34-63.

Ragsdale, B. D., & Lehmer, L. M. (2012). A knowledge of bone at the cellular (histological) level is essential to paleopathology. *A companion to paleopathology*, 227-249.

Rahman, I., & Adcock, I. M. (2006). Oxidative stress and redox regulation of lung inflammation in COPD. *European respiratory journal*, *28*(1), 219-242.

Ranere, A.J. (1980). The Re-excavation and reinterpretation of Cerro Mangote: A Preceramic shellmidden in Central Panama. Unpublished report.

Raqib, R., Alam, D. S., Sarker, P., Ahmad, S. M., Ara, G., Yunus, M., ... & Fuchs, G. (2007). Low birth weight is associated with altered immune function in rural Bangladeshi children: a birth cohort study. *The American journal of clinical nutrition*, *85*(3), 845-852.

Reichel-Dolmatoff, G. (1976). Training for the Priesthood among the Kogi of Colombia. *UCLA Latin American Studies Series and Latin American Studies Los Angeles*, 37: 265-288.

Reichel-Dolmatoff, G. (1985). *Los kogi: una tribu de la Sierra Nevada de Santa Marta, Colombia* (Vol. 2, 1949-1950). Bogotá: Procultura.

Reid, N. (1994). A conversation with sir david cox. *Statistical Science*, *9*(3), 439-455.

Reid, D. J., & Dean, M. C. (2006). Variation in modern human enamel formation times. *Journal of human evolution*, *50*(3), 329-346.

Reilly, J. J., Armstrong, J., Dorosty, A. R., Emmett, P. M., Ness, A., Rogers, I., ... & Sherriff, A. (2005). Early life risk factors for obesity in childhood: cohort study. *Bmj*, *330*(7504), 1357.

Reitsema, L. J., Vercellotti, G., & Boano, R. (2016). Subadult dietary variation at Trino Vercellese, Italy, and its relationship to adult diet and mortality. *AJPA*, *160*(4), 653-664.

Ritter, M. A., and Crispe, I. N., (1992). *The Thymus.* Oxford: IRL Press.

Roberts, C. A., & Manchester, K. (2007). *The archaeology of disease*. Cornell University Press.

Rojas-Sepúlveda, C. M., Rivera-Sandoval, J., & Martín-Rincón, J. G. (2011). Paleoepidemiology of pre-Columbian and Colonial Panamá Viejo: a preliminary study. *Bulletins et mémoires de la Société d'anthropologie de Paris*, *23*(1-2), 70-82.

Rose, J. C., Armelagos, G. J., & Lallo, J. W. (1978). Histological enamel indicator of childhood stress in prehistoric skeletal samples. *AJPA*, *49*(4), 511-516.

RStudio Team (2020). RStudio: Integrated Development for R. *RStudio*, PBC: Boston.

Sahlins, M. D. (1961). The Segmentary Lineage: An Organization of Predatory Expansion 1. *American anthropologist*, *63*(2), 322-345.

Salin, K., Kankaanpää, A., Hirvensalo, M., Lounassalo, I., Yang, X., Magnussen, C. G., ... &

Tammelin, T. H. (2019). Smoking and physical activity trajectories from childhood to midlife. *International journal of environmental research and public health*, *16*(6), 974.

Sato, K., & Takayanagi, H. (2006). Osteoclasts, rheumatoid arthritis, and osteoimmunology. *Current opinion in rheumatology*, *18*(4), 419-426.

Sattar, N., McConnachie, A., O'Reilly, D., Upton, M. N., Greer, I. A., Smith, G. D., & Watt, G. (2004). Inverse association between birth weight and C-reactive protein concentrations in the MIDSPAN Family Study. *Arteriosclerosis, thrombosis, and vascular biology*, *24*(3), 583-587.

Scheuer, L., & Black, S. (2004). *The juvenile skeleton*. New York: Elsevier.

Schillaci, M. A., & Stojanowski, C. M. (2003). Postmarital residence and biological variation at Pueblo Bonito. *AJPA*, *120*(1), 1-15.

Schneider, D. M. (1984). *A Critique of the Study of Kinship*. University of Michigan Press.

Scott, G. R., & Turner, C. G. (1997). *Anthropology of modern human teeth* (pp. 382-p). Cambridge: Cambridge University Press.

Seckl, J. R., & Holmes, M. C. (2007). Mechanisms of disease: glucocorticoids, their placental metabolism and fetal'programming'of adult pathophysiology. *Nature clinical practice Endocrinology & metabolism*, *3*(6), 479-488.

Sharpe, A. E., Smith-Guzmán, N., Curtis, J., Isaza-Aizpurúa, I., Kamenov, G. D., Wake, T. A., and Cooke, R. G. (2021). Preliminary Multi-Isotope Assessment of Human Mobility and Diet in Pre-Columbian Panama. *Journal of Archaeological Science: Reports 36: 102876.*

Shaw, A. C., Joshi, S., Greenwood, H., Panda, A., & Lord, J. M. (2010). Aging of the innate immune system. *Current opinion in immunology*, *22*(4), 507-513.

Shirtcliff, E. A., Coe, C. L., & Pollak, S. D. (2009). Early childhood stress is associated with elevated antibody levels to herpes simplex virus type 1. *Proceedings of the National Academy of Sciences*, *106*(8), 2963-2967.

Sjøvold, T. (1976). A method for familial studies based on minor skeletal variants. *Ossa*, *3*(4), 97-107.

Sjøvold, T. (1977). *Non-metrical divergence between skeletal populations: the theoretical foundation and biological importance of CAB Smith's mean measure of divergence.* (Doctoral dissertation, Stockholm University).

Smith-Guzmán, N.E., and Berger, S.M. N.d. Genetic anomaly or congenital syphilis? Supernumerary occlusal cusps in a molar from an early pre-Columbian burial at Cerro Juan Díaz, Panama.

Smith-Guzmán, N.E., and Cooke, R.G. (2018). Interpersonal violence at Playa Venado, Panama (550-850 AD): A reevaluation of the evidence. *LAA*, pp. 1-18.

Smith-Guzmán, N. E., & Cooke, R. G. (2019). Cold-water diving in the tropics? External auditory exostoses among the pre-Columbian inhabitants of Panama. *AJPA*, *168*(3), 448-458.

Smith, A. K., Reitsema, L., Fornaciari, A., and Sineo, L. (2019). A multifactorial approach to the developmental origins of infectious disease: comparing results from dental histology, paleopathology, and stable isotope analysis. *AJPA,* 171, 265-266.

Staquet, M. J., Durand, S. H., Colomb, E., Romeas, A., Vincent, C., Bleicher, F., ... & Farges, J. C. (2008). Different roles of odontoblasts and fibroblasts in immunity. *Journal of dental research*, *87*(3), 256-261.

Steeve, K. T., Marc, P., Sandrine, T., Dominique, H., & Yannick, F. (2004). IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. *Cytokine & growth factor reviews*, *15*(1), 49-60.

Stein, Z., Susser, M., Saenger, G., & Marolla, F. (1975). Famine and human development: The Dutch hunger winter of 1944-1945.

Stevens, J. P. (2012). *Applied multivariate statistics for the social sciences*. New York: Routledge.

Stiemsma, L. T., & Michels, K. B. (2018). The role of the microbiome in the developmental origins of health and disease. *Pediatrics*, *141*(4).

Stirling, M. W. (1949). Exploring the past in Panama. *National geographic*, *95*(3), 373-400.

Stojanowski, C. M. (2003). Matrix decomposition model for investigating prehistoric intracemetery biological variation. *AJPA*, *122*(3), 216-231.

Stojanowski, C. M. (2005). The bioarchaeology of identity in Spanish colonial Florida: Social and evolutionary transformation before, during, and after demographic collapse. *American Anthropologist*, *107*(3), 417-431.

Stojanowski, C. M. (2007). Comment on "Alternative dental measurements" by Hillson et al. *AJPA*, *132*(2), 234-237.

Stojanowski, C. M., & Carver, C. L. (2011). Inference of emergent cattle pastoralism in the southern Sahara desert based on localized hypoplasia of the primary canine. *International journal of paleopathology*, *1*(2), 89-97

Stojanowski, C. M., & Larsen, C. S. (2013). *Mission cemeteries, mission peoples: Historical and evolutionary dimensions of intracemetery bioarchaeology in Spanish Florida*. University Press of Florida.

Stojanowski, C. M., & Hubbard, A. R. (2017). Sensitivity of dental phenotypic data for the identification of biological relatives. *IJO*, *27*(5), 813-827.

Stojanowski, C. M., & Schillaci, M. A. (2006). Phenotypic approaches for understanding patterns of intracemetery biological variation. *AJPA*, *131*(S43), 49-88.

Stout, David (1947). *San Blas Cuna Acculturation: An Introduction.* New York: Viking Fund.

Takayanagi, H. (2007). Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nature Reviews Immunology*, *7*(4), 292.

Takayanagi, H. (2012). New developments in osteoimmunology. *Nature Reviews Rheumatology*, *8*(11), 684.

Tayles, N., and Oxenham, M. (2006). Introduction: Southeast Asian bioarchaeology past and present. In Bioarchaeology of Southeast Asia (Vol. 43), eds. M. Oxenham and N. Tayles, 1-30. Cambridge: Cambridge University Press.

Temple, D. H. (2014). Plasticity and constraint in response to early-life stressors among late/final jomon period foragers from Japan: Evidence for life history trade-offs from incremental microstructures of enamel. *AJPA*, *155*(4), 537-545.

Temple, D. H. (2019). Bioarchaeological evidence for adaptive plasticity and constraint: Exploring life-history trade-offs in the human past. *Evolutionary Anthropology: Issues, News, and Reviews*, *28*(1), 34-46.

Thayer, Z., Barbosa-Leiker, C., McDonell, M., Nelson, L., Buchwald, D., & Manson, S. (2017). Early life trauma, post-traumatic stress disorder, and allostatic load in a sample of American Indian adults. *AJHB*, *29*(3), e22943.

Thayer, Z. M., & Kuzawa, C. W. (2014). Early origins of health disparities: Material deprivation predicts maternal evening cortisol in pregnancy and offspring cortisol reactivity in the first few weeks of life. AJHB, 26, 723–730.

Thompson, A. L. (2012). Developmental origins of obesity: early feeding environments, infant growth, and the intestinal microbiome. *AJHB*, *24*(3), 350-360.

Tice, K. E. (1995). *Kuna crafts, gender, and the global economy*. Austin: University of Texas Press.

Torné, J. M., Torné, C. M., Bueno, M. G., Herrera, M. Á. H., & López, J. H. (2020). Approach to the Study of the Phenomenon of Multiple Burials at El Caño, Panama. *LAA*, *31*(1), 20-39.

Tsukasaki, M., & Takayanagi, H. (2019). Osteoimmunology: evolving concepts in bone–immune interactions in health and disease. *Nature Reviews Immunology*, *19*(10), 626-642.

Tucker, A., & Sharpe, P. (2004). The cutting-edge of mammalian development; how the embryo makes teeth. *Nature Reviews Genetics*, *5*(7), 499-508.

Turner, C.G., Nichol, C.R., and Scott, G.R. (1991). Scoring procedures for key morphological traits of the permanent dentition: The Arizona State University Dental Anthropology System. In *Advances in Dental Anthropology*, eds. C.S. Larsen, M.A. Kelley, Wiley-Liss, pp. 13-31.

Urlacher, S. S., Ellison, P. T., Sugiyama, L. S., Pontzer, H., Eick, G., Liebert, M. A., ... & Snodgrass, J. J. (2018). Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. *PNAS*, *115*(17), E3914-E3921.

Vach, W., & Alt, K. W. (1993). Detection of kinship structures in prehistoric burial sites based on odontological traits. *Computing the past. Oxford: Alden Press. p*, 287-21.

van Buuren, S., and Groothuis-Oudshoorn, K. (2011). mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software, 45(3), 1-67.

Van den Bergh, B. R., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., ... & Schwab, M. (2017). Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. *Neuroscience & Biobehavioral Reviews*.

Van Dyke, T. E. (2020). Shifting the paradigm from inhibitors of inflammation to resolvers of inflammation in periodontitis. *Journal of Periodontology*, *91*, S19-S25.

Vaupel, J. W. (1986). How change in age-specific mortality affects life expectancy. *Population studies*, *40*(1), 147-157.

Vaupel, J.W., Manton, K.G. and Stallard, E., (1979). The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography*, *16*(3), pp.439-454.

Velasco, M. C. (2018). Open sepulchers and closed boundaries? Biodistance analysis of cemetery structure and postmarital residence in the late prehispanic Andes. *AJPA*, *166*(4), 906-920.

Vercellotti, G., Piperata, B. A., Agnew, A. M., Wilson, W. M., Dufour, D. L., Reina, J. C., ... & Sciulli, P. W. (2014). Exploring the multidimensionality of stature variation in the past through comparisons of archaeological and living populations. *AJPA*, *155*(2), 229-242.

Vermunt, J. K., & Magidson, J. (2005). A comparison between traditional and latent class. *New developments in categorical data analysis for the social and behavioral sciences*, 41-62.

Wade, W. G. (2013). The oral microbiome in health and disease. *Pharmacological research*, *69*(1), 137-143.

Wadhwa, P. D., Buss, C., Entringer, S., & Swanson, J. M. (2009, September). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. In *Seminars in reproductive medicine* (Vol. 27, No. 5, p. 358). NIH Public Access.

Wadsworth, M. E., Cripps, H. A., Midwinter, R. E., & Colley, J. R. (1985). Blood pressure in a national birth cohort at the age of 36 related to social and familial factors, smoking, and body mass. *Br Med J (Clin Res Ed)*, *291*(6508), 1534-1538.

Waldron, T. (2009). *Paleopathology.* Cambridge: Cambridge University Press.

Walsh, M. C., Kim, N., Kadono, Y., Rho, J., Lee, S. Y., Lorenzo, J., & Choi, Y. (2006). Osteoimmunology: interplay between the immune system and bone metabolism. *Annu. Rev. Immunol.*, *24*, 33-63.

Walsh, M. C., Takegahara, N., Kim, H., & Choi, Y. (2018). Updating osteoimmunology: regulation of bone cells by innate and adaptive immunity. *Nature Reviews Rheumatology*, *14*(3), 146.

Warinner, C., Speller, C., & Collins, M. J. (2015). A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1660), 20130376.

Warner, D.F. and Brown, T.H., 2011. Understanding how race/ethnicity and gender define age-trajectories of disability: An intersectionality approach. *Social Science & Medicine*, *72*(8), pp.1236-1248.

Waterland, R. A., & Michels, K. B. (2007). Epigenetic epidemiology of the developmental origins hypothesis. *Annu. Rev. Nutr.*, *27*, 363-388.

Watts, R. (2011). Non-specific indicators of stress and their association with age at death in medieval york: using stature and vertebral neural canal size to examine the effects of stress occurring during different periods of development. *International Journal of Osteoarchaeology*, *21*(5), 568-576.

Watts, R. (2013). Childhood development and adult longevity in an archaeological population from Barton-upon-Humber, Lincolnshire, England. *International Journal of Paleopathology*, *3*(2), 95-104.

Watts, R. (2015). The long-term impact of developmental stress. Evidence from later medieval and post-medieval London (AD1117–1853). *AJPA*, *158*(4), 569-580.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., ... & Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature neuroscience*, *7*(8), 847-854.

Weisensee, K. E. (2013). Assessing the relationship between fluctuating asymmetry and cause of death in skeletal remains: a test of the developmental origins of health and disease hypothesis. *AJHB*, *25*(3), 411-417.

Wells, J. C. (2010). Maternal capital and the metabolic ghetto: an evolutionary perspective on the transgenerational basis of health inequalities. *AJHB*, *22*(1), 1-17.

Wells, J. C. K. (2007). The thrifty phenotype as an adaptive maternal effect. *Biological Reviews*, *82*(1), 143-172.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

White, T. D. (1978). Early hominid enamel hypoplasia. *AJPA*, *49*(1), 79-83.

Willey, G. R., McGimsey, C. R., & Greengo, R. E. (1954). *The Monagrillo culture of Panama: with an appendix on Archaeological marine shells*. Peabody Museum of Archaeology and Ethnology, Harvard University.

Williams, A. D., Blackwell, S. E., Mackenzie, A., Holmes, E. A., & Andrews, G. (2013). Combining imagination and reason in the treatment of depression: A randomized controlled trial of internet-based cognitive-bias modification and internet-CBT for depression. *Journal of consulting and clinical psychology*, *81*(5), 793.

Wilson, J. J. (2014). Paradox and promise: Research on the role of recent advances in paleodemography and paleoepidemiology to the study of "health" in Precolumbian societies. *AJPA*, *155*(2), 268-280.

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., ... & Wilkinson, R. G. (1992). The osteological paradox: problems of inferring prehistoric health from skeletal samples [and comments and reply]. *Current anthropology*, *33*(4), 343-370.

World Health Organization. (2015). *World health statistics 2015*. World Health Organization.

Worthman, C. M., & Kuzara, J. (2005). Life history and the early origins of health differentials. AJHB, 17, 95–112.

Yajnik, C. S. (2004). Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. *The Journal of nutrition*, *134*(1), 205-210.

Yaussy, S. L. (2019). The intersections of industrialization: Variation in skeletal indicators of frailty by age, sex, and socioeconomic status in 18th-and 19th-century England. *AJPA*, *170*(1), 116-130.

Yaussy, S. L., & DeWitte, S. N. (2018). Patterns of frailty in non-adults from medieval London. *International journal of paleopathology*, *22*, 1-7.

Zohar, I., and G. Cooke, R. G. (1997). The Impact of Salting and Drying on Fish Bones: Preliminary Observations on Four Marine Species from Parita Bay, Panama. *Archaeofauna* 6, 59–66.